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Biodiversity and Environmental Adaptation in Small Ruminants: Mapping Temporal and Spatial Genomic Changes in Italian Sheep and Goats

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ABSTRACT

Livestock biodiversity represents a critical resource for ensuring food security, cultural heritage, and resilience in the face of global change. Yet, this biodiversity is under increasing threat from genetic erosion, unsustainable management, and the accelerating impacts of climate change. Preserving the adaptive potential of small ruminants is particularly urgent, as these species play key ecological and socio-economic roles in many regions. This thesis addresses these challenges by combining ecological, demographic, and genomic perspectives to explore the diversity, adaptation, and vulnerability of goats and sheep, with a special focus on the Italian context.

The first part (Chapter 2.1) examines the domestication and evolutionary history of goats. Reviewing archaeological, historical, and genomic evidence, it traces the species' trajectory from early domestication events to modern breeding practices. This overview provides the conceptual background needed to understand how past processes have shaped present-day genetic diversity, and why a long-term perspective is essential when studying current patterns of adaptation and resilience.

The second part (Chapters 3.1–3.2) provides a comprehensive assessment of the geographical distribution, environmental context, and potential vulnerabilities of Italian sheep and goat breeds, addressing priorities highlighted by the FAO. For the first time, detailed maps were created for all Italian breeds based on the geolocalisation of over 3,700 individual farms, allowing a breed-specific characterisation of breeding ranges and their exposure to climatic and environmental risks. Chapter 3.1 integrates geolocated breed data with seven decades of summer climatic information, revealing a clear increase in average temperatures, temperature–humidity index, and heat waves over the last decades. Data revealed that the greatest changes occurred in northern Italy, while southern breeds, though raised in harsher environments, experienced relatively more stable conditions, highlighting their value as case studies for

resilience. Chapter 3.2 extends this approach by evaluating exposure to natural hazards together with future climate projections and landscape characterisation. Results reveal that Italian small ruminants are especially exposed to seismic risk, mainly in southern regions, and to increasingly warmer and drier conditions, threatening in particular Alpine breeds adapted to cold environments. The identification of breed-specific landscape patterns also underscores that the resilience of local livestock depends not only on climate, but also on broader ecological and socio-environmental contexts. This integrated approach enables the identification of disparities in exposure to different environmental factors at the breed or regional level, as well as the investigation of the interactions between small ruminants and the landscapes they inhabit, providing essential information to guide conservation and adaptive management strategies in accordance with FAO priorities.

Another essential aspect of safeguarding local breeds is the accurate recognition and characterisation of their originality and adaptive potential by integrating historical, phenotypic, and genomic information. Chapters 4.1 and 4.2 focus on the Sicilian local goat breed, the Comune di Sicilia (Mascaruna), a population long known to farmers and documented in historical records but not formally recognised as a distinct breed. Chapter 4.1 combines morphological and genomic analyses to characterise the population, demonstrating its distinctiveness while also detecting candidate genomic regions linked to specific phenotypic traits. Chapter 4.2 situates the Comune di Sicilia within a broader Mediterranean and African context, identifying putative Greek ancestry. Selection signature analyses highlighted genes associated with production traits, body size, fertility, coat colour, fat deposition, and horn and ear development, reflecting the breed's unique history and adaptive profile. Together, these studies provided the scientific evidence required for this breed's formal recognition, which was approved in 2025, and will contribute to developing strategies to preserve its genetic variability.

Chapters 4.3 and 4.4 extend the investigation to the national scale, providing a comprehensive genomic assessment of Italian sheep and goat biodiversity. These studies integrate population structure, genetic diversity, and genomic background analyses with temporal comparisons using historical samples, offering a novel approach to detect recent evolutionary shifts and understand local breed dynamics over the past two decades. When interpreted alongside demographic data and the broader historical, management, and socio-cultural context of Italian livestock, the results reveal how past practices and recent changes have shaped breed variability and resilience.

Specifically, Chapter 4.3 focuses on Italian sheep, highlighting critical situations in terms of population viability, with many breeds showing declining demographic trends and reduced genetic variability. While many breeds remain clearly distinguishable, strong introgression from foreign breeds (e.g., Île-de-France in Merinizzata Italiana) and increased admixture in others (e.g., Gentile di Puglia and Nera di Arbus) were detected. Temporal comparisons over twenty years identified genomic regions with the highest allele shifts, including loci related to production, reproduction, immunity, and adaptation. Integrating climatic data enabled correlations between these shifts and environmental changes, identifying SNPs associated with genes previously linked to sheep adaptation, as well as relevant pathways including immunity, oxidative stress response, alarmones, body growth, water balance, circadian rhythm, energy metabolism, and cardiovascular function.

Chapter 4.4 examines Italian goats, revealing a clear distinction between northern populations, which were generally well differentiated, and central-southern populations, which were often more admixed and shared a common ancestry. Temporal comparisons showed that most breeds maintained their genomic backgrounds, with notable exceptions such as Bianca Monticellana and Capestrina, which exhibited increased homogeneity and inbreeding. Landscape genomic approaches identified genomic regions putatively associated with environmental adaptation. Three genes were detected across multiple methods—*KPNA1*, *PARP9*, and *LRP8*—involved in immunity, fat metabolism, neuronal and behavioural development, and oxidative stress response. Additional signals included genes linked to adaptive morphological traits, immunity, and the development of urinary and digestive systems. For the first time, genomic offset analyses were applied to Italian goats to predict vulnerable areas and populations under projected climate scenarios (2080–2100), highlighting northern Alpine fringes, the eastern Po Valley, and the Murgia-Gargano area, home to the Garganica breed.

Taken together, the studies presented here provide an integrated understanding of the biodiversity of goats and sheep, from their domestication to contemporary diversity and potential future vulnerabilities. By combining historical, ecological, and genomic approaches, the work illustrates how past trajectories, current environmental conditions, and human management interact to shape the genome, drive adaptation, and influence the vulnerability of small ruminant populations, with Italy providing a particularly informative context due to its ecological heterogeneity and rich cultural traditions. These findings underscore that all these aspects should be taken into consideration to effectively guide conservation priorities and adaptive management strategies, thereby supporting the long-term survival and adaptive potential of livestock genetic resources. The results also reveal opportunities for further research, which could take advantage of emerging tools and complementary techniques—such as epigenomic profiling, sequencing approaches, or experimental validation. For example, populations identified as especially interesting from an adaptive perspective provide targets for fine-scale genomic investigations, which could refine our understanding of their ability to persist in their environment and inform tailored conservation strategies.

Finally, beyond advancing scientific knowledge, the findings provide practical tools to support the conservation, management, and sustainable use of local breeds. Close collaboration with Asso.Na.Pa. ensured that the results can directly inform the design and implementation of effective safeguarding strategies, including prioritisation of breeds for conservation, planning of breeding programs to maintain genetic variability, and monitoring of population trends over time. By bridging research and applied conservation, these outcomes offer breeders and associations concrete guidance to make evidence-based decisions that enhance the long-term resilience of Italy's small ruminant genetic resources.

RIASSUNTO

La biodiversità zootecnica rappresenta una risorsa fondamentale per la sicurezza alimentare, la conservazione del patrimonio culturale e la resilienza del settore agroalimentare di fronte ai cambiamenti in atto su scala globale. Tuttavia, questa biodiversità è in crescente pericolo a causa di fenomeni di erosione genetica, pratiche di gestione insostenibili e cambiamenti climatici. I piccoli ruminanti assumono, in molte aree del mondo, un ruolo chiave sia dal punto di vista ecologico che socioeconomico, rendendo la loro salvaguardia un tema di estremo interesse. Pertanto, la presente tesi si prefigge di affrontare tali sfide integrando studi di carattere ecologico, demografico e genomico al fine di studiare la diversità, l'adattamento e la potenziale vulnerabilità delle specie ovina e caprina, con particolare attenzione al contesto italiano.

La prima parte della tesi (Capitolo 2.1) tratta della domesticazione e della storia evolutiva delle capre, ripercorrendo il percorso di questa specie dagli esordi della sua domesticazione fino alle più moderne pratiche di allevamento, mediante l'analisi di studi archeologici, storici e genomici presenti in letteratura. Questa panoramica fornisce una base concettuale fondamentale per comprendere come gli eventi intercorsi durante l'intera storia evolutiva della specie abbiano contribuito a plasmarne l'attuale diversità genetica, influenzando lo sviluppo delle popolazioni, la selezione antropica e l'adattamento alla moltitudine di ambienti in cui questi animali vengono allevati.

Seguono i Capitoli 3.1 e 3.2, in cui si fornisce una descrizione dettagliata della distribuzione geografica, del contesto ambientale e delle potenziali vulnerabilità che caratterizzano le razze ovine e caprine italiane, in linea con quanto annoverato tra le priorità strategiche della FAO per la preservazione delle risorse genetiche animali. In particolare, questa rappresenta la prima realizzazione di mappe dettagliate della geolocalizzazione su suolo nazionale di oltre 3.700 allevamenti, il che consente una caratterizzazione precisa degli areali di allevamento di tutte le razze ovicaprine italiane e della loro esposizione a diversi fattori climatici e ambientali. Il Capitolo 3.1, in particolare, integra queste informazioni con dati climatici

relativi ai mesi estivi degli ultimi settant'anni, evidenziando un chiaro aumento delle temperature medie, dell'indice temperatura–umidità e delle ondate di calore negli ultimi decenni. Inoltre, viene riscontrato un più marcato cambiamento soprattutto nelle aree settentrionali del nostro Paese, mentre le razze meridionali, sebbene allevate in ambienti complessivamente più ardui, hanno sperimentato condizioni relativamente più stabili, diventando pertanto ottimi modelli per lo studio della resilienza ambientale. Il Capitolo 3.2 amplia questo approccio valutando l'esposizione delle razze ovine e caprine italiane a vari rischi naturali, integrando anche proiezioni climatiche future e la caratterizzazione del territorio dove le diverse popolazioni insistono. I risultati mostrano una notevole esposizione al rischio sismico, in particolare tra le popolazioni meridionali, e all'avvicendarsi di condizioni climatiche sempre più calde e secche, di particolare rilevanza per le razze alpine, storicamente adattate a climi più freddi. La caratterizzazione paesaggistica delle diverse popolazioni sottolinea come l'adattamento delle razze locali al proprio ambiente di allevamento debba tenere in considerazione non solo i fattori climatici, ma anche il più ampio contesto ecologico e socio-ambientale. Combinando tutti questi aspetti è quindi possibile, da un lato, identificare a quali differenti fattori sono diversamente esposte le regioni geografiche e le razze ovicaprine italiane, e dall'altro comprendere meglio come gli animali interagiscano con l'ambiente in cui vivono— e viceversa—, informazioni fondamentali per lo sviluppo di strategie efficaci di conservazione e gestione delle popolazioni zootecniche.

Un altro aspetto cruciale per salvaguardare le razze locali è la caratterizzazione e il riconoscimento della loro originalità e del loro potenziale adattativo sulla base dell'integrazione di informazioni storiche, fenotipiche e genomiche. In questo contesto vengono riportati due studi (Capitoli 4.1 e 4.2) relativi alla capra Comune di Sicilia (o Mascaruna), una popolazione da tempo nota agli allevatori e documentata in fonti storiche, ma mai ufficialmente riconosciuta come razza. Il Capitolo 4.1 combina analisi morfologiche e genomiche atte a caratterizzare questa popolazione, evidenziandone l'unicità e identificando regioni genomiche associate a suoi tratti fenotipici peculiari. Il Capitolo 4.2 colloca la Comune di Sicilia in un contesto geografico più ampio, suggerendone una possibile ascendenza greca. Tale studio, inoltre, analizza firme di selezione specifiche—legate a caratteristiche produttive, dimensioni corporee, fertilità, colore del mantello, deposito di grasso e sviluppo di corna e orecchie—, risultato del lungo processo di evoluzione, selezione e adattamento di questa razza. Nel loro insieme, questi due studi hanno fornito le evidenze scientifiche necessarie al riconoscimento della capra Comune di Sicilia, ottenuto nel 2025, e contribuiranno a guidare la costruzione di strategie per preservarne la variabilità genetica.

I Capitoli 4.3 e 4.4 estendono l'analisi a livello nazionale, offrendo una valutazione genomica completa della biodiversità delle pecore e delle capre italiane. Questi studi combinano analisi di struttura di popolazione, diversità genetica e background genomico con comparazioni su scala temporale, proponendo un approccio innovativo per la rilevazione di cambiamenti evolutivi recenti delle razze locali negli ultimi due decenni. Considerati insieme ai dati demografici e al più ampio contesto storico, manageriale e socioculturale, i risultati mostrano come pratiche passate e cambiamenti recenti abbiano plasmato la variabilità e la resilienza dei piccoli ruminanti.

In particolare, il Capitolo 4.3 analizza le pecore italiane, evidenziando che molte razze mostrano trend demografici in diminuzione e ridotta variabilità genetica, suscitando seri dubbi sulla loro possibilità di sopravvivenza. Seppur nella maggior parte dei casi sia possibile identificare chiaramente le diverse popolazioni, si rileva, in alcune, una marcata introgressione da parte di razze estere (ad esempio Île-de-France nella Merinizzata Italiana) e/o altre razze italiane (ad esempio Gentile di Puglia e Nera di Arbus). I confronti con campioni risalenti a circa vent'anni fa hanno inoltre consentito di rilevare quali regioni genomiche siano state sottoposte a una più marcata variazione, molte delle quali risultano essere associate a tratti produttivi e riproduttivi, alla risposta immunitaria e/o all'adattamento ambientale. Un ulteriore approfondimento su quest'ultimo aspetto, possibile grazie all'integrazione di dati relativi al cambiamento di numerose variabili climatiche nel corso del tempo, ha portato all'identificazione di SNP associati a geni noti per il loro ruolo nell'adattamento e a pathway potenzialmente rilevanti, come immunità, risposta allo stress ossidativo, crescita corporea, equilibrio idrico, ritmo circadiano, metabolismo energetico e funzionalità cardiovascolare.

Il Capitolo 4.4 si concentra sulle capre italiane, i cui dati genomici rivelano una chiara distinzione tra popolazioni settentrionali, generalmente ben differenziate, e centro-meridionali, spesso più *admixed* e caratterizzate da un pool ancestrale condiviso. I confronti temporali mostrano che la maggior parte delle razze ha mantenuto il proprio background genomico nel corso degli ultimi vent'anni, con eccezioni quali Bianca Monticellana e Capestrina, che presentano maggiore omogeneità e inbreeding rispetto al passato. Grazie a un approccio di *landscape genomics*, inoltre, si sono individuate regioni genomiche potenzialmente associate all'adattamento ambientale. In particolare, incrociando i risultati di più metodi, sono emersi tre geni—*KPNA1*, *PARP9* e *LRP8*— coinvolti nel funzionamento del sistema immunitario, nel metabolismo dei grassi, nello sviluppo neuronale e comportamentale e nella risposta allo stress ossidativo. Ulteriori segnali includono geni legati a tratti morfologici adattativi, immunità e sviluppo degli apparati escretore e digerente. In questo studio, per la prima volta, è stato stimato un valore di *genomic offset* per le capre italiane, con l'obiettivo di prevedere quali aree geografiche e popolazioni possano risultare più vulnerabili ai futuri scenari climatici, attesi per il 2080–2100. In particolare, tale analisi ha evidenziato che il confine settentrionale della regione alpina, la porzione orientale della Pianura padana e l'area della Murgia e del Gargano, dove viene allevata la razza Garganica, rappresentano le aree a maggior rischio.

Nel complesso, gli studi presentati in questa tesi forniscono quindi una comprensione multidisciplinare della biodiversità delle specie caprina ed ovina, a partire dalla loro domesticazione fino alla diversità attuale e alle possibili vulnerabilità future. Combinando approcci storici, ecologici e genomici, la presente tesi mostra come traiettorie passate, condizioni ambientali attuali e gestione antropica interagiscano per modellare il genoma, guidare l'adattamento e influenzare la vulnerabilità delle popolazioni di piccoli ruminanti in un contesto, quello italiano, particolarmente informativo, data la sua eterogeneità ecologica e le ricche tradizioni culturali che lo caratterizzano. Questi risultati evidenziano inoltre come tutti questi aspetti vadano considerati al fine di orientare le priorità conservazionistiche e le strategie di gestione delle popolazioni, sostenendo la sopravvivenza a lungo termine e il potenziale adattativo delle risorse genetiche animali. Inoltre, questi studi forniscono un punto di partenza per ulteriori indagini, che potrebbero fare uso di tecniche complementari ed innovative, come epigenomica, sequenziamento completo e validazione sperimentale. Ad esempio, le popolazioni identificate come di particolare interesse dal punto di vista adattativo possono rappresentare target per studi genomici mirati, utili per approfondire la comprensione dei meccanismi che hanno consentito loro di persistere nel loro ambiente e per sviluppare strategie di conservazione ad hoc.

Infine, i dati qui presentati offrono strumenti pratici per supportare la conservazione, la gestione e l'uso sostenibile delle razze locali. La stretta collaborazione con Asso.Na.Pa. ha infatti permesso di tradurre le evidenze scientifiche in strategie concrete, tra cui la priorizzazione delle razze per la conservazione, la pianificazione di programmi di allevamento per mantenere la variabilità genetica entro e tra popolazioni e il monitoraggio dei trend demografici. Colmando il divario tra ricerca e applicazione, questi risultati forniscono agli allevatori e alle associazioni indicazioni concrete per prendere decisioni basate su dati obiettivi che possano potenziare la resilienza a lungo termine delle risorse genetiche dei piccoli ruminanti italiani.

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1 INTRODUCTION

1.1 BIODIVERSITY AND LOCAL LIVESTOCK BREEDS

The term *biodiversity* was first introduced by Walter G. Rosen in 1986 as a contraction of "biological" and "diversity" [1]. Since then, several definitions have been proposed, each capturing different nuances of the concept. For instance, biodiversity has been described as "the number, abundances, functional variety, spatial distribution, and interactions of genotypes, species, populations, communities, and ecosystems" [2].

Biodiversity is commonly conceptualised as consisting of three main pillars [3]. The broadest of these is *ecosystem diversity*, which refers to the variation among biomes and ecological processes. On a narrower scale, *species diversity* concerns the number and abundance of species in a given area, contributing to ecological balance. Finally, *genetic diversity* refers to the variety of genetic traits within and between populations. This last component is essential at all levels of biological organisation—from individuals to ecosystems. Genetic diversity also plays a key role in how populations respond to environmental change. When the conditions of a habitat shift, populations may either migrate to track their climatic niche or persist locally. The latter is only possible through phenotypic plasticity and/or genetic adaptation, both of which rely on the availability of genetic variation. When neither migration nor adaptation is feasible, populations face demographic decline and an increased risk of extinction [4].

If until the late 1980s, biodiversity conservation was primarily concerned with wild species and natural ecosystems, the concept of *agricultural biodiversity* (or *agrobiodiversity*), defined as the "variety and variability of animals, plants and micro-organisms that are necessary for sustaining key functions of the agro-ecosystem, including its structure and processes for, and in support of, food production and food security" [5], emerged in the early 1990s, when the Convention on Biological Diversity (CBD) recognised its importance and brought it into the global agenda [3].

Globally, around 40 mammal and poultry species have been domesticated and are currently bred to meet diverse human needs, including food, fibre, draught power, recreation, and income. *Livestock biodiversity* represents a remarkable case of intra-species diversity, currently comprising about 8,500 distinct breeds, 87% of which are considered local (DAD-IS, accessed 18/07/2025). These breeds—recognised as Animal Genetic Resources (AnGR)—are the product of centuries of natural and artificial selection, genetic admixture, evolutionary processes, and adaptation to changing environments and production demands [6,7].

1.1.1 Biodiversity crisis

Species evolution and extinction are natural processes. However, numerous studies have shown a significant acceleration in extinction rates over the past century compared to background levels. This alarming trend is largely attributed to human activities, the introduction of invasive species, and climate change [6,8,9].

Similarly, livestock biodiversity has always been dynamic, shaped by the emergence of new breeds, the disappearance of others, and the ongoing evolution of existing populations in response to changing environments and societal demands. Yet today, even livestock genetic resources are facing a biodiversity crisis. According to the most recent data from DAD-IS (Domestic Animal Diversity Information System, maintained by FAO; accessed 18/07/2025), 8% of mammalian and avian breeds are already extinct, and approximately 30% are classified as at risk. However, the status of 43% of breeds remains unknown, particularly in developing countries, where data collection is limited. Figure 1.1.1 reports the risk status and trends for local breeds of major domesticated mammalian species.

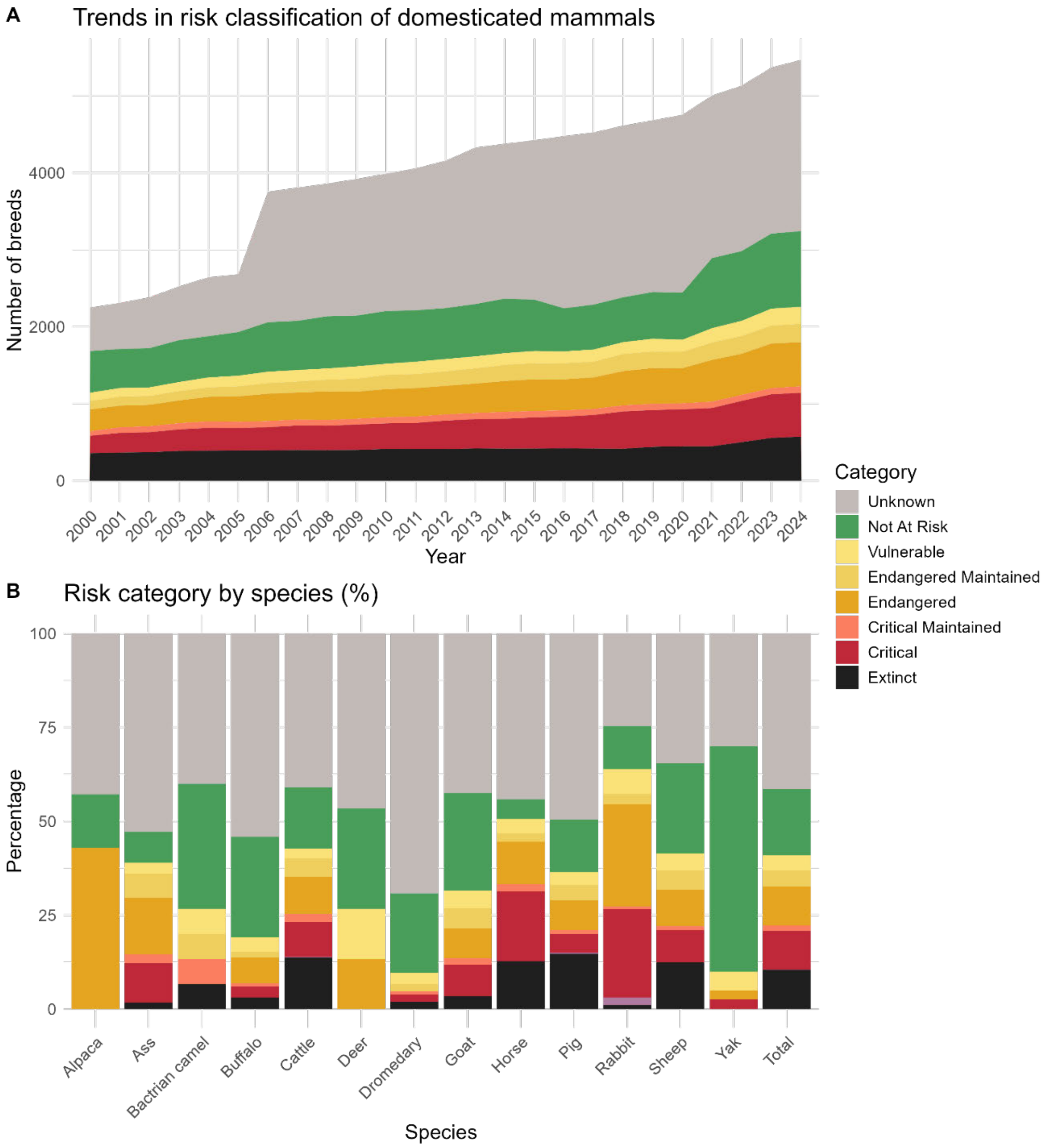

Figure 1.1.1 Risk status for local mammal breeds: trend (A) and current status by species (B). Data were retrieved from DAD-IS (accessed 18/07/2025).

The main driver of this decline is the global spread of a few highly specialised, cosmopolitan breeds [10,11]. In response to increasing demand for food and market uniformity, these high-input, high-output breeds are often favoured over local breeds, despite the latter being better adapted to their native environments—many of which are characterised by low-input, harsh conditions. As a result, local breeds are frequently replaced by specialised ones or subjected to indiscriminate crossbreeding. This process contributes to genetic erosion and, in some cases, leads to the complete loss of unique AnGR [12].

This erosion is not limited to intensive farming systems. In marginal areas, biodiversity loss is further exacerbated by the abandonment of traditional husbandry practices [13,14]. Technological advances have also contributed to this shift: they enable the use of exotic breeds by mitigating the negative effects of environments they are poorly adapted to. Furthermore, mechanization has reduced the need for animal power for draught, leading to a dramatic decline in the breeding of entire species in some parts of the world, such as donkeys, or to a shift in the use of certain breeds—for instance, cattle originally bred for triple-purpose (milk, meat, and work) are now often selected for single production traits [12].

Another major concern is the erosion of genetic diversity within populations. Intensive selection for productivity and uniformity—especially in commercial breeds—combined with widespread use of artificial insemination and a limited number of elite sires, has led to increased genetic homogeneity and reduced effective population sizes. However, it is important to acknowledge that the relevance of maintaining genetic diversity within breeds has gained increasing recognition in recent years, both in international policy frameworks and within the practices of breeding organisations. This shift is reflected in recent guidelines and breeding strategies, which increasingly incorporate the conservation of within-breed diversity as a key objective for ensuring sustainable breeding programs and long-term population viability [15]. On the other hand, many local breeds are maintained in small, often isolated populations. These small population sizes, as well as limited connectivity among farms, elevate the risk of inbreeding, which can reduce fitness through inbreeding depression and further compromise long-term adaptability [12,16–18].

Climate change represents an additional threat to livestock biodiversity. It is already affecting the distribution, abundance, and seasonal patterns of livestock, and is expected to further reshape farming systems globally [11,19]. Highly productive commercial breeds, optimised for controlled environments, are particularly sensitive to heat and nutritional stress, often suffering from reduced fertility, milk yield, and survival under such pressures [20,21]. However, adaptation strategies are often implemented in intensive farms to mitigate the negative effects of unfavourable environmental conditions on livestock. These include adjustments to housing and management systems, the integration of technologies that improve microclimatic conditions, nutritional interventions, and, more recently, the adoption of precision livestock farming systems that monitor animal welfare and environmental parameters in real time, enabling timely and targeted responses [22,23]. On the other hand, local breeds tend to be more resilient to harsh environments due to long-term adaptation; however, because they are generally reared in extensive or low-input systems with minimal infrastructure, they are also more exposed to climatic stressors which might exceed their adaptive capacity [24–26]. Additionally, climate change is driving shifts in land use and grazing patterns, altering traditional farming systems and forcing farmers to abandon or replace local breeds that are no longer economically viable under new conditions [26].

1.1.2 Recognising and preserving animal genetic resources

In a context of increasing demand for food [27] and accelerating climate change, agricultural biodiversity is becoming ever more important. It underpins the production of food and a wide range of goods and services across diverse biophysical environments. Moreover, biodiversity is the primary source of the adaptive potential of agricultural systems, enabling them to respond to ongoing and future challenges [28].

In particular, the conservation and knowledge of AnGR is gaining prominence. The characterisation, inventory, and monitoring of local livestock populations—and the risks they face—have been included among the FAO's strategic priorities as essential steps for guiding biodiversity conservation strategies [26].

There are several compelling reasons to intensify efforts in this direction [29,30]:

- A strong level of genetic diversity forms the foundation for effective selection and long-term adaptability. It serves as an insurance policy against future threats and uncertainties—not only in climate and disease but also in market conditions and changes in husbandry practices. Even considering present needs, maintaining a variety of breeds is essential to support the diversification of animal production systems and products.
- Local livestock populations are often well adapted to harsh environmental conditions and are typically more resilient, hardy, and tolerant or resistant to stressors, pathogens, and parasites. These traits have sparked growing interest in their physiological and genomic profiles, shedding light on the mechanisms underlying environmental adaptation. Additionally, in this context, local breeds may carry unique genetic variants of high potential value, which could prove critical for future breeding goals or resilience strategies.
- In regions where climatic and environmental constraints prevent the use of specialised breeds, local populations hold significant socioeconomic value. They often represent the only sustainable means of food production and livelihood for local communities.
- Local breeds are deeply intertwined with their surrounding landscapes. When managed properly, they contribute positively to their ecosystems by providing environmental services such as pasture maintenance and biodiversity preservation [31–33]. This is particularly evident in marginal areas, where traditional livestock farming is often the only viable activity; without it, such areas would risk abandonment and ecological degradation [34].
- Finally, local breeds possess significant cultural and historical value. They represent the interface between human societies and ecosystems and reflect a long-standing co-evolutionary relationship between people and domesticated animals. As such, they can be considered cultural assets in their own right, documenting the agricultural heritage

of rural communities and offering non-material benefits in addition to their role in subsistence [35].

The formation of breeds is a complex process shaped by a long history of interplay between artificial and natural selection. However, the definition of a breed has always been—and still is—a subject of debate. A broad definition considers a breed to be any group of animals recognised as distinct by the communities that maintain them, without necessarily requiring genetic isolation or uniformity. In contrast, a more conservative definition describes a breed as a group of animals that are recognizably different from other members of the same species and that reliably reproduce these distinguishing traits when interbred. This latter definition, therefore, implies a certain degree of genetic uniformity and predictability [11]. Besides the scientifical debate, the legal definition of a breed should also be taken into account, especially in the context of breed recognition processes. According to Regulation (EU) 2016/1012, a breed is defined as: "a population of animals sufficiently uniform to be considered to be distinct from other animals of the same species by one or more groups of breeders which have agreed to enter those animals in breeding books with details of their known ascendants for the purpose of reproducing their inherited characteristics by way of reproduction, exchange and selection within the framework of a breeding programme." As this definition shows, a breed is recognised as such only when a breeding book is established, and while it emphasises uniformity and distinctiveness as perceived by breeders, it does not explicitly require genomic assessment.

A looser form of breed can be described as a landrace or ecotype—animal populations that have diverged from others through a unique combination of geographical and reproductive isolation, as well as specific natural and artificial selection pressures, but that are not formally recognised or managed through structured breeding programs. Nevertheless, these populations can be considered AnGR and contribute significantly to livestock biodiversity.

The recognition of an AnGR can be summarized in three steps: discover, secure, and sustain [11]. The discovery phase involves determining whether a candidate landrace population qualifies as a genetic resource, i.e., whether it deserves recognition as a breed. Since landraces are often found in remote locations, it is especially important to foster collaboration between academic researchers and local communities. Assessing the breed status of a population can be based on phenotypic evaluation, understanding of its historical background—which often mirrors phenotypic traits, as repeated introductions from external populations typically lead to increased variability, while long-term isolation fosters uniformity—and genetic analysis. A candidate population is considered successful in this phase if it proves distinguishable from other breeds and displays a good level of within-population similarity. The next step is to carefully define the criteria for including animals in the breed, which should reflect both its historical context and overall phenotype. The conservation population should include all animals showing the phenotypic and genomic traits typical of the breed, while excluding individuals with clear signs of introgression from other sources. When genomic data are used, particular attention must be paid to the initial sampling pool, as inadequate sampling may lead to the exclusion of certain subpopulations, thereby narrowing the genetic base available for future breeding.

The second phase is to secure the population, which may involve rescuing animals from precarious or unsustainable situations.

Finally, to ensure the long-term survival of the population, it must be sustained in two key ways. First, by managing its population genetics to maintain a balance between preserving its unique traits and ensuring a sufficient level of genetic variability. Second, by ensuring that the breed's rearing is economically viable—or ideally profitable—through the development of demand for its products and/or services.

From a legislative perspective, the establishment of breeding programs for the creation of new breeds is regulated by Regulation (EU) 2016/1012 and, in Italy, by Legislative Decree No. 52/2018. For example, with regard to sheep and goats, the recognition procedure starts with an expression of interest by the breeders for the recognition of the breed and the activation of a related breeding program. This triggers the collection of historical records and bibliographic documentation and the realisation of scientific studies that demonstrate the distinctiveness and originality of the population. Although not formally required, genomic results can strongly support this step.

These findings, together with a definition of the breed standard, are then presented to the *Commissione Tecnica Centrale* (Central Technical Commission) of Asso.Na.Pa., which will judge the request. Among the information required by the law for drafting a breeding program are: the name of the breed; characterization of the breed's essential traits; information on the geographical territory where the breed is managed; the system for identification of individuals and registration to the program; and the detailed circumstances justifying the establishment of the breed.

If approved, the resolution of the *Commissione Tecnica Centrale*, together with the breed standard, is sent to the *Ministero delle Politiche Agricole, Alimentari e Forestali* (Ministry of Agricultural, Food and Forestry Policies), which will decide on the definitive recognition of the breed and, in case of a positive outcome, will issue a dedicated Ministerial Decree.

1.1.2.1 Livestock biodiversity conservation

The main strategies adopted in conservation programs include *in vivo* conservation, through the strategic management of live animal populations either *in situ*—within their traditional production systems—or *ex situ*—in controlled environments—and *in vitro* cryoconservation of biological material such as gametes or embryos stored in biobanks. While cryoconservation serves as a powerful long-term backup for safeguarding genetic diversity, it provides only a static snapshot of the genetic makeup at a specific point in time. In contrast, *in vivo* conservation preserves living, evolving populations, allowing them to continue adapting to environmental changes. For this reason, *in situ* conservation is generally preferred whenever feasible, as it allows animals to remain within the ecosystems and cultural contexts that shaped them. Nevertheless, cryopreserved material can play a crucial complementary role, especially in supporting *in vivo* efforts or rescuing endangered traits or populations [36].

These conservation methods typically involve the adoption of breeding strategies aimed at minimising inbreeding and retaining genetic variability. They may also be designed to preserve specific traits, safeguard the cultural value of a breed, or conserve its adaptive potential to a particular environment. In extreme cases, where the effective population size and genetic variability are insufficient to ensure the survival of a population, genetic rescue may be necessary. However, any conservation plan should follow a holistic, multidisciplinary approach that involves livestock keepers, breeders, and local communities. These stakeholders can offer indigenous knowledge that would otherwise be difficult to access, and they ultimately play a crucial role in implementing conservation plans [36,37].

One important consideration when designing biodiversity conservation strategies is that available resources are often insufficient to protect all existing breeds. This leads to the so-called "Noah's Ark problem" [38]. As a result, objective and scientific prioritisation criteria must be established to determine which breeds to focus conservation efforts on. These criteria typically aim to: i) preserve as much genetic diversity as possible—both within and between breeds; ii) take into account phenotypic performance traits relevant to productivity and adaptation; and iii) evaluate breed demography, including population size and trends, geographical distribution, degree of crossbreeding, level of farmer organization, and the socio-cultural significance of each breed. In this context, molecular genetic data can be extremely useful in refining prioritization criteria and assessing the urgency of conservation [29].

A foundational approach was developed by Weitzman [39,40], who proposed a general theory of diversity that prioritizes breeds based on a conservation potential index that derives from a combination of genetic distance matrices and estimates of extinction probabilities over a given time horizon. However, this method has a technical limitation: it tends to prioritise genetically homogeneous breeds. Moreover, it fails to account for other critical factors, such as a breed's unique traits, productivity, genomic variability for key characteristics, or its cultural relevance.

For this reason, alternative methods have been proposed that incorporate additional elements believed to influence extinction risk—such as population size, geographic range, and socio-cultural farming contexts—, historical and projected future persistence of the population, and expected loss of genetic variation over time [29]. Weitzman himself later expanded his model to include terms reflecting breed utility and the cost of conservation programs, in addition to genetic distinctiveness [38]. A summary of recommendations by Kristensen [41]—based on genetic diversity, uniqueness, adaptability, and demographic data—is reported in Table 1.1.1.

Table 1.1.1 Criteria to define conservation priority in livestock breeds.

	Genetic uniqueness	Adaptation	Genetic variation	Recommendation	Conservation priority
Scenario 1	Genetically unique	Highly adapted to local conditions	High	If Ne > 500–1000 and not decreasing: no need to change management strategy.	High
Scenario 2	Genetically unique	Highly adapted to local conditions	Limited	Gene flow from populations to increase Ne and genetic variation.	High
Scenario 3	Genetically unique	Maladapted to local conditions	High	Characterise the cause of maladaptation. Start to select for increased local adaptation. Gene flow from populations adapted to similar environments if no response to selection.	Intermediate
Scenario 4	Not unique	Adapted to local conditions	Low	Increase Ne. Gene flow from populations adapted to similar environments.	Low (if there are other available conspecific populations)
Scenario 5	Not unique	Maladapted to local conditions	Low	Gene flow from populations adapted to similar environments.	Low

However, it should be noted that the formal implementation of such prioritization methods remains limited in practice, for several reasons. Many local breeds—particularly in developing countries—have never been genetically characterised, resulting in a lack of molecular data to assess their diversity. Furthermore, conservation programs are sometimes guided by alternative priorities, such as promoting breeds of national significance or favouring those receiving institutional funding. Lastly, the absence of a clear consensus on the most appropriate method for breed prioritization has also hindered the adoption of these strategies [29].

1.1.3 Italian small ruminants: an excellent example of biodiversity and adaptation

Small ruminants, particularly sheep and goats, are of strategic importance in global livestock systems, especially in marginal and resource-limited areas. Their ability to survive and produce under harsh environmental conditions with minimal inputs makes them central to the sustainability of agriculture in challenging contexts [12,42].

Following their domestication, these species spread alongside human migrations, demonstrating remarkable ecological plasticity and adapting to a wide range of climates and farming systems. Through centuries of interaction with both local environments and traditional management practices, a great diversity of genetically and phenotypically distinct breeds has emerged [12]. Compared to other livestock, small ruminants—especially goats—show higher tolerance to diseases and environmental stressors such as heat, feed scarcity, and limited water availability [43–45]. These adaptive traits are particularly evident in many local breeds, which are increasingly recognised as critical genetic reservoirs for breeding programs aimed at enhancing resilience to climate change [25,26,42].

Despite their value, small ruminant genetic resources remain under threat due to demographic decline, indiscriminate crossbreeding, intensification, and insufficient policy support [12,42]. DAD-IS (accessed 18/07/2025) reports 1,162 and 667 local sheep and goat breeds, respectively, which together represent over one third of the world's domestic mammalian AnGR, along with 239 and 87 transboundary breeds, all widely distributed across the globe (Figure 1.1.2). However, nearly one third of these populations are reported at risk of extinction, and 27% are classified as having unknown risk status. This loss of biodiversity undermines the very traits that underpin resilience and adaptability to environmental variability.

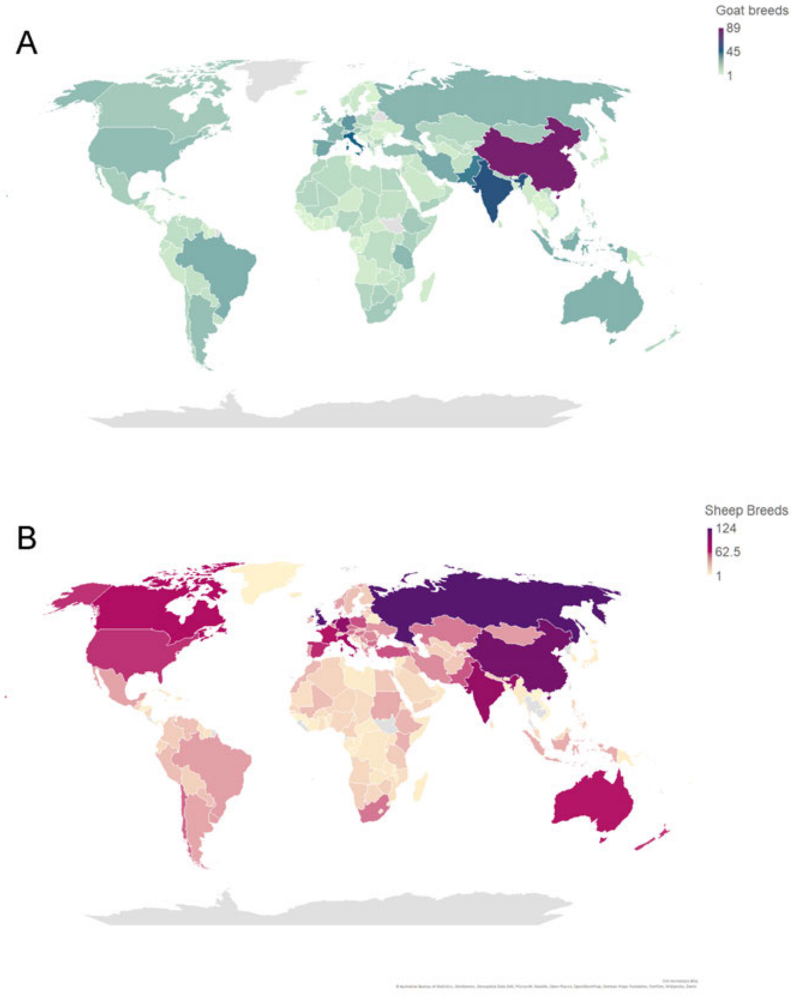

Figure 1.1.2 Number of goat (A) and sheep (B) breeds in the world according to DAD-IS (accessed 18/07/2025). Grey countries reported no data. Italy ranks 3rd for number of goat breeds and 7th for number of sheep breeds.

In recent years, small ruminants have attracted growing scientific attention, particularly in studies on genetic diversity, local adaptation, and evolutionary processes. Their genomes serve as key resources for understanding how livestock species respond to diverse environmental pressures, making them valuable models for climate adaptation research and conservation genetics [46,47].

This global perspective sets the stage for examining the Italian context, where small ruminants—especially local and native breeds—play an essential role in agricultural biodiversity, cultural heritage, and the sustainability of extensive farming systems. Italy's unique geographic and climatic diversity has fostered the development of numerous autochthonous breeds, many of which are adapted to specific regional conditions. Of these local breeds, 45 and 71 are recorded for goat and sheep species, respectively, in DAD-IS (Figure 1.1.2, accessed 18/07/2025), and 47 and 63 are currently officially recognised and managed by the *Associazione Nazionale della Pastorizia* (Asso.Na.Pa.; www.assonapa.it, accessed 10/09/2025), the sole authorized breed society that manages sheep and goat breeding books in Italy (D.Lgs 11/05/2018 n.52 and Regulation (EU) 2016/1012). However, many other populations and ecotypes are present that are not yet officially recognised [48].

Small ruminant farming in Italy has deep historical roots, dating back to the Neolithic period. Archaeological and historical records highlight its centrality in the Roman economy, where sheep and goats were reared for meat, fibre, skin, and milk, and the first landraces were developed [49,50]. Over the centuries, small ruminants, particularly sheep, have remained a fundamental part of Italy's agro-pastoral systems, especially in mountainous and marginal areas [51].

Despite its long history, the sector has undergone profound structural transformations in the modern/contemporary era. Since the beginning of the 19th century, sheep farming began to decline in the northern regions, where cattle husbandry started to prevail, and crossbreeding with foreign Merino breeds was attempted to increase production. In contrast, sheep farming remained widespread in the mountainous areas of central–southern and insular Italy. Goat breeding, however, became increasingly confined to marginal contexts, in arid and harsh mountainous areas, mostly within small family-run farms. By the end of the 19th century, the sector as a whole was in decline. Factors contributing to this downturn included urbanisation and the consequent abandonment of marginal lands and traditional husbandry practices, restrictions on pastoral movement and transhumance, reduction of pasture areas, and—especially in the sheep sector—the crisis of the wool market [52].

Census data over the last century show an overall negative trend, with the exception of a temporary increase between the 1970s and 1990s [53]. In recent decades, the total number of animals—approximately 6 million sheep and 1 million goats—has slightly decreased. However, there has been a marked reduction in the number of farms, a trend indicative of the sector's ongoing intensification [54]. Indeed, while many farms remain small-scale—around 28% of the approximately 110,000 are considered family farms—over half of all small ruminants are now housed in large farms with more than 300 heads [54,55]. These represent only about 5% of all farms and are predominantly located in Sardinia, which alone hosts about half of the animals, followed by Sicily, Lazio, and Tuscany [54]. Among sheep, the Sarda breed is predominant, representing about half of the total sheep stock, followed by Valle del Belice and Bergamasca—Italy's main meat sheep breed, prevalent in north-central regions—and the foreign Lacaune [55].

Goat distribution differs from that of sheep: while Sardinia still hosts the majority of goats—about one quarter, mainly of the Sarda breed—the rest of the caprine stock is divided between southern regions—especially Calabria and Sicily, which mainly rely on the extensive or semi-extensive farming of local breeds [55,56]—and Alpine areas, particularly Lombardy and Piedmont, where traditional systems, often still practising vertical transhumance, coexist with more intensive dairy farms of specialised breeds such as Saanen and Camosciata delle Alpi [55,57].

Italy's small ruminant sector is predominantly oriented toward milk production, which accounts for approximately 80% of animals (considering both milk-only and mixed systems). The milk is mainly transformed into cheese, with annual production exceeding 80,000 tons (approximately 75,000 tons for sheep and 7,000 for goats). These figures make Italy the leading EU producer of sheep cheese and the third-largest for sheep milk, accounting for 33% and 20%

of total EU production, respectively. In contrast, goat milk products represent a much smaller share. Around 40% of these cheeses are PDO-certified, including Pecorino Romano DOP, which alone accounts for 80% of all sheep cheeses and is largely exported, especially to the USA [54,58].

Meat production plays a secondary role, involving around 17% of small ruminants. It is largely seasonal, concentrated during religious holidays such as Easter and Christmas, with lamb representing over two-thirds of slaughtered animals. In recent years, there has been a moderate decline in the number of slaughtered heads, a reduction more pronounced than that observed in overall production—likely due to increased animal weights and a gradual destagionalization of consumption. However, this has been accompanied by rising imports of live animals and meat [54].

Although the sector contributes only about 1.4% of total agricultural value, its territorial, environmental, and social functions are disproportionately important [54]. In many remote and marginal areas, such as the mountainous and hilly zones of the Alps or southern and insular regions, small ruminants often represent the only viable form of agricultural activity [32,59,60]. Their ability to exploit low-productivity land helps preserve rural landscapes, prevent land abandonment, and mitigate fire risk, especially in shrub- and forest-covered areas. While it is true that mismanagement—such as overstocking—can lead to environmental degradation and biodiversity loss [61,62], proper grazing management has been shown to enhance biodiversity, improve soil conditions, and contribute to carbon sequestration [31–33]. These benefits are especially notable when local breeds are reared in their native environments [63].

In addition to these ecosystem services, the cultural value of local small ruminant populations, as well as the traditional farming practices in which they are reared, deserves to be acknowledged and preserved. The UNESCO recognition of transhumance as intangible cultural heritage is an example of recognition of the socio-cultural value of these systems [64]. In particular, two transhumance systems have developed in Italy. The vertical system (*alpeggio*), mainly practised in the Alps, involves moving animals to alpine pastures during summer [57,65,66]. In central-southern Italy, vertical transhumance was typically combined with horizontal transhumance, with shepherds moving between the Apulian plains in winter and the Abruzzo mountains in summer [67]. Unfortunately, the decline of transhumance, the reduced attractiveness of traditional and less predictable activities, shrinking grazing lands, and the wool market crisis have led to the abandonment of many traditional practices and placed several breeds at risk of extinction [48,68,69].

Over the past years, substantial national and international efforts have been dedicated to advancing our understanding of sheep and goats also from a genomic perspective. These initiatives have led to the development of extensive ovine and caprine genomic datasets, which have greatly contributed to reconstructing the history and evolution of these species worldwide. Importantly, many of these datasets have been made publicly available, fostering further research and collaboration. In sheep, a key role has been played by the International Sheep Genome Consortium (www.sheephapmap.org), which, through the use of the Illumina OvineSNP50 BeadChip, produced the Sheep HapMap dataset, encompassing 74 breeds from across the globe [70]. More recently, the consortium has been involved in the third run of the 1000 Genomes Project for sheep, which has already included over 3,500 genomes sequenced using short-read whole-genome sequencing. For goats, the International Goat Genome Consortium (www.goatgenome.org), together with the Feed the Future program, the NextGen project, and the 3SR project, promoted the ADAPTmap initiative, leading to the publication of a dataset covering 148 breeds from 35 countries, all genotyped with the Illumina GoatSNP50 BeadChip [71]. More recently, the VarGoats Consortium has taken advantage of advances in sequencing technologies to assemble a large dataset of short-read whole-genome sequences from more than 1,300 subjects representing 133 local and transboundary domestic populations and 8 wild goat species [72]. At the national level, Italy has also developed significant genomic resources for small ruminants. The Biovita project, for instance, produced SNP data for 20 sheep populations [73] while the Italian Goat Consortium generated genomic data for 32 goat populations [74].

1.2 CLIMATE CHANGE AND THE LIVESTOCK SECTOR

Climate change is progressing rapidly, with rising temperatures, sea levels, and the frequency of extreme weather events such as heatwaves, floods, and droughts [24,75]. The Mediterranean area, including Italy, has proven to be highly exposed to such changes. For example, the Alpine region has experienced an increase in surface air temperature of about 2°C over the last 40 years—nearly twice the global average—and higher elevations appear to be more vulnerable to this warming. Heatwaves are increasing and are expected to grow in frequency, intensity, and duration. Precipitation patterns have changed differently across the country, with an average increase in the northwest and decreases in the southeast, especially during the summer. However, of greater concern is the increase in extreme precipitation events, such as heavy rainfall and storms, as well as the opposite phenomenon of seasonal droughts. Unfortunately, these trends are expected to continue and intensify over time [75–77]. Additionally, Italy is highly susceptible to geological, hydrological, and hydraulic instability, which climate change further exacerbates [78].

Unsurprisingly, these changes are severely impacting the agricultural sector, which depends on environmental conditions and the availability of natural resources. Severe effects on all types of crop production are already being observed and are expected to increase, with obvious financial consequences and implications for food security. For example, economic losses expected for the Italian agricultural sector range between €12 and €30 billion by 2050, with a temperature increase of 2°C or 4°C, respectively [76].

Climate change also affects the livestock sector in multiple ways, both directly (e.g., impacts on animal physiology and behaviour) and indirectly [20]. A well-documented effect is the decline in production performance, both in quality and quantity, especially in high-yielding animals due to their higher metabolic heat production. Heat stress impacts animals' metabolism, reducing feed intake and diverting nutrients and energy from growth or milk synthesis to thermoregulation [79–82]. Fertility is severely affected by high temperatures, with negative impacts on ovarian function, semen quality, and embryo development [83].

Animal health is also compromised. Climate change impairs immune defences directly, such as through heat stress, increasing the incidence of mastitis, gastroenteritis, and respiratory diseases. Indirectly, changing climate conditions affect the distribution of pathogens and pests, with warmer conditions generally favouring vectors and parasites [26,80,84], such as the Bluetongue virus [85]. In addition, severe heatwaves and extreme weather events can cause the death of thousands of animals in a short period [26,86,87].

Another indirect effect is the change in feed quality, quantity, and availability due to climate impacts on forage crops, pastures, and grasslands [75,77,80]. The availability of water resources, crucial for livestock, is also compromised. Global warming, altered CO_2 levels, and altered hydrological cycles and precipitation patterns are already reducing crop yields and quality in some regions. These changes also require shifting certain crops to new regions, adopting more resilient varieties, and combating the spread of parasites. Extreme events further exacerbate these problems. Increased weather variability also leads to volatility that can impact animal feeding. Wildfires, storms, droughts, or floods can suddenly and severely reduce pasture availability or destroy forage crops, with long-term consequences for soil [75,76,88].

These trends raise serious concerns for both human health and livestock production systems. Although sheep and goats are relatively heat-tolerant and may have an adaptive advantage, increased thermal stress—especially when rapid or prolonged—still compromises productivity and animal welfare, particularly in extensive systems with limited infrastructure to mitigate heat effects [19,24,75,89]. Water scarcity, along with degradation, shrinkage, and shifts in the composition of grasslands, poses additional challenges for pastoral systems that rely minimally on feed integration. These systems are also directly affected by climate-induced restrictions on animal mobility, often leading to the abandonment of traditional migratory routes essential to seasonal grazing [90]. Furthermore, existing sociopolitical and economic pressures exacerbate the vulnerability of pastoralists and their capacity to maintain traditional practices [90,91]. Since most local breeds are raised within these extensive systems, their resilience is tightly bound to the viability of pastoralism. In addition, at the level of the animals themselves, the selection pressure exerted by climate change will likely decrease the genomic variability of populations that have to adapt to the new conditions to survive. When environmental conditions within a breed's traditional range change beyond its adaptive capacity—either related to animal biology or management practices—, the risk of displacement or even extinction increases [92]. Shift in species and breed distribution due to climate change has, in fact, already been reported in several occasions [12,93]. Moreover, extreme events such as prolonged droughts, floods, or disease outbreaks—although sometimes spatially limited—can devastate breeds with narrow geographic distributions, potentially leading to their irreversible loss [93].

Nonetheless, livestock biodiversity may be key to coping with these challenges. Animal genetic resources offer a reservoir of resilience and adaptive traits that may become essential for future animal farming [12]. Beyond implementing temperature-mitigation systems and technologies, sustainable livestock production under climate change could greatly benefit from the use of local populations, through:

- Species or breed substitution, replacing commercial breeds unable to maintain production with locally adapted breeds or more resilient species. However, these breeds are generally less productive and may not fully adapt to new environments, despite similarities—for instance, due to different feed resources or disease pressures. In addition, for a successful outcome, farmers must be involved in the development of the plan and provided with adequate training to properly manage the newly introduced population [92,94–96].
- Targeted and strategic crossbreeding between specialised and adapted breeds, to develop animals with good productivity under harsh conditions. A possible drawback is the introgression of commercial genes into marginal environments, which may cause genetic erosion of local breeds and reduce their adaptive ability [92,94,96].

- Development of selection programs for thermal tolerance and resilience to new endemic diseases. This requires identifying complex traits, major genes, or developing accurate estimated breeding values, supported by extensive genetic, epidemiological, phenotypic, and environmental data. For these reasons, such programs are expected to be feasible in the short term only for a limited number of well-characterized species and breeds, and only in a few countries [93–95,97].
- Use of biotechnologies to insert specific adaptation-related genes into commercial breeds [93].

Given these considerations, it is not surprising that research on local breeds and their adaptive mechanisms has expanded significantly in recent decades, moving beyond basic physiological studies toward integrated approaches incorporating genetic, ecological, and environmental data [98]. This multidisciplinary research approach is indispensable to dissect the complex genetic architecture of adaptation, to systematically identify and evaluate resilience traits, and to translate these findings into practical applications for evidence-based decisions in breeding management and conservation policy.

1.3 GENOMIC TOOLS TO STUDY BIODIVERSITY AND ADAPTATION

1.3.1 Evolution and application of genomic tools in the livestock sector

Animal breeding and selection date back to the time of domestication and the earliest stages of herding activity, although carried out empirically, if not involuntarily, and mainly based on phenotypes, whether performance, reproduction, and/or morphology related.

More organised forms of breeding practices were established in the late 17th century, but an artificial selection theory was developed and diffused only starting from the following century. In particular, two names emerged in that period: Robert Blackwell and Sir John Seabright. Bakewell's approach emphasised the careful selection of both males and females through the consistent evaluation of offspring performance, anticipating some of the principles of modern quantitative genetics, as well as the use of inbreeding and the avoidance of outcrosses [99]. On the other hand, Seabright, despite agreeing with most of these principles, asserted that: i) the choice of good parents does not always provide for good progeny; ii) inbreeding could have several drawbacks and should be carefully managed; and iii) ancestry breeding should be preferred over progeny tests [100]. In the meantime, the contemporaneous rise of Thoroughbred horse breeding introduced the concept of purity through pedigree and public studbooks, fostering a purebred culture that implied an absolute connection between genealogy, and thus purity, and quality [101].

19th-century naturalists and early geneticists were increasingly interested in understanding inheritance mechanisms through plant and animal experiments, laying the groundwork for Mendelian genetics and the concept of hybridisation. However, this period witnessed the consolidation of this kind of purebred breeding as the dominant paradigm. While these systems provided a framework for breed standardisation, they also entrenched attitudes that sometimes resisted integration with scientific approaches to heredity. Interestingly, another system was also developed over the late 19th and the 20th centuries, despite being limited to a minority of species—such as horses and dogs—, namely the so-called standardbred breeding, which aimed to produce animals meeting well-defined and pre-set standards [101].

The rediscovery of Mendelian genetics around 1900 introduced a new conceptual framework for heredity. However, the discrete inheritance patterns described by Mendel were initially difficult to reconcile with the continuous variation typical of most economically important traits in livestock, leading to the insurgence of the debate between Mendelism and Darwinism, linked to the biometric approach [102]. The synthesis of these two approaches led to the development of population and quantitative genetics, particularly through the work of R.A. Fisher—who described the infinitesimal model of inheritance of quantitative traits—, Sewall Wright—who theorized the possibility of use inbreeding to generate genetic shift of livestock populations and also proposed a measurable inbreeding coefficient—, and J.L. Lush—who combined Fisher and Wright thinkings into an applicable formal structure, the livestock genetics—. Together, these scientists demonstrated that continuous traits could be modelled as the result of many genes of small effect, interacting with the environment. Their theories provided a foundation for breeding value estimation, heritability analysis, and selection response prediction—key tools that underpin modern breeding programs [101,103–106]. Despite these theoretical advances, practical breeding remained largely impervious to formal genetic input until the mid-20th century, after the widespread use of artificial insemination and the establishment of progeny testing schemes.

From the 1980s onward, the development of molecular genetics and subsequently genomics brought about a profound transformation in animal breeding. At the time, two geneticist groups were present: molecular geneticists, who focused on locating genes, and quantitative geneticists, who applied statistical techniques to predict the physical outcome of gene action. The development of the Restriction Fragment Length Polymorphism (RFLP) technology, paired with the theory of linkage disequilibrium (LD), established the base for the research on the genome-wide scan for quantitative trait loci (QTL) and the development of marker-assisted selection. However, it was the advent of genomic selection, as proposed by Meuwissen *et al.* (2001) [107], that marked a paradigm shift. This approach, which aimed to predict the genetic merit of individuals for selection, was based on two main assumptions, met only in later times: the possibility of cost-effective genotyping of thousands of markers located across the whole genome, and the availability of a marker density sufficient for all genes responsible for a trait to be in LD with flanking markers [108]. By using dense SNP data to predict genomic estimated breeding values, selection could be applied earlier and more accurately, even in young animals without performance records. However, it has to be acknowledged that the success of genomic selection, especially in dairy cattle, was made possible by the huge efforts of previous times in the building of an infrastructure of data through quantitative genetic programs and traditional purebred recordings [101].

The integration of genomics into breeding programs has enabled the rapid identification of animals carrying favourable alleles, reduced generation intervals, and improved the accuracy of selection across traits that are difficult to measure. Moreover, the development of high-throughput genotyping technologies and the increasing availability of reference genomes have expanded the potential of breeding beyond traditional selection, opening new possibilities for functional genomics, genomic prediction, and gene editing.

Currently, three main types of genomic data are widely used in livestock breeding and research: microsatellites, single-nucleotide polymorphism (SNP) arrays, and whole-genome sequencing (WGS).

Microsatellites, also known as short tandem repeats, are repetitive DNA sequences consisting of 2–6 base pair motifs. They gained prominence in the 1990s and have been extensively applied in parentage verification, individual traceability, QTL mapping, and the study of genetic diversity and population structure [109]. Despite being largely superseded by SNP technologies, microsatellites remain in use for specific applications, particularly for parentage testing and in forensic contexts, and in countries or species where SNP tools are not yet fully established.

SNPs are the most abundant form of genetic variation in the genome, typically occurring at a frequency of approximately one SNP per kilobase in most mammalian genomes. They result from single-base substitutions and are generally biallelic, making them suitable for high-throughput, automated genotyping platforms. SNP arrays have been developed for a wide range of domestic species and are typically classified by density: low-density (<20,000 SNPs), medium-density (~50,000 SNPs), medium-high density (~150,000–200,000 SNPs), and high-density (>500,000 SNPs). These arrays are constructed based on high-quality, highly informative SNPs selected from diverse populations and breeds. In cattle, a variety of commercial arrays are available across all density levels, whereas small ruminants have more limited resources. For sheep, five medium-density arrays, one low-density array (~11,000 SNPs), and one high-density array (~600,000 SNPs) are available [110]. In goats, only medium-density SNP arrays are currently accessible [110], although efforts to develop higher-density chips are underway [111]. While SNP arrays offer several advantages over microsatellites—such as genome-wide distribution, stability, location within or near coding regions or QTLs, and scalability for large datasets—they are susceptible to ascertainment bias, particularly when applied to populations not included in the discovery panel (e.g., local or endangered breeds) [109].

WGS currently represents the most comprehensive genomic approach, enabling the analysis of the complete DNA sequence of an individual. Both short-read and long-read platforms are used, offering distinct advantages. WGS allows for the discovery of both common and rare variants, including SNPs, insertions/deletions, structural variants, and copy number variations, without the bias introduced by predefined marker sets. As such, it is a powerful tool for population genomics, evolutionary studies, and genomic selection. Recent developments in graph-based reference genomes and pangenomes aim to overcome the limitations of using a single linear reference by incorporating structural and allelic diversity from multiple individuals. This approach improves variant discovery and alignment accuracy, particularly in regions of high diversity or structural complexity [112]. However, several challenges still hinder the widespread adoption of WGS in livestock: cost remains a significant barrier, particularly for sequencing large numbers of individuals needed for population-level analyses or routine breeding programs; moreover, bioinformatics expertise and computational requirements for storing, processing, and analysing large-scale sequencing data are substantial. Despite these constraints, the decreasing cost of sequencing and the increasing availability of cloud-based and open-source bioinformatics tools are making WGS more accessible, and its use in livestock genomics is expected to grow rapidly in the coming years.

The main applications of SNP panels in livestock breeding have expanded significantly over the last two decades, providing valuable tools for genetic improvement, population management, and traceability. Key uses include [113,114]:

- Parentage verification and pedigree correction: SNP panels are routinely used to verify parentage and correct pedigree errors, which is essential not only for accurate genetic evaluations but also for informed mating decisions and more precise estimation of inbreeding levels.
- Animal and product traceability: Genomic tools enhance the traceability of both animals and derived products, providing transparency and assurance to consumers. Moreover, they play a critical role in legal contexts such as forensic investigations and in the unambiguous identification of animals in research settings.
- Detection of chromosomal abnormalities: SNP genotyping can assist in the detection of specific chromosomal aberrations, such as aneuploidies, chimerism, and cattle Robertsonian translocation 1;29 [115–117], although traditional cytogenetic analysis remains the gold standard for this task.
- Sex determination.
- Breed composition assessment and individual assignment: SNP data enable the estimation of breed composition and the assignment of individuals to specific breeds during registration processes.

- Trait association and identification of major genes: SNP genotyping supports genome-wide association studies (GWAS) to detect loci associated with economically important traits. It also facilitates the identification of causal mutations responsible for specific phenotypes or inherited disorders, such as resistance to scrapie in sheep or susceptibility to *Theileria* infection [118].
- Mating plan optimisation: Genomic information is used to design mating schemes that maximise genetic gain while controlling the rate of inbreeding, a strategy known as *optimal contribution selection* [119].
- Genomic selection: By integrating genomic data into prediction models, it is possible to estimate genomic estimated breeding values more accurately and at an earlier age, thereby accelerating genetic progress and aligning selection with market demands.

1.3.2 Genomic study of biodiversity

As described above, genomics plays a pivotal role in animal breeding, enhancing the ability to select and manage livestock populations. However, it also serves several more specific purposes in the investigation and conservation of livestock biodiversity [110,120,121]:

- Improving knowledge of domestication, breed development, and evolution: Genomic tools enable the reconstruction of relationships among populations and can trace the history of domestication, as well as how breeds were formed and evolved under natural and artificial selection pressures.
- Breed characterisation and recognition: As previously noted, molecular characterisation is a crucial step in assessing the breed status of a population and identifying the most appropriate individuals for breeding. This supports both official breed recognition and effective breed management.
- Population management: Genomic data allow breeders and conservationists to monitor inbreeding levels and relatedness among individuals. This supports informed

mating decisions aimed at minimising inbreeding depression and maintaining genetic diversity, which is especially important in small or endangered populations.

- Prioritisation of local breeds for conservation: In contexts where conservation resources are limited, it may be necessary to identify which populations warrant the greatest attention. Genomic tools provide critical information on genetic distinctiveness, private alleles, and adaptive potential, all of which can inform prioritisation strategies. Additionally, genomics facilitates the more accurate delineation of conservation units, distinguishing between populations that are genetically distinct versus those that are part of a broader gene pool [122].
- Biobank creation: Biobanks preserve genetic material (e.g., semen, embryos) for long-term conservation of animal genetic resources. When financial or logistical constraints exist, genomic analyses can help prioritise individuals or breeds to include, ensuring the selection of a "safe set" with minimal relatedness within and between breeds [123,124] .
- Detection of hybridisation and outbreeding depression: Genomic analyses are useful for identifying past or ongoing hybridisation events between breeds or species. This is essential for managing unintended gene flow, particularly in conservation programs. Additionally, genomics can help detect early signs of outbreeding depression, which may occur when individuals from genetically divergent populations are crossed, due to genic incompatibilities or reduced adaptation to the local environment [122].
- Development of crossbreeding programs: Locally adapted breeds often carry valuable alleles for traits such as disease resistance, heat tolerance, or reproductive efficiency. Genomics can help identify donor individuals that will contribute beneficial traits to commercial breeds while minimising the risk of inbreeding or loss of unique genetic variation.
- Development of genetic rescue programs: In small or declining populations, the translocation of individuals carrying beneficial alleles can increase genetic diversity and

reduce the risk of extinction. Genomic data help identify optimal donor individuals or populations, maximising adaptive potential while minimising the risk of outbreeding depression or genetic swamping [41,122].

Most of these tasks rely on the analysis of individual and population-level genetic diversity, the study of relationships among breeds and, to a broader extent, species, and the identification of genomic regions under selection.

1.3.2.1 Genomic diversity

Genetic diversity assessment provides valuable information on the risk of inbreeding depression and population extinction, the effects of crossbreeding on breed composition, and demographic events such as bottlenecks that may have occurred during domestication, migration, or subsequent breed history [110].

Typically, genetic diversity is measured through the amount of heterozygosity or homozygosity in the genome. In particular, observed heterozygosity (Ho) refers to the occurrence of two different alleles at a given locus in a diploid organism, while expected heterozygosity (He) is the proportion of loci expected to be heterozygous under Hardy-Weinberg equilibrium, i.e. in a large, panmictic population not subject to evolutionary forces [125].

Various parameters related to inbreeding can also be calculated. An inbreeding coefficient measures the probability that two alleles at a locus are identical by descent from a common ancestor [126]. Traditionally, inbreeding estimation has relied on pedigree data. However, pedigree-based inbreeding coefficients estimate the expected proportion of the genome identical by descent between two parents and do not account for variation introduced by Mendelian sampling or linkage during gamete formation. Furthermore, they assume that pedigree founders are unrelated, which is rarely the case, often resulting in an underestimation of relatedness. Pedigrees may also be incomplete or inaccurate, particularly in species such as small ruminants [127–129]. Genomic data offer the advantage of estimating realised individual inbreeding and kinship, even in the absence of pedigree information. They also allow identification of genomic regions with elevated inbreeding levels [120].

One of the earliest genomic inbreeding coefficients, proposed by Wright in 1951 [130], is F_{IS}, derived from He and Ho. A positive F_{IS} value may indicate inbreeding or genetic subdivision (the Wahlund effect), while negative values are usually associated with crossbreeding [131].

Another widely used genomic inbreeding estimator is based on runs of homozygosity (ROHs), defined as contiguous homozygous segments in the genome [132]. The F_{ROH} coefficient is calculated as the proportion of the autosomal genome covered by ROHs relative to the total length of the genome assessed by SNPs [133]. F_{ROH} has been extensively applied in livestock populations due to its advantages over SNP-by-SNP approaches: it captures inbreeding from both recent and ancient events—whereas F_{IS} is mainly sensitive to recent events—and it shows a stronger correlation with the recessive mutation load [110,120,134]. Moreover, ROH length can be used to infer the timing of inbreeding: long ROHs are the result of recent inbreeding events, as they have not yet been fragmented by recombination, while shorter ROHs reflect more distant ancestry. For example, assuming 1 cM ≈ 1 Mb, ROHs of approximately 16.6 Mb, 10.0 Mb, or 5.0 Mb correspond to inbreeding events that occurred about 3 (6 meioses), 5 (10 meioses), or 10 (20 meioses) generations ago, respectively [120,135]. However, a major drawback of using ROHs is the lack of consensus on the parameters used to define them across different species and SNP densities, which makes it difficult to compare results across studies. Indeed, ROH detection is highly sensitive to several factors, including minor allele frequency (MAF) thresholds, LD pruning, and the specific settings applied—such as minimum ROH length, SNP density, maximum gap between SNPs, and the number of allowed missing or heterozygous genotypes. Additionally, species-specific features like recombination rate, LD patterns, and mutation rates, as well as chip density, can significantly influence ROH identification. For these reasons, it is essential that all studies clearly and explicitly report the parameters used for ROH detection [136–138].

A further key parameter for assessing genetic diversity is the effective population size (Ne). Ne is defined as the number of individuals in an idealised population—mating randomly and unaffected by selection or migration—that would experience the same rate of genetic drift as the actual population [139]. In simpler terms, it represents the number of individuals who effectively contribute offspring to the next generation [140]. Ne is generally smaller than the census size, particularly in domesticated species where non-random mating, imbalanced sex ratios, and reproductive technologies are common [41,137]. A reduced Ne is associated with diminished adaptive potential and increased extinction risk. For instance, the 50/500 rule proposed by Franklin (1980) [141] considers populations with Ne < 50 at immediate risk, 50–500 at long-term risk, and Ne > 500 as relatively safe. Although Ne is a widely used indicator of population viability, it is prone to estimation biases and should be interpreted with caution, ideally in combination with other parameters [110,142]. For instance, the FAO currently recommends a multidimensional risk classification based on numerical scarcity (e.g., number of breeding females), inbreeding rate (which is inversely proportional to Ne), and the presence of active conservation programs [36].

Several methods are available for estimating Ne. Traditional estimates are based on census or pedigree data, such as the commonly used Wright's formula based on sex ratio [143,144]. However, genome-wide marker-based approaches have proven more accurate. Among genomic methods, approaches include heterozygosity excess relative to Hardy-Weinberg equilibrium (though less effective unless Ne is very small), estimation from frequencies of full- and half-siblings in population samples, temporal methods tracking allele frequency changes over time (requiring samples from different generations), and LD-based methods, which are currently among the most widely applied. LD-based Ne estimation is grounded in the observation that, in a finite population, genetic drift increases the correlation between alleles at linked and even unlinked loci [145,146].

1.3.2.2 Population structure and between-breed relationships

Genomic characterisation of populations usually involves datasets composed of multiple breeds or populations. One of the primary aims of these studies is to understand population structure and explore how these groups are genetically related to each other.

One of the most widely used methods to represent both intra- and inter-population genetic diversity is principal component analysis (PCA). PCA is a statistical procedure that reduces the dimensionality of the data while preserving as much of its variance as possible, allowing for intuitive visualisation of large datasets, including genomic ones. It is a model-free analysis that transforms potentially correlated variables into a new set of uncorrelated variables, known as principal components (PCs), which are linear combinations of the original variables. These PCs are ordered by the proportion of explained variance, and typically, only the first few components are visualised to capture the main patterns of genetic variation in the dataset. Plotting individuals based on the eigenvectors of the first PCs allows the investigation of the genetic structure of individuals and, by extension, populations. For instance, in scenarios where population structure reflects a continuous isolation-by-distance model, the first PCs often depict genetic clines that correlate with geographical distribution [147]. PCA can also suggest past admixture events, as admixed individuals tend to be positioned between their parental populations on the plot. However, since only a limited portion of total genetic variation is captured by any single PC, fine-scale relationships between individuals may not be visible. Caution is also required when interpreting results, as unbalanced sample sizes can strongly affect group positions in the PCA plot. Additionally, populations that are substantially different from the rest—often due to high inbreeding levels—may dominate the major PCs, skewing interpretation [109,110]. A similar approach is multidimensional scaling (MDS), which reduces the dimensionality of a dataset while preserving the pairwise distances—or dissimilarities—between points in a lower-dimensional space. MDS can be applied to various distance measures, such as identity-by-state (IBS), F_{ST}, or Hamming distance (i.e., the number of differing alleles between two individuals), as implemented in the PLINK software. When the distance configuration is Euclidean, MDS typically yields results equivalent to PCA [148].

Another approach to characterising population structure is through pairwise genetic distance matrices among individuals, such as measures of kinship or identity-by-state, or population. Various formulas have been developed to calculate genetic distances between breeds, using either allele frequencies (Euclidean or angular) or allele size distributions. Each method has its own strengths, limitations, and assumptions [149,150]. A commonly used metric is the Reynolds distance [151], a good estimator of Wright's F_{ST} (a well-known measure of inbreeding due to population subdivision) [130,152] and is independent of the marker type or panel. Reynolds distance assumes genetic drift as the primary force of breed differentiation and is linearly related to divergence time. Therefore, it is particularly suited for representing recent divergence events, such as breeding differentiation [109,110]. Another widely used measure is the Nei distance [153], which preserves a linear relationship with divergence time but includes the effects of both genetic drift and mutation. For this reason, Nei distance is better suited when the comparison is done over long evolutionary timescales or when mutation plays a significant role.

These genetic distance matrices are typically visualised as dendrograms or phylogenetic trees. Given the complexity of breed histories, the neighbour-joining (NJ) algorithm [154] is generally preferred over UPGMA or cladogram-based methods, as it does not assume uniform evolutionary rates among lineages. NJ trees produce phylograms where branch lengths correspond to genetic distances, thus better reflecting breed-specific drift [110,155,156]. However, to capture more complex relationships, reticulation can be introduced. For instance, the NeighbourNet algorithm, implemented in SplitsTree [157], generates network trees that, like NJ trees, represent terminal branches and clear breed clusters but replace often misleading bifurcations with more informative network structures [110]. Another widely used tool is Treemix [158], which constructs maximum-likelihood trees based on allele frequency covariance. Treemix allows for the inclusion of migration events, visualised as arrows between tree branches. Nevertheless, inferred migration patterns should ideally be validated through formal tests of allele frequency covariance, such as f3 and f4 statistics [110].

A third common approach to characterise population structure is the use of clustering methods. The first of these was developed by Pritchard, Stephens, and Donnelly (2000) [159] and implemented in the software STRUCTURE . STRUCTURE assumes Hardy-Weinberg and linkage equilibrium among SNPs and models the dataset as composed of K genetic clusters. Using a Markov Chain Monte Carlo (MCMC) algorithm, it estimates the proportion of each individual's genome that originates from each cluster. In unsupervised clustering, both the number and composition of clusters are inferred without prior knowledge. In contrast, supervised clustering involves defining ancestral clusters in advance and estimating admixture proportions of additional individuals relative to them. Several alternative programs have since been developed, such as FRAPPE [160], ADMIXTURE [161], and sNMF [162]. These tools offer faster computation and, in the case of sNMF, are less affected by inbreeding and more scalable to large datasets [110].

Lastly, identity-by-descent (IBD) haplotype sharing can be used to explore population structure. IBD segments are genomic regions inherited from a common ancestor without recombination. There are multiple methods to detect IBD, based on either shared genotype/haplotype frequencies and lengths or on probabilistic approaches, with or without consideration of LD. Probabilistic methods typically offer higher accuracy and lower false-positive rates but are more computationally intensive [163]. IBD analysis has a wide range of applications, including detection of natural selection, IBD mapping for disease variants, pedigree reconstruction and relationship inference, phasing and imputation, and heritability estimation in founder populations [163,164]. Additionally, IBD-based sharing provides insight into population demography and recent admixture. Closely related individuals share longer and more numerous IBD segments than distant relatives, as recombination has not yet broken them apart. Although it is challenging to infer pairwise relationships between distant relatives, patterns of IBD sharing across populations can reveal features such as population size, growth, and the timing and depth of subdivision [164]. For example, it has been observed that the total length of shared haplotypes between two breeds correlates linearly with the time since admixture or divergence from a common ancestor [165].

Taken together, the methods described each reveal different aspects of population structure and demographic processes. As each approach is influenced by specific assumptions and potential biases, their complementary use is advisable to achieve a more accurate and comprehensive reconstruction of genetic relationships among populations.

1.3.2.3 Selection signatures

The genome of livestock populations has been shaped by domestication, adaptation, breed differentiation, and selective breeding, all of which have left specific genetic patterns referred to as selection signatures [110]. A population may be subject to different forms of selection: positive, negative, and balancing. Positive selection, whether natural or artificial, increases the frequency of beneficial alleles. However, due to LD, neutral variants located near the target locus may also increase in frequency via the hitchhiking effect. As a consequence, genetic variation is reduced in the selected region, while differentiation between populations exposed to different selective pressures increases. Negative or purifying selection acts against deleterious alleles. Although it does not directly affect linked neutral variants, the ongoing removal of harmful alleles—known as background selection—results in highly conserved genomic regions. Conversely, balancing selection maintains multiple alleles at a locus, leading to high local genetic diversity. This may result from mechanisms such as heterozygote advantage or frequency-dependent selection [110,166].

Because positive selection tends to leave more detectable traces in the genome and is also the primary form of selection involved in artificial selection and local adaptation, numerous methods have been developed to identify its signatures at both macroevolutionary (e.g., among taxa or species) and microevolutionary (e.g., among breeds or populations) levels. In many cases, these signatures take the form of selective sweeps, which can be classified as hard, when a novel advantageous mutation rapidly reaches fixation, or soft, when selection acts on standing variation or multiple beneficial alleles.

To detect selection at the microevolutionary scale, analyses can be conducted either within a single population or between populations. Intra-population methods aim to detect selection signatures by analysing genetic patterns in individual populations and are typically grouped into three categories.

The first includes approaches based on the site frequency spectrum (SFS), which exploit the tendency of selective sweeps to increase the number of rare alleles while reducing intermediate-frequency variants. This results in a skewed frequency distribution, as captured by statistics such as Tajima's D [167]. However, SFS-based methods are not optimal for genome-wide SNP datasets.

The second group comprises LD-based methods, which detect extended regions of LD. Indeed, due to the hitchhiking effect, selected alleles remain in high LD with neighbouring loci until disrupted by recombination. Therefore, unusually long or frequent haplotypes suggest recent positive selection [168]. One of the earliest LD-based methods is the relative extended haplotype homozygosity (rEHH), which measures the probability that two chromosomes sharing a core region, randomly chosen within the population, remain IBD across increasing physical distances from that region [168]. Another widely used statistic is the integrated haplotype score (iHS), which considers recombination rates to assess whether haplotype structures surrounding a given SNP are unusually long relative to the genomic background. This method is particularly suited for detecting selected alleles at intermediate frequencies and is less sensitive than rEHH to demographic events, thus reducing the likelihood of false positives [169].

The third category includes approaches that identify genomic regions with reduced local variability, such as those characterised by low pooled heterozygosity or the presence of long ROHs.

Inter-population methods assess locus-specific allele frequency differences among two or more populations. These can be applied to single markers or haplotypes. Among single-marker methods, one of the most widely used is F_{ST}, which measures allele frequency differentiation between populations at each locus, allowing for outlier detection [170]. Several related statistics have been proposed to refine and complement F_{ST}. One such method is FLK, which compares observed F_{ST} values to those expected under neutrality using a variance ratio test. Importantly, FLK incorporates a phylogenetic estimation of population kinship matrix, making it more robust to demographic history and thus less prone to false positives than F_{ST} [171]. Haplotype-based methods, which help reduce SNP ascertainment bias, include cross-population EHH (XP-EHH), an extension of the EHH framework designed for pairwise population comparisons [172], and hapFLK, which applies the FLK model to haplotype data to detect selection in structured populations [173].

Because different approaches capture distinct genomic signals and are influenced by different assumptions and biases, combining them can enhance both detection power and resolution. Composite methods have been developed to either aggregate scores from the same test across multiple adjacent loci—thus reducing false positives—or to integrate scores from independent tests at the same locus. Alternatively, different methods may be applied independently, and loci identified by more than one method can be considered candidate regions under selection [166].

The detection of selection signatures has been widely explored across livestock species, as it plays an important role both in selective breeding, by facilitating the identification of genes or QTLs associated with economically important traits, and in conservation genomics, by revealing genomic regions involved in adaptation to challenging environments. Identifying such adaptive features can inform conservation priorities, particularly for breeds with traits of potential value in future production systems [110,174].

1.3.3 Genomic environmental adaptation

The genome of a population is shaped by evolutionary forces such as mutation, genetic drift, gene flow, and both natural and artificial selection. Among these, spatially variable environmental pressures can drive local adaptation, whereby populations evolve genetic variants that enhance fitness in their native habitats. This form of divergent selection acts differently across geographic locations, targeting traits related to survival and reproduction, and over time leads to distinct allele frequency patterns that reflect adaptation to local environmental conditions [175–177].

The detection of local adaptation relies on several ecological conditions. First, selection must be strong and persistent over time to counteract the homogenising effect of gene flow. Second, locally optimal genotypes must confer a fitness advantage in their native environment while being less advantageous elsewhere. Third, the evolution of adaptive phenotypic plasticity must be constrained by physiological costs or evolutionary limits; otherwise, genetic differentiation would not be necessary. Finally, population sizes should be large enough for the effects of genetic drift to be negligible [176,178].

In general, the genetics of local adaptation can be studied using either a top-down or bottom-up approach. The top-down approach starts by identifying candidate demes suspected of being locally adapted, followed by measuring adaptive traits, and finally conducting reciprocal transplant experiments to assess performance in native versus non-native habitats. If evidence of local adaptation is observed, GWAS or QTL mapping can be used to identify the genotypes associated with the measured traits [179]. However, this approach presents several logistical and biological limitations: transplant experiments are often infeasible for animals; relevant phenotypes may be complex or difficult to measure; environmental adaptation is typically polygenic; and both phenotyping and genotyping a sufficient number of individuals can be prohibitively expensive [94].

The bottom-up approach, by contrast, uses genomic data directly to detect adaptive genetic variation, which is then related to evolutionary processes or environmental gradients. This approach does not require phenotypic data and is therefore more broadly applicable. Two main types of bottom-up analyses are commonly used: population genomics and landscape genomics.

Population genomics aims to detect genomic regions showing elevated genetic differentiation among populations living in different environments. It typically uses the same methods described for identifying selection signatures, such as F_{ST} outlier tests. While this can reveal regions under positive selection, it is important to note that polygenic local adaptation often results in subtle allele frequency shifts across many loci, which may not be detected by outlier-based methods. Furthermore, in livestock populations, it is difficult to distinguish local adaptation from artificial (anthropogenic) selection, which may act on similar traits and leave overlapping genomic signatures.

Landscape genomics, on the other hand, explicitly integrates environmental and genomic data to identify environmental factors that have shaped adaptive genetic variation, as well as the genes and alleles underlying local adaptation, by directly associating habitat variables with genomic variation. This field, which merges principles from landscape genetics and population genomics, was initially introduced by Joost [180]. Since then, it has grown rapidly, spurring the development of increasingly sophisticated analytical tools and a large body of empirical studies across a wide range of species.

1.3.3.1 Landscape genomics and genotype-environment associations

When preparing the groundwork for a landscape genomic study, four main steps should be carefully planned and evaluated: i) an appropriate sampling design, which should capture both intraspecific genetic variability and relevant environmental variation; ii) geo-referenced environmental data that effectively describe the putative selective pressures acting on the studied populations; iii) high-quality genome-wide data; and iv) appropriate statistical tools to correlate genomic response variables with environmental predictors while accounting for possible confounding effects such as neutral genetic structure [181].

When selecting animals to genotype and the source of environmental data to analyse, it should be considered that environmental and genomic datasets should match in spatial resolution, that the geographic scale must match the ecology of the studied species, and that the time lag between the environmental selective pressure and the resulting genetic response must be acknowledged. Different sampling designs, each with advantages and limitations, can be adopted (Figure 1.3.1).

Uninformed sampling designs do not take into account prior knowledge of environmental or genetic structure. These designs typically involve sampling individuals or populations on a regular grid (regular sampling), randomly across a wide area (scattered sampling), or along linear geographic gradients (transect sampling), assuming to be able to capture both genetic and environmental variability across the species' range. Environment-informed designs, on the other hand, select samples based on environmental variability. For instance, individuals may be sampled along an environmental gradient (e.g., temperature), allowing for focused investigation of specific selective pressures. However, this approach is difficult to replicate and does not easily accommodate the integration of additional environmental variables at a later stage. Categorical sampling represents another strategy, usually involving populations that live in contrasting environments (e.g., hot vs. cold, wet vs. arid), to facilitate pairwise comparisons. While useful, this approach limits the range of analyses that can be performed and requires more replicates to achieve statistical power. The most suitable sampling strategy is one that is both genetics- and environment-informed. This involves stratified sampling to ensure an equivalent number of individuals per environmental category and per genetic cluster [177,179].

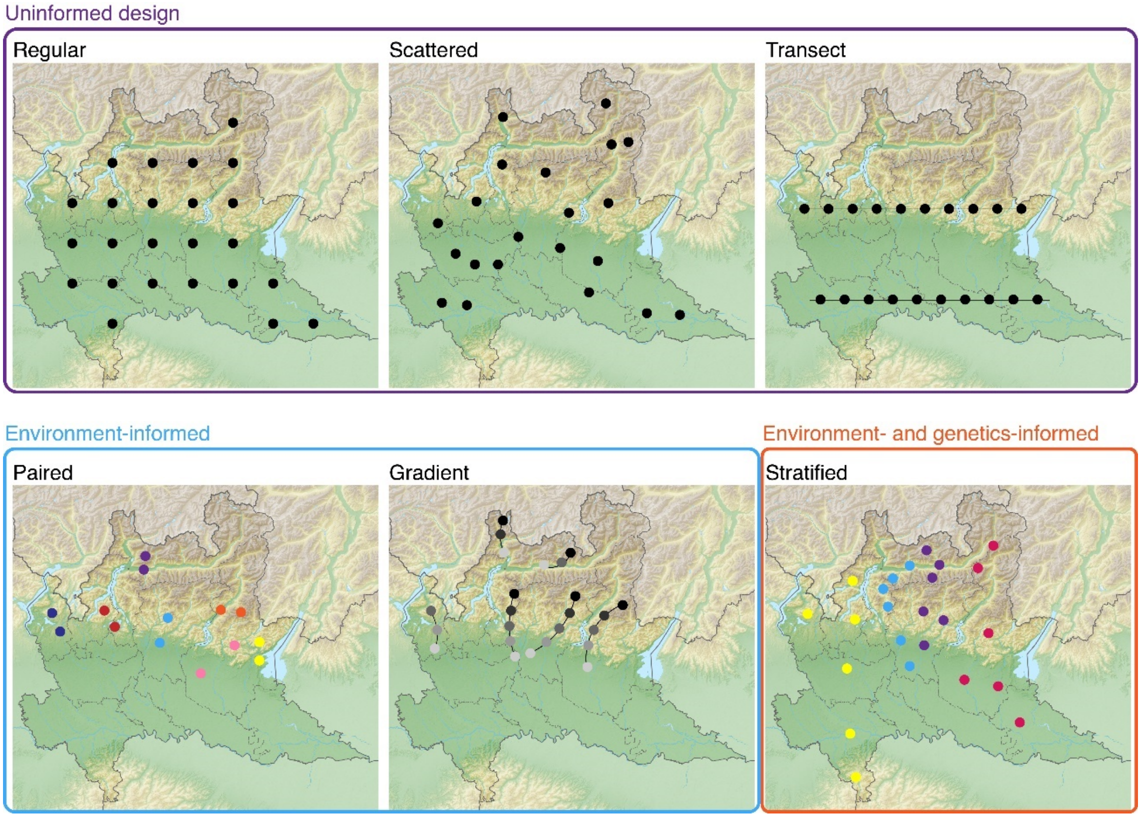

Figure 1.3.1 Examples of sampling designs for landscape genomic studies as proposed by Dauphin *et al.* (2023).

Collecting and managing environmental data properly is essential for obtaining robust results from landscape genomic analyses. Most studies use data from public databases, which provide interpolated environmental variables over large areas or at regional scales. While convenient and accessible, such data often have limitations: they are typically restricted to specific time periods, and their coarse spatial resolution may fail to capture environmental differences between nearby locations. Alternatively, remote sensing or in situ measurements may be used to acquire local conditions during specific years. However, these approaches may inadequately reflect long-term environmental trends that are often more relevant to genomic adaptation.

Another critical consideration is the inter-correlation among environmental variables. Many environmental predictors are highly correlated, and testing all of them increases the number of statistical tests and the risk of false positives. Several methods can be applied to reduce collinearity and dimensionality: for instance, by excluding highly correlated variables based on pairwise correlation matrices, by selecting those with the greatest contributions to the first few axes of a PCA, or by filtering based on variance inflation factors (VIF) [182]. It should be noted, however, that even excluded variables—although not directly tested—should still be considered during interpretation. An alternative strategy is to use linear combinations of variables, such as PCs from a PCA, in the analysis. While this simplifies modelling, it may render the biological interpretation of the results less straightforward [177,179].

Before proceeding with the actual genotype–environment association (GEA) analyses, a final consideration is necessary: neutral evolutionary processes, such as genetic drift, gene flow, and mutation, can mimic the genomic patterns expected under divergent selection, potentially leading to false positives [177,179,183]. For this reason, the neutral genetic structure of the populations should be incorporated into the analyses to correct for these confounding effects. Earlier GEA studies accounted for such structure indirectly by including spatial autocorrelation variables, such as geographic coordinates or pairwise distances between sampling locations. However, it is now recommended to incorporate genetic structure directly. Ideally, this should be estimated using a subset of known neutral markers [184]. although these are typically not known a priori. More commonly, researchers use either a large number of markers distributed across the genome—assuming that loci under selection are rare enough not to bias global estimates—or select markers presumed to be neutral (e.g., non-outlier loci, synonymous SNPs, or those in non-coding regions). Neutral genetic structure can then be estimated using global or pairwise fixation indices such as F_{ST} or kinship, or via matrix factorization methods such as PCA or clustering algorithms [177,179]. Additionally, some analytical frameworks integrate neutral genetic structure directly into the model during GEA testing.

The first GEA method developed was the Spatial Analysis Method (SAM) [180]. which uses multiple univariate logistic regressions to test whether an environmental variable is associated with the presence or absence of a specific allele or genotype. However, since SAM does not account for neutral genetic structure, it is prone to a high rate of false positives, particularly in cases involving complex demographic histories. An extended version, Samβada [185], was developed to address this limitation. Samβada also incorporates multivariate analyses and supports parallel computing, improving computational efficiency and reducing spurious associations.

Another widely used framework involves general linear models, in which allele frequencies are treated as response variables and modelled as a function of environmental predictors and neutral genetic structure. Mixed-effect models, in particular, allow the inclusion of neutral genetic structure as a random effect, while environmental variables are treated as fixed effects. Among these, BAYENV [186] evaluates correlations between allele frequencies and environmental variables while controlling for population structure, modelled through a covariance matrix derived from genome-wide allele frequencies. It also accounts for uncertainty due to uneven sampling. An updated version, BAYENV2 [187], adds the ability to perform non-parametric tests, increasing robustness in more diverse datasets. A related method, BayPass [188], builds on BAYENV's Bayesian framework and further improves the modeling of allele count data, accounting for sampling variance and uneven coverage, can compute both GEAs and XtX, a Bayes-based analogue to F_{ST}, and introduces alternative modelling strategies to deal with population-specific covariables. BayeScEnv [189] is another Bayesian method, based on F model, which considers simultaneously locus-specific effects due to divergent selection and to other processes that jointly tests for F_{ST} outliers and environment-driven selection.

Latent Factor Mixed Models (LFMMs), introduced by Frichot *et al.* (2013) [190], are another widely used approach. LFMMs model neutral genetic structure through the estimation of latent factors, which are introduced as random effects and are inferred simultaneously with the GEA analysis using a Markov Chain Monte Carlo algorithm. The number of latent factors (K) must be defined by the user, typically based on prior analysis of population structure (e.g., from PCA or clustering algorithms). Environmental predictors are treated as fixed effects, and regression coefficients quantify the strength of association between environmental covariates and genetic markers. LFMMs have proven to be computationally efficient, with low false-positive and false-negative rates, and are robust across a wide range of sampling designs and demographic scenarios.

Multivariate models offer an alternative that better accommodates the polygenic nature of adaptation. For instance, Canonical Correlation Analysis (CCA) identifies maximally correlated linear combinations of multiple genetic and environmental variables, which are then tested for statistical significance [191]. Another powerful method is Redundancy Analysis (RDA), a form of constrained ordination that models multivariate linear relationships between genotype and environment. It constructs canonical axes by performing PCA on the fitted values of genetic data constrained by environmental predictors. When a partial RDA (pRDA) is applied, neutral genetic structure can be included as a conditioning variable. These ordination techniques are especially robust in scenarios involving isolation by distance or low dispersal capability, and they reduce the risk of spurious results due to spatial autocorrelation or population structure [192].

Recently, machine learning methods have begun to emerge as promising tools in GEA. One example is Gradient Forest [193], an extension of Random Forest that models non-linear changes in allele frequencies along environmental gradients. It allows for variable importance ranking and identification of thresholds in environmental responses, but currently does not incorporate neutral genetic structure, which limits its ability to distinguish between adaptive and neutral variation.

Regardless of the method employed, GEA analyses face several challenges—most notably, a high rate of false positives. This arises from multiple sources. First, as already mentioned, geographic and demographic processes can generate allele frequency patterns that mimic those produced by selection. While accounting for neutral genetic structure mitigates this risk, in scenarios such as range expansion that creates a cline in allele frequency along and environmental gradient or strong isolation by distance, these corrections may still be insufficient [194]. Second, GEA typically involves multiple hypothesis testing, which increases the chance of spurious associations. As such, false discovery rate (FDR) control procedures, such as those proposed by Benjamini and Hochberg (1995) [195] or Storey and Tibshirani (2003) [196], should always be applied. Third, linkage disequilibrium can lead to false associations if linked, non-causal variants hitchhike with selected loci. Fourth, extreme or coincidental outlier values in both environmental and genomic data can produce artificial correlations. To reduce this, individuals from habitats with extreme environmental values should be avoided where possible, and rank-based, non-parametric tests may offer more robust alternatives. Lastly, spurious associations can arise when a locus under true selection is misattributed to a covarying environmental variable that was included in the analysis, while the actual selective pressure remains unmeasured.

In addition to these considerations, validation strategies can improve the reliability of GEA analysis results. One approach is to combine multiple GEA methods: loci identified by several independent approaches are less likely to be false positives, although this may increase the risk of missing weaker selection signals. Another strategy is to examine the biological relevance of identified loci, for instance by investigating their functional annotation, gene ontology (GO) categories, or GO enrichment tests. It is also useful to determine whether identified polymorphisms are synonymous or non-synonymous. Finally, the most direct form of validation is through replication in independent datasets or experimental testing, which remain the most convincing evidence for a true GEA [179,197].

1.3.3.2 Genomic prediction of maladaptation

Given the magnitude of forecasted climate change, a prominent interest in genomic research lies in predicting how these changes will impact populations. It is expected that climate change will disrupt, to varying degrees, the balance between existing genotypes and the optimal fitness they have evolved under their native environmental conditions. The ability to predict how populations will respond to these changes—or which populations may be most vulnerable—could support targeted conservation strategies, as well as guide the development of restoration or crossbreeding programs using the most suitable breeds in the case of livestock [198–200].

To this end, different approaches have been developed to quantify the genetic distance between the current genomic composition of a population and the composition that would be optimal under a future or alternative environmental scenario. This concept is commonly referred to as genomic offset (g.o.) (Figure 1.3.2).

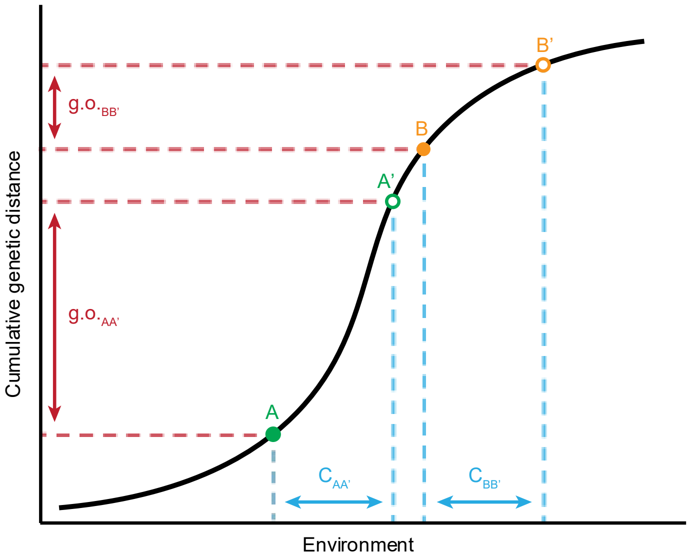

Figure 1.3.2 Conceptual illustration of genomic offset. Points A and B represent populations at different positions along an environmental gradient (horizontal axis). A projected environmental shift (from A to A′, or from B to B′) is associated with a corresponding change in the predicted adaptive genetic component (vertical axis), typically measured as cumulative allele frequency change or allelic turnover. This change defines the genomic offset (g.o.). For the same magnitude of environmental change (C), the genetic response differs: the shift from A to A′ occurs in a region of steep allelic turnover, resulting in a larger genomic offset ($g.o._{AA'}$), while the shift from B to B′ produces a smaller genomic offset ($g.o._{BB'}$).

The estimation of g.o. relies on a preliminary identification of putatively adaptive loci through GEA analyses, which will be used in downstream models. GEA results must also be generalized in space, to provide continuous predictions of allele frequencies, including in locations where environmental data are available but individuals have not been genotyped [200].

Genomic vulnerability can be predicted by integrating GEA-derived data with spatial or temporal climate projections. Two related but distinct metrics are used for this purpose: the Risk of Nonadaptedness (RONA) and genomic offset (g.o.). Both aim to quantify the genomic mismatch expected under future environments. RONA estimates the allele frequency shift required at each locus and aggregates it into a composite index. In contrast, g.o. accounts for the polygenic nature of adaptation by summarizing the combined turnover of multiple adaptive alleles along environmental gradients.

Various approaches exist to estimate g.o. or RONA. Gradient-based methods include Generalized Dissimilarity Modeling (GDM) and Gradient Forest (GF). GDM estimates the expected disruption of local adaptation by modeling genetic differentiation (by using F_{ST}) as a function of environmental distance between present and future conditions [201]. GF, an extension of the random forest algorithm, models the change in allele frequency at each locus along environmental gradients and summarizes this change across loci [201,202].

A multivariate approach is based on RDA, which decomposes the genotype–environment relationship into orthogonal axes representing independent patterns of covariation. More specifically, adaptive loci identified through GEA are used as response variables in a constrained ordination model, with environmental predictors as explanatory variables. For each significant RDA axis, a genetic-based index of adaptation is computed by summing, for each environmental variable, the product of its loading on that axis and its standardised value at a given location. The difference in these indices between current and future environments provides an estimate of g.o. [192].

The Spatial Areas of Genotype Probability (SPAG) framework offers another multivariate approach. It applies logistic regression to project the spatial distribution of adaptive genotypes under current and future climates. Several SPAG variants exist: univariate SPAG, which considers individual genotypes; I-SPAG, which models the probability of carrying all genotypes in a given set (for traits with additive effects); U-SPAG, which models the presence of at least one adaptive genotype (suited for epistatic traits); and K-SPAG, which calculates the probability of carrying at least K% of a set of adaptive loci (applicable when multiple genotypes jointly contribute to fitness, until reaching a K threshold) [203].

Finally, Genome-Wide Environment Selection (GWES) extends traditional GWAS to explicitly incorporate environmental gradients as predictors of fitness [204]. However, this method requires phenotypic data and common garden or reciprocal transplant experiments, which limits its applicability in many systems.

Despite the promise of these methods, the estimation of g.o. and RONA relies on multiple biological and statistical assumptions, which can limit their accuracy and the interpretation of results.

First, these models assume that current allele frequencies reflect local adaptation and optimal fitness under present environmental conditions. However, populations may already be maladapted, and local adaptation should be validated before predictions are made. Moreover, the substitution of spatial for temporal patterns assumes that genotype–environment relationships remain constant over time, despite the potential for novel environmental conditions, demographic shifts, or genotype–environment interactions to alter these dynamics.

Current offset models do not account for evolutionary processes such as mutation, recombination, gene flow, genetic drift, or adaptive evolution, treating environmental change as instantaneous. Additionally, most models assume uniform selection pressure across populations and do not account for spatial heterogeneity in selective forces.

GEA and offset approaches also typically ignore complex genetic architectures. They assume additive, independent effects of alleles and overlook non-additive interactions, pleiotropy, trait correlations, and phenotypic plasticity. Yet many adaptive traits are polygenic, redundant, and governed by small-effect loci that are difficult to detect. Moreover, loci are often assumed to respond to a single selective pressure, while in reality, alleles may affect multiple traits and respond to multiple interacting environmental drivers.

The interpretation of genomic offset as a proxy for fitness decline is another critical assumption. Offset magnitude reflects allele frequency shifts rather than direct fitness effects and is influenced by the shape of allele frequency gradients. Consequently, large offsets may not always correspond to high vulnerability, and environmental change may even increase fitness in some populations.

Finally, not all methods correct for population structure, which can bias GEA results. The inclusion of mixed-effect models or latent factor corrections is essential where structure is present. Demographic processes such as migration and changes in population size should also be considered. Importantly, prediction uncertainty must be quantified to assess model reliability and inform practical applications.

Given these constraints and limitations, caution should be exercised in the interpretation of results. Moreover, this underscores the importance of always quantifying and reporting measures of predictive uncertainty. Equally critical is the correction for the confounding effect of neutral genetic structure, which, if unaccounted for, can lead to spurious associations and misleading predictions [199,205]. Ideally, findings from genomic offset analyses should be validated using controlled experiments, such as common garden trials, where forecasted responses can be compared against empirical data [205]. However, such approaches are rarely feasible, especially for long-lived species or complex traits. An alternative strategy is to incorporate temporal data, for example by calculating GEA models on historical cohorts and testing whether the predicted beneficial alleles have increased in frequency over time. Similarly, g.o. can be estimated for past cohorts under present environmental conditions and compared with contemporary genomic data to assess the coherence and plausibility of the predictions [199].

2

THE HISTORY OF GOAT DOMESTICATION AND SELECTION

Domestication is one of the most significant human-driven evolutionary processes, transforming wild species into populations adapted to life under human management. It involves a combination of natural and artificial selection, demographic bottlenecks, and gene flow, resulting in profound changes in behaviour, morphology, and physiology. In livestock, domestication was not a single event but a long, dynamic process shaped by environmental constraints, cultural practices, and technological innovations [206,207].

The initial stages of animal domestication could occur through different pathways, reflecting the nature of early interactions between humans and wild species. Larson and Fuller (2014) [206] proposed three primary pathways, which are not mutually exclusive and may overlap in time or context. These pathways describe the ecological and behavioural circumstances that facilitated the transition from wild to domestic populations:

- Commensal pathway: This route applies to species initially attracted to human settlements, often in search of food resources such as refuse or stored crops, or for protection from predators. Over time, individuals with reduced flight responses and increased tolerance to human presence were favoured, leading to a gradual process of habituation and eventual domestication. This pathway is typically unintentional and may precede the development of agriculture, as in the case of the dog derived from wolves or the domestic cat.
- Prey pathway: Domestication arises from the progressive management of species previously hunted for subsistence. Early humans may have practised selective harvesting or seasonal culling, later transitioning to active herding, selective breeding,

and protection from predators. This pathway generally coincides with the shift to sedentary or semi-sedentary lifestyles in the Neolithic. Herbivores such as cattle, sheep, and goats exemplify this pathway, reflecting the gradual transition from hunting to farming economies in the Fertile Crescent.

- Directed pathway: This pathway involves the deliberate domestication of a species to fulfil a specific role or produce a particular product. Unlike the other two routes, it is typically intentional from the outset and occurs after humans have already acquired animal management skills. Examples include the domestication of the horse for transport and warfare, the camel for desert travel, and various fibre-producing species.

Domestication imposes a first form of anthropic selection, as only a small subset of the wild population is captured and bred, resulting in a reduction in genetic diversity relative to the ancestral species [208,209]. On the other side, genetic and phenotypic variation are also shaped by the concurrent relaxation of natural selective pressure, allowing new variants and traits to appear, and positive selection resulting from intentional or unintentional human preferences [206,210]. Nevertheless, during these early stages, recurrent interbreeding with wild ancestors was still common [209].

Subsequent reductions in genetic diversity were further influenced by human-driven dispersal from the centres of origin [211,212]. Typically, only a subset of the original stock accompanied humans during migrations, leading to differentiation of emigrated populations along geographic gradients [11]. Populations distant from the domestication centre retained only a fraction of the original genetic variation and sometimes incorporated alleles from local wild relatives through admixture, in what can be called “introgressive capture”, facilitating adaptation to novel environments [206,213].

Over time, founder effects, isolation from wild populations, demographic bottlenecks, natural selection for local adaptation, and human-directed selection for desirable traits promoted differentiation into distinct landraces adapted to specific ecological niches or production systems [11].

Later phases involved the formalisation of breeding practices. From the 19th century onwards, breeders of specific landraces established breed standards, implementing isolation by design through closed populations to maintain desired phenotypes [11,212]. While this approach further reduced within-breed genetic diversity, it allowed breeds to expand beyond their original geographic ranges, since isolation was determined by human rules rather than natural barriers [208]. In the following century, intensified artificial selection for high-yield traits led to the creation of synthetic composite breeds and, eventually, industrial commercial strains [11].

Small ruminants such as sheep and goats clearly illustrate this trajectory. They originated via the prey pathway, underwent early selection for general productivity and adaptability, diversified into regionally adapted types under traditional management, and more recently experienced strong directional selection for traits such as milk yield, wool or fibre quality, and growth rate.

Studying the genomic signatures of domestication and selection in sheep and goats sheds light on their evolutionary history and the mechanisms driving adaptation to human-managed environments [214,215]. This approach reveals how ancient domestication shaped key traits and how modern breeding has further intensified selection for production, often narrowing genetic diversity. By linking past and present processes, these insights not only enhance evolutionary and archaeological understanding but also inform conservation strategies aimed at preserving the adaptive potential of contemporary livestock. [212,213,215].

Building on this framework, the work presented in this chapter (2.1) investigates the history and genomic footprints of domestication and selection in goats, tracing patterns from their initial domestication to recent breeding practices, with the aim of linking historical processes to present-day genetic diversity and adaptive potential.

2.1 WHERE DO GOATS COME FROM?

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2.1.1 Abstract

This chapter describes the history and biodiversity of the goat species, whose worldwide distribution demonstrates its great adaptability to a wide range of environmental conditions. We explain the genomic evidence clarifying the contribution of wild ancestors, the dynamics of the domestication process, and the post-domestication evolutionary history of this species, also reviewing the signals that both natural and human-mediated selection have left in goats' genomes. Animal welfare is related to the mental and physical state of a single subject, whereas genomic tools deal with populations. Hence, in the context of genetics, the concept of "fitness" depends on the interaction between genotype/phenotype and environments and is estimated as the individual's contribution of descendants to the next generation. During the domestication process, goats adapted to a new environment created by human activities and that differed substantially from the natural conditions in which they evolved. Similar to what happens in the natural context, also under anthropogenic conditions an increase in fitness comes at a cost, i.e., the morphological and physiological changes induced by the adaptation to human management and to the new environmental and nutritional conditions, which likely generated stress in the animals, particularly at the beginning of the process.

2.1.2 The origin of domestic goats

The bezoar, *Capra aegagrus*, is the progenitor of domestic goats, *Capra hircus*. Available evidence indicates that, after a demographic expansion in the Late Pleistocene (ca. 250,000 years ago), wild goat populations inhabiting south-western Asia underwent strong reductions in census size during the last two glacial periods, i.e. 130–160,000 and 12–71,000 years ago [216,217]. During these glacial periods, the nuclei of individuals which survived in refugial areas in south-western Asia remained separate for thousands of years and became genetically differentiated. Thus, the wild goat populations that were involved in the domestication process were already highly differentiated at the level of both nuclear and uniparental (i.e. mitochondrial DNA, Y-chromosome) markers [216,218,219].

Today the bezoar, which is classified as a near-threatened species by IUCN (International Union for Conservation of Nature), lives in the shrubland and mountainous areas of central-southern Asia, over a range spanning from the south-east of Turkey to Pakistan [220] (Figure 2.1.1). The bezoars are bigger than the domestic goats and present a coat with variable colouration, usually grey or brown, with darker areas on the muzzle, chest, back, and limbs, and a lighter abdomen. Both sexes have beard and horns, which in males are particularly long, curved like a scimitar, and with a sharp inside edge. Does and kids live in packs of about 50 individuals, separated from bucks, which stay alone or form packs of males only [221].

Figure 2.1.1 Present-day distribution area of the bezoar, *C. aegagrus*, the wild ancestor of domestic goats. Data from NatureServe and IUCN (2020).

According to zooarchaeological and molecular evidence, goat domestication began during the Neolithic around 11,000 years ago in south-western Asia, over an area located north of the Fertile Crescent and spanning across the Zagros Mountains of north-western Iran, the Caucasus, and south-eastern Anatolia [216,218,222–224]. Palaeoclimatic studies report that, starting from the peak of the last glacial period (ca. 14,000 years Before the Common Era, BCE) until the beginning of the Holocene (ca. 9750 BCE), this area was characterized by an arid and cold steppe climate, followed by a slow transition to warmer and wetter conditions [225]. The climate change occurring in this area at the end of the Pleistocene is widely accepted as one of the main drivers of the transition from the human hunting-gathering culture to agriculture and herding: the harsh environmental conditions, paired with an excessive hunting pressure, led to a reduction in the inhabitable territories as well as in the number of wild ungulates. This made it necessary for humans to develop new ways to obtain and preserve food [226]. Therefore, the goat domestication process can be considered as the outcome of a gradual passage from active hunting to captivity and management of wild animals [207,223,227–229]. Assemblages of bone remains deriving from hunting activities are, in fact, mainly attributable to large adult animals, especially males, which maximize the return in terms of meat. On the other hand, the archaeological assemblages found in Iraq and Iran and dating back to 9900 BCE mainly consisted of young males and adult females, indicating a transition to herding where females are slaughtered after finishing their productive career and excess males are culled at a young age. At that point, goats were still very similar to their wild counterparts, but some domestication-induced morphological changes, such as a reduction in body size and the alteration of horn shape and size, appeared about 500–1000 years later when the herds were moved to Iran lowlands, which were characterized by a hotter and dryer climate [227,228].

There are different reasons why goats and sheep were among the first domesticated species, whereas other ungulates sharing the same territories, such as deer and gazelles, did not undergo the same process. Among these reasons, the natural social structure of sheep and goats plays a role of primary importance: these animals follow a single dominant leader and, despite having a home range, namely a restricted area where they live and search for food, they are not territorial. This made them suitable to be led by herdsmen and to be gathered in compact and large groups. Certainly, goats' great resilience and varied diet also contributed to their success as domesticated animals; indeed, it has been suggested that Neolithic farmers might have taken advantage of goats' activity of clearing land after the forest was burnt or cut down [226].

As previously mentioned, domestic goats derive from multiple bezoar nuclei, as first suggested by the occurrence of six highly divergent mitochondrial haplogroups (i.e. groups of haplotypic sequence variants sharing a common ancestor), whose origin clearly predated the Neolithic age [216,222,230–232]. This hypothesis was later confirmed by the analysis of ancient goat genomes spanning from the Palaeolithic to the Middle Ages and covering a large area surrounding the Fertile Crescent [218]. According to this latter study, these genetically and geographically differentiated early domestic goat nuclei of the Neolithic contributed differentially to the gene pools of modern goat populations of the major continents, i.e., Africa, Asia, and Europe when, after domestication, goats followed the humans along the migration routes which spread agriculture and farming out of the Fertile Crescent [218]. The traces of this pre-domestication population structure and ancient migration events are still clearly detectable in the distribution of genomic diversity of present-day goat populations, where the occurrence of distinct continental gene pools is still evident [218,233,234].

Hybridization with wild relatives is another evolutionary force which seems to have impacted profoundly on the genetic makeup of domestic goat populations [234,235]. Nine wild goat species of the genus *Capra* are currently extant, with limited distribution areas scattered across Eurasia and North Africa [236] (Figure 2.1.2). Being interfertile with *C. hircus*, many of these wild species can cross with domestic goats [237–239] and seem to have contributed differentially to pre- and post-domestication evolution through the so-called adaptive introgression phenomenon. This occurs when, after a hybridization event, natural selection leads to the retainment of genetic variants derived from a different species or source population and conferring a selective advantage and increased fitness [240].

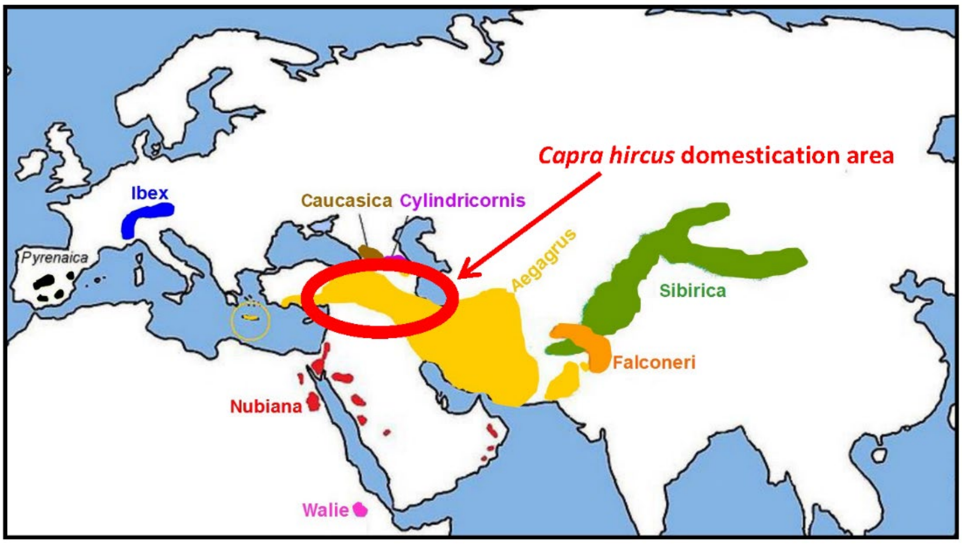

Figure 2.1.2 Present-day distribution areas of the nine extant wild goat species and indication of the *C. hircus* domestication site. *Capra pyrenaica* (black) is present in the Iberian Peninsula; *Capra ibex* (blue) in the Alpine region; *Capra caucasica* (brown) and *Capra cylindricornis* (purple) on Great Caucasus Mountains; *C. aegagrus* (yellow) across Southwestern Asia and the Caucasus region as far as southwestern Turkey; *Capra sibirica* (green) in Central Asia, as far north as southern Siberia; *Capra falconeri* (orange) in Afghanistan, Pakistan, Tajikistan, Uzbekistan, and India; *Capra nubiana* (red) in Egypt, Ethiopia, and Arabian Peninsula; and *Capra walie* (pink) in Ethiopia. Modified from Amills *et al.* (2017) [236].

Evidence exists of hybridization between bezoars and a species sister to the present-day Caucasian turs (*Capra caucasica* and *Capra cylindricornis*) having occurred in the Caucasus region before the onset of the domestication process [234,235]. Later crossing of domestic goats and ibexes in the Alps (*C. hircus* x *Capra ibex*) and the Pyrenees (*C. hircus* x *Capra pyrenaica)* occurred when the early domestic populations reached Southern Europe [237,241]. This interspecific gene flow seems to have been bidirectional and may account for the discrepancies found between phylogenies based on different loci [239,242].

The high rusticity and capability to adapt even to harsh environments made goats a key resource during both the agricultural transition of the Neolithic and the diffusion of agriculture and farming in the subsequent millennia [243]. Local populations of this species became adapted to a large variety of different environmental conditions, often contrasting in terms of extremes of temperature, humidity, water and feed availability, and diseases [244].

Early after domestication (i.e. 11,000 years ago), human-mediated selection began and started affecting morphological and production traits [218]. In time, this led to the formation of phenotypically distinct breeds with clearly different production purposes and adapted to a variety of different environments.

2.1.3 The effects of domestication and selection

2.1.3.1 Understanding the origin and diversity of goats: DNA-based tools

Recent advances in genomic techniques have provided useful tools for understanding the origin and genetic diversity of domestic goats. The international scientific community has focused widely on studying the genome of several goat breeds around the world, to understand the molecular mechanisms underlying their ability to adapt to the different climatic and environmental contexts in which local goat populations are bred since the post-Neolithic expansion.

The evolution of techniques now offers molecular markers, such as single nucleotide polymorphisms (SNPs), or complete genome sequencing, that permit evaluation of similarities and differences between and within breeds. This allows us to reconstruct phylogenetic relationships and to identify genomic regions that have been affected by natural or anthropogenic selection in relation to environmental and climatic conditions, or to selection goals established by humans [245].

Selection, indeed, induces specific changes in the patterns of variation among selected loci and in neutral loci linked to them. These genomic footprints are known as selection signatures and can be used to identify loci subjected to natural and artificial selection. A reduction in genetic variation in the genomic regions adjacent to the selected beneficial mutation is caused by the rapid fixation of that mutation after some generations [245]. The phenomenon by which a new beneficial mutation increases in frequency in a population and thereby reduces variability in the associated neutral sites is termed as “selective sweep” or “hitchhiking effect” (e.g. [246,247]). The identification of selection signatures has been a key interest of animal geneticists and breeders in the last decades as it can be used to identify genes and beneficial mutations that may provide a selective advantage to a particular livestock population [248].

2.1.3.2 Domestication syndrome: selection for behaviour?

Domesticated species share many morphological, behavioural, and physiological traits, collectively referred to as the “domestication syndrome”. In animals, selection for tameness is believed to have been fundamental to the establishment of the human–animal relationship [249]. It has led to changes in the levels and patterns of animal development with the maintenance of neotenic features and the appearance of common phenotypic traits such as white patches, drooping ears, and curled tails. The expression of genes with pleiotropic effects, i.e., affecting several traits at the same time, related to the development of the nervous, immune, and endocrine systems (particularly adrenocortical hormones secretion), or involved in the fear/flight reaction and pigmentation, is well known in many domesticated animals [250].

Selective pressure during the initial stages of domestication has targeted behaviour, specifically the reduction of fearful or aggressive responses to humans. The decrease of acute fear and long-term stress is a prerequisite for assuring high welfare levels, as well as successful reproduction in captivity. The diminution in the size and function of the adrenal glands, which leads to a decrease of the physiological reaction to fear and stress, is also considered a fundamental step that occurred during domestication. Moreover, this goes along with a slower maturation of the adrenal gland and the consequent extension of the socialization window. During this period, kids are exposed to human presence and interaction and, thus, get familiar with them [250].

Adrenal gland development is closely linked to neural crest cells (NCCs), a specific class of stem cells that appear during early embryogenesis at the level of the dorsal "crest" of the neural tube and then migrate ventrally through the body, both in the head and trunk. NCC-derived tissues include much of the head, sympathetic ganglia, adrenal medulla, pigment-related melanoblasts in both the head and trunk, and tooth precursors. Traits associated with the domestication syndrome, such as behavioural and morphological neotenic characters (e.g. reduction in skull and body size, drooping ears, disappearance of homochromia typical of the wild species, and appearance of white patches) are all variations associated with migration and developmental patterns of NCCs [250].

Signatures have been detected in the genomes of several domesticated species pointing at selective pressures acting on genes with pleiotropic effects involved in the pathways described previously. In the following section, these signatures of selection identified in goats will be described.

2.1.3.3 Early changes in the goat genome

In addition to these direct effects of domestication, further changes in the genome, and thus in the phenotype, of goats can be traced back to the earliest stages of the herding activity, and to the strong selective pressure exerted by the abrupt changes in lifestyle and feeding regimes. The comparison between genomes of Neolithic goats and modern bezoar allowed for the identification of some loci that showed high divergence and reduced variability. In particular, two pigmentation loci (*KIT* and *KITLG*), related to piebaldism, i.e., the absence of melanocytes producing pigment in certain areas of skin and hair, might have been favoured either indirectly within the context of the domestication syndrome or directly by humans as a way to better distinguish animals belonging to different herds. Other identified loci were related to a reduction of height at withers (*SIRT1*) and kidding interval (*EPGN*), as well as enhanced mammary gland development (*STAT1*). Moreover, domestic goats seem to have developed higher resistance to some xenobiotics (external substances), including the ones produced by fungi contaminating the cereals that were probably used to feed livestock, thus showing an adaptation to their new diet [218]. Further molecular evidence pointed at selection signals in genes related to behaviour and resistance to gastrointestinal pathogens and parasites. Particularly, a favourable mutation in the *MUC6* gene conferring higher tolerance towards nematodes might have resulted from adaptive introgression from a wild species related to the West Caucasian tur followed by natural selection [234].

While domestication has triggered positive selection for many traits in livestock species, it has also led to a relaxation of selection over traits of lesser importance in anthropogenic conditions, such as camouflage colouration, sexual selection, twinning, and the ability to escape or avoid predators [251].

2.1.3.4 Back to the wild

As reported before, the core of the bezoar's current distribution area is central-southern Asia; however, at present, some goats displaying bezoar-like phenotypic features are found also on different Mediterranean islands, like Crete, Youra, Montecristo, and Antimilos. The current opinion is that these populations are not native of these regions, but derive from domestic goats or early-domesticates introduced by humans in ancient times, from the Neolithic period onwards [252–254].

Due to the lack of *ad hoc* genomic investigations, it has not yet been clarified to what extent the bezoar-like populations of the different islands derive from already domesticated goats or from wild taxa released on the islands as “living larders”, or from the interbreeding of the starting wild stock with domesticated animals introduced in later times [252,253]. It is evident that these insular wild goats share phenotypical similarities with both the bezoars (e.g. coat pattern and horn shape) and domestic goats (e.g. smaller size, even though this could also be related to the so-called insular dwarfism).

Crete island hosted the largest population of free-living goats (*C. a. cretica*, often referred to as Agrimi). These animals used to live in all the mountainous regions of the Isle, but their range has since contracted [252,253]. Recent scientific literature about their current distribution is lacking, but they are reported to be found only on the three small islands of Dia, Thodorou, and Agii Pontes just off the shore of Crete (Colli, personal communication).

The island of Montecristo, located in the Tuscan Archipelago, is home to the homonymous goat. The origin of this population has not yet been clarified: some authors suggest it was introduced during the Neolithic, while others date this event between the sixth and thirteenth centuries and attribute it to the activities of the monk community that was present on the island at that time. During the last two centuries and until the establishment of a natural reserve in 1971, Montecristo island has been extensively used as a game reserve, which led to the necessity to restock the goat population with domesticated animals imported from the mainland, as indicated by a shared common ancestry highlighted by molecular evidence [254].

2.1.3.5 Adaptation and selection

The earliest type of human-mediated selection, i.e., for tameness, was likely an involuntary process which was then followed by an active but empirically performed selection carried out for millennia to improve production traits [226]. Later, phenotypic traits such as coat colours, body shape, and size started to be selected, when the standardization began in England during the Victorian era and subsequently spread mainly to Europe and the developed countries. To maintain a standardized appearance, between-breed crossbreeding was prevented which made it possible to push selection over performance characteristics further, such as meat, milk, or fibre production. Consequently, highly standardized breeds often became specialized for only one attitude, although many local breeds, especially in low-income countries, are still bred for multiple purposes [12].

In the case of goats, differently from most livestock species, the extensive and nomadic or semi-nomadic breeding system has maintained the animals exposed to the pressures from the natural environment over time. Goat breeds have evolved to survive, breed, and produce in a variety of different and often harsh environments, and their genomes are likely to have segregated unique allele variants underlying adaptation to such diverse production regimes and natural conditions. At the same time, over the centuries, goat breeds have been selected for different economically important traits, such as growth, milk and meat production, and coat/wool features and quality. Environmental- and production-driven selective pressures are strongly interconnected: animals well adapted to their living conditions will perform better with respect to production, and therefore will be more likely selected as breeders by the farmer. This is especially true in extensive management systems, in which animals must cope largely with natural environmental conditions [233,244]. Even though most local goat populations are still bred this way, it should also be noted that intensive breeding farms are growing in number in most developed countries [255]. The industrial and highly specialized breeds reared in these facilities, such as the Saanen, are little subjected to the external environment, and their selection is aimed at maximizing their production potential.

2.1.3.5.1 Environmental adaptation

Environmental factors are one of the key forces influencing agricultural and livestock productivity, exerting a strong selective pressure over domestic animal populations. The responses to this type of pressure include not only coping with extreme climates but also with poor forage and scarce water availability. Goat breeds and populations show great phenotypic variability, with peculiarities that can be related to the climatic condition of their breeding range. Recently, several genomic studies were carried out to detect selection signatures related to environmental adaptation by performing genome scans between groups of breeds with a limited geographic distribution and/or landscape genomic analyses in local goat breeds with a small census size. Several genes linked to circadian clock rhythms, coat colour, high-altitude adaptation, growth, immunity, and reproduction traits have been highlighted as under environmental selection [74,244,256,257].

The goat is recognized as the domestic ruminant species with the greatest ability to adapt to hot and arid environments [43]. From a morphological point of view, goat populations that had to cope with these conditions developed mechanisms to regulate body temperature and water loss, goals that have been achieved in different ways by different populations. Dwarf goats are widely spread in the African continent, especially in wet and hot tropical regions, and are bred in very low-input systems. Small size is generally associated with a greater surface-to-volume ratio, which contributes to increase heat dissipation. This evidence is supported by the general tendency of animal populations living in hotter climates to be smaller compared with those in colder areas [258]. Dwarf goats are also reported as particularly resistant to pathogens typically spread in humid environments, such as *Trypanosoma* and *Haemonchus*, and to gastrointestinal parasites [259]. Thus, it is likely that different features within the same environment differentially exerted selective pressures on both size and pathogen resistance.

On the other hand, goat populations that developed in hot but arid areas, such as Sudanese and Egyptian desert goats, are often large-sized but characterized by long and thin appendages which increase surface available for heat dissipation. Moreover, larger animals tend to have lower mass-specific metabolic rates, which also implies that they produce less metabolic heat. A large body size might also have been favoured in goats mainly intended for meat production. Coat colour and texture are also important for thermoregulation: a light-coloured coat reflects higher amounts of solar radiation and is therefore recognized as being advantageous in hot climates, especially when paired with highly pigmented skin that blocks UV radiation [82]. Also, high-producing animals, particularly dairy ruminants, are generally considered as more susceptible to heat stress, given the positive correlation between metabolic heat load and milk production [81]. Adaptation to harsh and arid environments has been extensively studied also from the genetic perspective (e.g. [244,260–263]). The identification of regions undergoing selection consistently pinpointed loci with effects on thermo-tolerance (melanogenesis), body size and development, energy and digestive metabolism, and autoimmune response. Candidate loci differentiated in response to heat stress and altitude are linked to specific metabolic pathways, such as the insulin and glucose signalling pathway, glycogen metabolism, lipid biosynthetic processes, oxidative stress, and regulation of vasodilation [244].

Tibetan animals are ideal case studies to investigate adaptation to high-altitude environments characterized by low oxygen levels, low temperatures, and intense solar radiation. Several species, including Tibetan cashmere goats, showed selection signatures in genes related to the response to hypoxia; in particular, the *EPAS1* gene was identified as under selection in several studies on different species living in the Tibetan plateau [257,264] , while a haplotype variant at the *PAPSS2* gene significantly associated to high-altitude adaptability in Tibetan goats likely derived from a recent hybridization between goats and a wild caprid species, the markhor, *C. falconeri* [265]. Cashmere goats present a double coat with strata of long and wavy hair. This coat likely evolved to help animals cope with cold climates, but was later actively selected by humans, becoming one of the main products of this breed. Consistently, some genes related to both high-altitude adaptation and fibre production, such as *FGF5*, were found to harbour signatures of selection in Cashmere goats [266].

2.1.3.5.2 Production

When we speak about livestock selection for production traits, we usually think of selection programmes based on knowledge of phenotypes and genotypes. However, we should not forget that herders have been selecting animals since the beginning of domestication, by choosing as breeders the ones they preferred, either for personal reasons like aesthetic preference, or for real advantages in terms of management and production, or both. For most local breeds, this still holds true today. For example, archaeologic findings indicate that twisted horns have been preferred over other shapes since the Bronze Age, but the reasons are unclear: perhaps herdsmen preferred them for aesthetic reasons, or because they caused fewer damages during fights between males, or maybe they were somewhat related to other phenotypical characteristics. However, there were, and there are, some populations with exaggeratedly long horns that surely were difficult to manage, but that were appreciated for their peculiarity as a sign of prestige [226]. The “modern” process of selection started to emerge only after the rediscovery of Mendel’s laws in the twentieth century, thus covering only a minimal, but surely fundamental, span of the 11,000-year-long history of goats’ selection and improvement.

The prolonged selection for performance traits left deep signatures in the goat genomes, which are clearly detectable in several populations around the world. In a large-scale investigation, a comprehensive screening of signatures of selection associated with external phenotypes (coat colour) and production traits (meat, milk, and wool) was carried out using different approaches in local and transboundary breeds sampled over five continents [244]. Several genes associated with milk production in dairy breeds like Alpine and Saanen have been identified as under selection. In particular, selective sweeps were found in both the genomic region containing the cluster of casein genes, which are known to play a major role in milk composition with respect to cheese production, and close to genes, such as *ABCG2*, previously associated with milk yield and composition in cattle and sheep [267,268]. Genes involved in fibre production in the Angora breed included *CUX1* and *PLOD3*, associated with hair development and texture. Interestingly, signatures on these genes were found only in Angora goats, which have a single coat made of curly hairs like sheep, and not in the Cashmere breed, which instead presents a completely different hair conformation, as previously explained. When breeds were contrasted based on meat production, some of the regions under selection contained genes associated with muscle formation, for example *AMPD1* and *NRAS* on chromosome 3. Surprisingly, the Boer goat, which is the main breed for meat production worldwide, showed no evidence of strong selection for this trait. A possible explanation is that variation in meat production traits in this breed could be highly polygenic (i.e. many alleles with small effects determine the trait) [244].

2.1.4 Present distribution

According to FAO data, the goat is one of the most diffused livestock species worldwide (http://www.fao.org/3/a-i4787e/index.html. Accessed 15/08/2022). The small size and lower management needs, if compared to other ruminants, allowed goats to follow humans during their migrations and movements. Thanks to their capability of withstanding harsh conditions in different types of ecosystems, goat populations spread and adapted to a large variety of different environments, in a process that is still underway and that will likely be pushed further by the ongoing climate changes: goats are, indeed, the ruminant species that can better adapt to the foreseen hotter and drier environmental conditions.

Collecting data about goat populations' consistency and diffusion might prove difficult, especially in countries in which they are bred in a context of subsistence, and sometimes nomadic, farming. The FAO database data indicate an increasing number of goats worldwide over the past 20 years (Figure 2.1.3, FAOSTAT data, https:// www.fao.org/faostat. Accessed 15/08/2022). In fact, even if goats are still less numerous than the two main farmed ruminant species, i.e., cattle and sheep, it is worth noticing that they showed the greatest growth in terms of number of heads, from ca. 760 million in 2000 to ca. 1.13 billion in 2020, with a net increase of about 370 million heads compared to the 200 million of cattle and sheep.

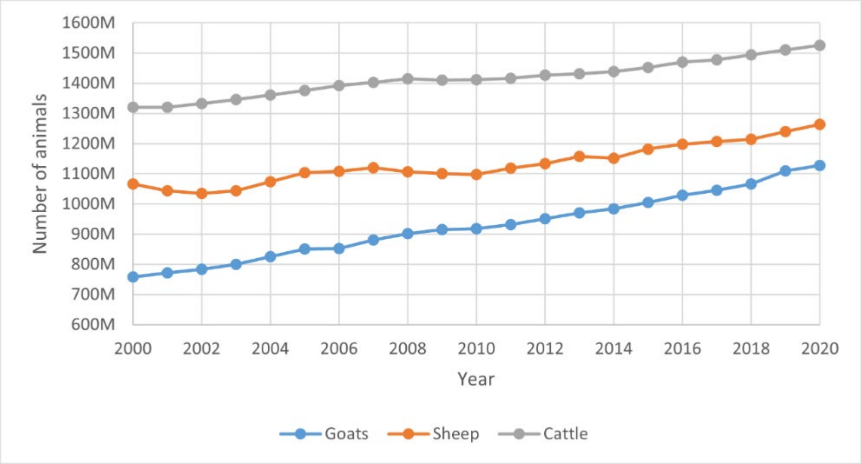

Figure 2.1.3 Trend in the evolution of the number of the worldwide goat, sheep, and cattle populations during the last twenty years (FAOSTAT-world; the Authors thank Hassan R. Ghulami for contributing to the analysis of these data).

When we consider that in the same period the human population has increased from 6.14 billion to 7.79 billion (data taken from: https://www.worldometers.info/world-population), we can estimate that, while in 2000 there was 1 goat for every 8 people, in 2020 the ratio became 1 for less than 7 people. However, if we consider that the rural population, which includes the majority of goat breeders, decreased by 9.5%, at present there is 1 goat for every 3 people in rural areas.

Considering worldwide data (Figure 2.1.4), goats are mainly distributed in poor or developing countries, where they constitute one of the main resources to fight poverty. Asia accounts for 51.4% of the total goat population, with a particularly high contribution from India and China. Recently, the goat population underwent a sharp increase in Africa, and at present the continent comprises 43.3% of the global population, with the highest number in Nigeria and Ethiopia. The remaining 5.3% of goats live in the Americas (especially in central-southern America), Europe (mainly in the Mediterranean countries), and Oceania.

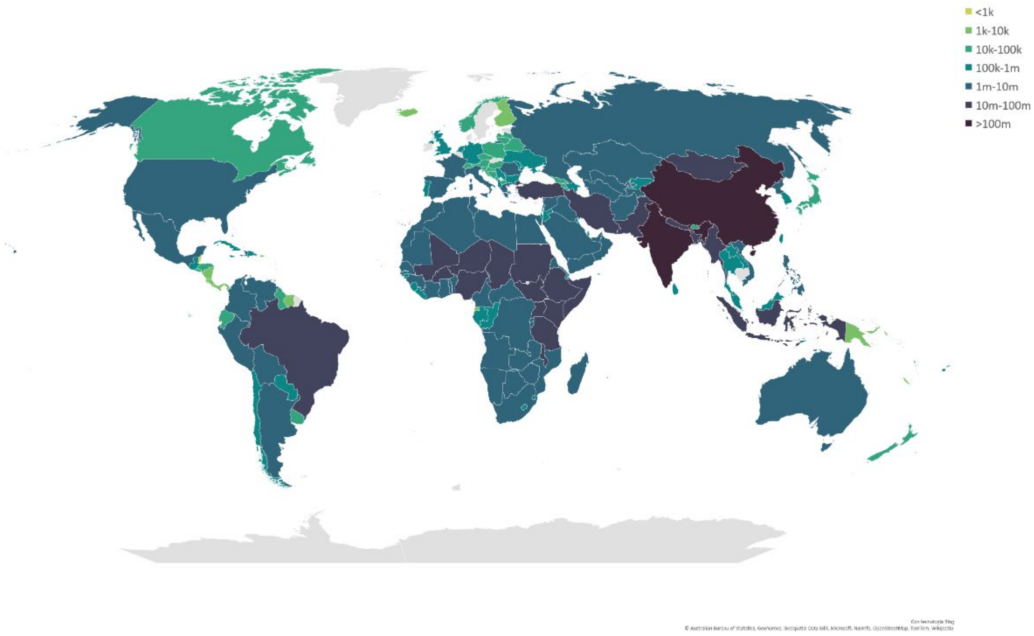

Figure 2.1.4 Goat worldwide distribution and population consistency by country (https://www.fao.org/faostat for the year 2020. Accessed 15/08/2022). The darkest colours correspond to the highest numbers, whereas light grey indicates countries with missing data.

2.1.4.1 Goat biodiversity: breeds and populations

According to FAO statistics [12], local goat breeds account for 21% of a total of 576 local ruminant breeds raised in the world.

From the list of breeds reported in the Domestic Animal Diversity Information System (DAD-IS) database, we reconstructed the distribution of the overall number of breeds reared in different countries of the world (Figure 2.1.5), finding the highest numbers in China, followed by India and Italy. However, FAO database information can be incomplete, as it is managed independently in each country and is provided and updated on a voluntary basis: for example, only some countries include wild species, thus not providing a complete picture of their distribution.

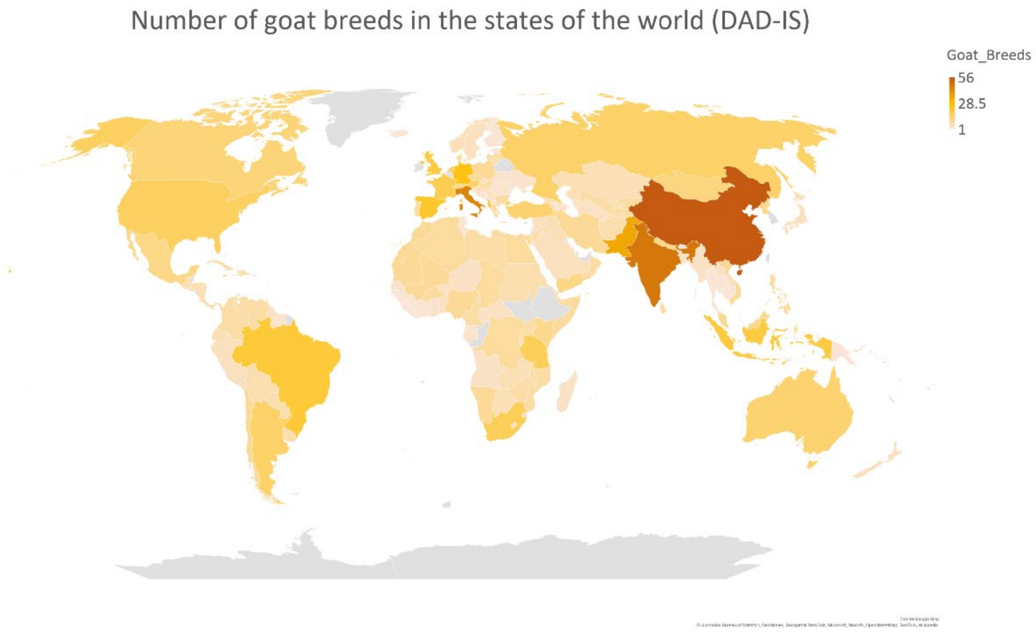

Figure 2.1.5 Number of goat breeds recorded per country (data from DAD-IS).

Data about wild species can be found on the IUCN Red List website (www.iucnredlist.org). The bezoar (*C. aegagrus*) at present inhabits a discontinuous territory comprised between Turkey and Pakistan (Figure 2.1.1 and Figure 2.1.2) and, with an estimated census size of about 70,000 mature goats, it is considered "near threatened" [220]. The markhor (*C. falconeri*) is classified as "near threatened" as well, numbering less than 6000 adults, all living in the mountainous regions of central Asia [269] (Figure 2.1.2). The Alpine ibex (*C. ibex*) is listed as "least concern" species after risking extinction in the early nineteenth century. At present, it counts approximately 53,000 mature subjects and is endemic to Europe (Figure 2.1.2), but it has also been introduced in Bulgaria and Argentina [270]. Other wild goat species include the Siberian ibex (*C. sibirica*), the Ethiopian Walia ibex (*C. walie*), the Eastern tur (*C. cylindricornis*) living in the Great Caucasus, and the Iberian wild goat (*C. pyrenaica*) (Figure 2.1.2).

Among the local goat breeds (Figure 2.1.5), 50 are bred in different countries and are therefore referred to as "transboundary". The largest number of transboundary goat breeds is found in Africa, followed by Europe and Asia (Figure 2.1.6). In addition, a small number of breeds can be considered "cosmopolitan", being bred on all continents. Among these there are two highly specialized dairy breeds, Saanen and Alpine, both native to Switzerland, as well as another important dairy breed, the Toggenburg, which is now bred in most of the world. The Boer goat, instead, is the most widespread and productive meat breed. The intensive selection for this production goal was carried out during the twentieth century by the Dutch breeders living in South Africa, but formerly the breed was raised for milk production and it is recorded to have been crossed with Indian and European breeds during the eighteenth or nineteenth century to improve yields [271,272]. Anglo-Nubian and Nubian breeds are also widespread in more than 50 countries worldwide. The Anglo-Nubian, as its name implies, originated from a cross between Nubian and British breeds dating back to the mid-nineteenth century. Today it is recognized as a dual-purpose breed (meat-milk) particularly adapted to Australia's hot climates. In Africa, dwarf goats present a wide distribution and are used for multiple purposes, such as milk, meat, and leather. Regarding goats specialized for fibre production, Angora is the most widespread breed, followed by Cashmere and Mohair goats. Fibre production, and in particular Cashmere, is still a characteristic of nomadic cultures in many countries [273].

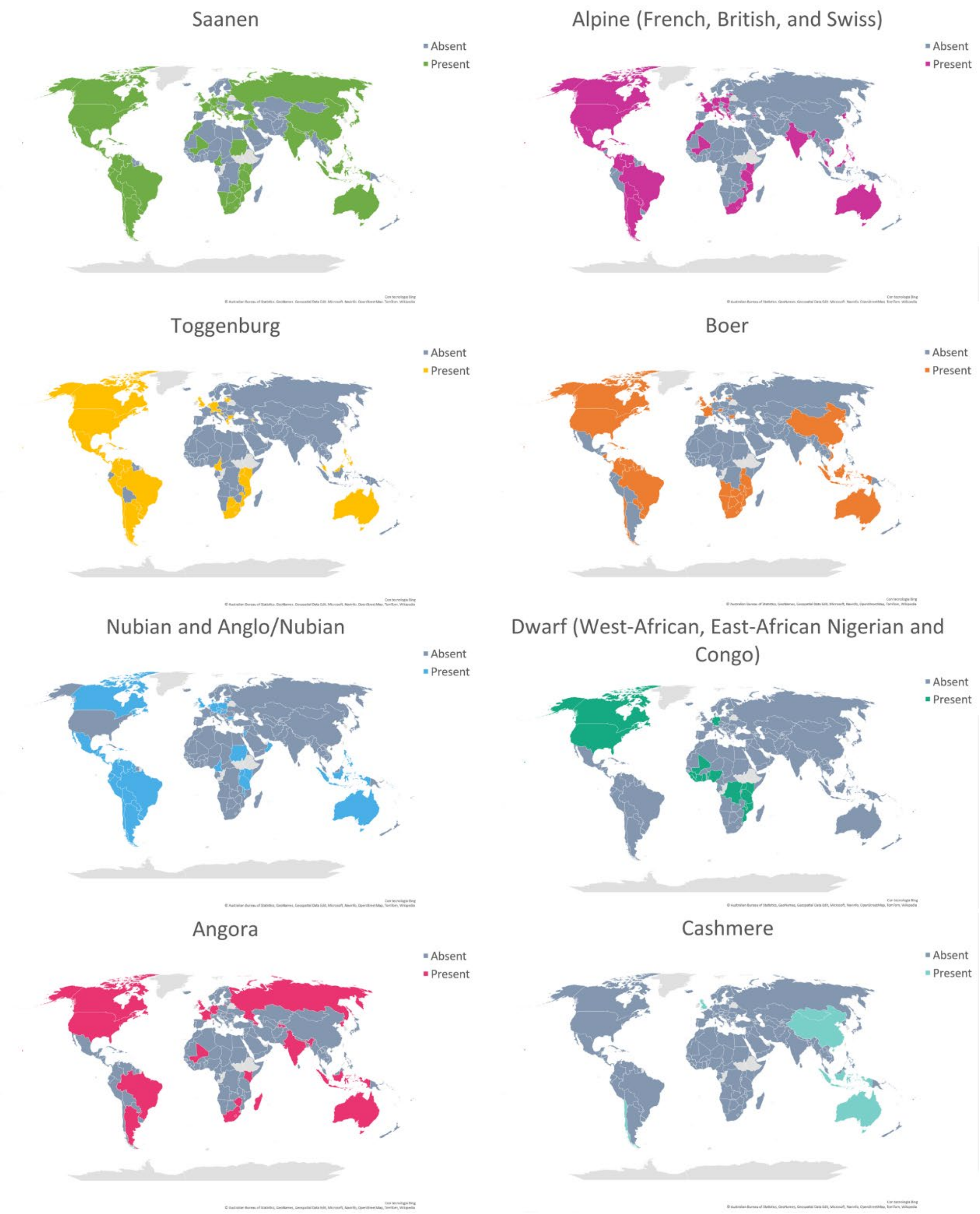

Figure 2.1.6 Maps showing the worldwide distribution and occurrence of cosmopolitan and transboundary breeds. Coloured areas correspond to the countries where the breed is reported according to the DAD-IS database of FAO.

The FAO database also includes information on the risk status of livestock breeds which adopted the following ranking categories similar to IUCN's ones: Unknown, Not at risk, Vulnerable, Endangered-Maintained, Endangered, Critical-maintained, Critical, Cryo-conserved only, and Extinct. Based on FAO report [12], the risk status is unknown for about

34% of the recorded goat breeds, 50% are not endangered, while 9% are endangered, and 5% are in critical status. For 1% of the breeds in critical status and 2% of those at risk, conservation programmes have been put in place. The growing awareness of the importance of biodiversity and its preservation recently led to the development of projects for the conservation and enhancement of native animal resources in many countries and to the establishment of biobanks to store biological material to prevent extinction. Due to climate changes and growing socio-political uncertainty in many areas of the world, the survival of many local breeds is strongly threatened by the increasing spread of extreme weather phenomena (floods, landslides), economic crises, and war. This places the genetic variability of the species at risk, and might have negative effects on animal welfare, due to the loss of specific traits that have evolved over time, improving goats' adaptation and ability to cope with different environments.

2.1.5 Conclusions

Overall, the history and spread of goats around the world, together with the currently available genomic information, show how natural and artificial selection often act in synergy and how it is sometimes difficult to disentangle the effects of the two selective pressures in livestock populations.

As with other domestic species, the domestication process in goats has brought about major genomic, physiological, and behavioural changes that, together with anthropogenic selection, have determined the success of this species in the world. Pastoralism, a common practice in small ruminant management, has probably contributed to the complexity of forces that have shaped and are shaping the goat genome by exposing the animals to a variety of conditions in terms of environmental features, disease occurrence, feeding regimes, and water availability.

The biological architecture that regulates the expression of genes involved in complex traits has started to be dissected more thoroughly in recent years due to the availability of increasingly powerful molecular and bioinformatic tools, which provided a genome-wide picture of the genes and genomic regions which were subjected to selection along the history of goats and their relationship with humans. Although the intricate network of interactions between genes directly or indirectly involved in the mechanisms of adaptation is far from being fully understood, several studies have helped pinpoint genomic regions that are important putative targets of anthropogenic and/or natural selection for traits related to domestication, animal behaviour, and welfare (e.g. animal hardiness and resilience).

It is also worth remembering that selection goals in livestock are in constant evolution and are strictly related to socio-cultural and economic needs, which change over time. For example, recently increasing attention is being paid to livestock environmental adaptation, given the threats that animals and breeders will likely have to face in the near future due to climate change. Moreover, in countries in which food availability and supply are ensured, animal welfare, health, and fitness are becoming new objectives of selection. Indeed, on the one hand, these elements are advantageous for the breeders, contributing to the reduction of breeding costs and the production of healthier and more natural products, and on the other hand they respond to the increasing consumers' awareness about livestock well-being. For these reasons, the development of breeding goals is now oriented towards increasing the welfare, resilience, and longevity of animals, in the face of a decreased emphasis on production-related traits.

3 CHARACTERISING DISTRIBUTION AND ENVIRONMENTAL CONDITIONS OF ITALIAN SMALL RUMINANT POPULATIONS

The effective and sustainable management and conservation of livestock genetic resources require the integration of multiple disciplines and diverse sources of information, ranging from breed characteristics such as productivity, morphology, and adaptability, demographic data, and genetic variability, to their geographical distribution, production environment, and socio-economic context [211].

Comprehensive knowledge of a breed's demographic parameters—such as population size, temporal trends, and population structure—is essential for assessing its conservation status and extinction risk. In particular, the estimation of effective population size (Ne) provides a key indicator of genetic viability, as low Ne values are associated with increased inbreeding, loss of genetic diversity, and a higher probability of extinction [36,211]. Equally important is the documentation of a breed's geographic distribution and the spatial extent of its breeding range. Breeds with restricted or highly fragmented distributions are indeed more vulnerable to stochastic events, including disease outbreaks, natural disasters, and sudden environmental changes, which can rapidly decimate small and isolated populations [142].

Integrating environmental information into these distribution datasets enhances our understanding of the reciprocal relationship between livestock populations—especially locally adapted breeds—and the ecosystems they inhabit. This relationship is inherently bidirectional.

On the one hand, livestock adapts over generations to the specific climatic, ecological, and management conditions of their regions. On the other hand, grazing and other management practices exert measurable influences on ecosystems, shaping vegetation dynamics, soil properties, and biodiversity, and affecting interactions with wildlife [274]. These processes can yield both positive and negative outcomes, from the provision of ecosystem services such as landscape maintenance, wildfire prevention, and nutrient cycling, to potential ecosystem degradation if grazing pressure is excessive [31,33,61].

Georeferencing breed populations and sampling locations is therefore a cornerstone for studying environmental adaptation. This approach enables the correlation of genetic and phenotypic diversity with environmental parameters, revealing the selective pressures—both natural and anthropogenic—that shape the evolutionary trajectories of populations [274,275]. While climatic data such as temperature, precipitation, and derived bioclimatic indices are frequently employed in this context, the environmental factors influencing livestock adaptation extend well beyond climate. Landscape features, including topography, vegetation cover, land use, and soil characteristics, also play a critical role in determining forage availability, shelter opportunities, exposure to disease vectors, and other ecological pressures that influence survival, reproduction, and productivity [276–278].

The inclusion of such environmental and landscape variables is particularly important in the context of climate change. Past, present, and projected future shifts in temperature regimes, precipitation patterns, and the frequency and intensity of extreme weather events are expected to alter the suitability of traditional breeding areas, potentially forcing changes in herd mobility, driving population displacement, or leading to local extinctions [26]. By integrating environmental mapping into breed distribution analyses, it becomes possible to identify risk factors, anticipate shifts in production environments, and develop appropriate mitigation and conservation strategies, such as targeted breeding for resilience traits, adaptive grazing management, or the establishment of genetic reserves.

Small ruminants are frequently cited for their ability to cope with challenging environments. Compared to larger livestock species, they generally exhibit greater tolerance to heat and water scarcity, owing to a combination of physiological, morphological, dietary, and behavioral adaptations. Nevertheless, this resilience is not uniform across all populations, and even locally adapted breeds can experience negative effects under harsh climatic conditions or rapid environmental changes, particularly when managed in extensive systems [82].

Mapping these environmental constraints in relation to breed distributions provides essential insight into which populations are most vulnerable, and which possess inherent resilience. This knowledge underpins the design of conservation priorities and adaptive management strategies. Indeed, the FAO identifies as strategic priorities the assessment of breed geographical distribution and production environments, the identification of potential climate change–related threats to specific animal genetic resources, and the characterisation of adaptive traits relevant to climate change adaptation [26]. The Organisation also emphasises that the absence of such information limits the capacity to evaluate climatic risks—particularly those posed by extreme weather events—and to plan effective responses [279]. Integrating geographic, environmental, and genetic data, therefore, represents not only a scientific necessity but also a practical tool for safeguarding small ruminant biodiversity in an era of rapid environmental change.

Italy represents an especially suitable setting for such investigations: its varied topography, marked climatic gradients, and coexistence of contrasting farming systems expose local breeds to a wide range of ecological conditions. This heterogeneity not only creates differences in vulnerability but also reveals the spectrum of adaptive responses that can develop within a single national context, making Italy a valuable case study for exploring livestock resilience.

Within this framework, the studies presented in this chapter provide a comprehensive assessment of the geographical distribution and environmental context of all registered local Italian breeds of sheep and goats. The first study (Chapter 3.1) analyses the spatial distribution of breeds alongside long-term climatic data, focusing on variables known to negatively affect animal welfare and productivity, such as temperature, temperature–humidity index (THI), precipitation, and the frequency of heat waves. Climatic trends over the past 70 years are examined to identify temporal changes, current conditions, and variability in exposure among breeds, thereby providing insights into differential vulnerability within the national genetic resource pool.

The second study (Chapter 3.2) expands this perspective by addressing environmental risks under both present and projected climate scenarios and incorporating potential threats from extreme events and natural disasters. In addition, it offers a detailed characterisation of the landscapes and socio-environmental contexts in which these breeds are raised, drawing on multiple dimensions such as land use, vegetation cover, topography, and human activity. This integrated approach captures the complex interplay between local breeds and their surrounding environments, reflecting not only how breeds adapt to ecological conditions, but also how their presence and management contribute to shaping these landscapes.

Together, these works provide an integrated view of breed distribution, environmental exposure, and ecosystem context, delivering essential information to guide the development of targeted conservation programmes and adaptive management strategies. By explicitly linking genetic resources to their climatic and landscape environments, they support actions that promote the long-term resilience of local sheep and goat breeds, while fostering a balanced coexistence between livestock production systems and natural ecosystems in the face of ongoing and future environmental change.

3.1 70 YEARS OF HEAT WAVES AND SUMMER CLIMATE CHANGE AFFECTING ITALIAN SMALL RUMINANT POPULATIONS

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3.1.1 Abstract

Climate change and heat stress pose significant challenges to livestock. Local breeds, particularly small ruminants, are gaining importance due to their adaptability to harsh climates. However, the extensive system they are commonly reared in leaves them exposed to the effects of climate change. This study aims to describe the distribution and climate-related challenges faced by registered Italian sheep and goat breeds over the past seven decades. Geolocalized data from all registered small ruminant farms were combined with climatic information retrieved from the "ERA-5-Land hourly data from 1950 to present" dataset. These data were used to calculate average daily temperature, temperature humidity index (THI), and total precipitation during summer. Additionally, THI-based heat waves (HWs) were examined, including the yearly number of HW days and mean THI during HW days. These data were analysed through linear regression models including region or breed, year, and their interaction as fixed factors. The climate data indicate a concerning trend of rising summer temperatures, THI, and HW frequency and intensity, particularly over the past three decades. Central-northern Italy, including the Po Valley and the Alpine Arch, is the most affected region, impacting breeds like Rosset and Brogne sheep, and Lariana and Frisa Valtellinese goats. This is of particular concern because these populations have not been selected for hot climates, and their already small population size exacerbates the problem. Conversely, southern Italy, characterized by hotter and drier temperatures, remained relatively stable. Breeds from this region, such as Girgentana and Nicastrese goats and Nera di Arbus sheep, might represent excellent case studies for climatic adaptation and potential resources for selection for resilience in the face of ongoing climate changes. The findings presented here are essential for the development of monitoring and intervention strategies for breeds facing future vulnerabilities, as well as for designing experiments to explore environmental adaptability in small ruminants.

3.1.2 Introduction

Climate change is one of the most significant challenges facing the world today. There is evidence of globally rising mean temperature [280,281], as well as an increase in frequency, duration, and intensity of extreme weather events, especially in heat waves (HWs) [25,280–283]. Moreover, the Mediterranean areas have shown to be particularly interested by these changes [284]. Numerous studies have reported HWs as the weather events associated with the highest number of human deaths [24,280], but there is also well-documented evidence of a significant increase in livestock losses during these phenomena [86,285,286].

The detrimental effect of heat stress on ruminants encompass various aspects of animal health, welfare, and production, and has been extensively documented [20,24]. Even though sheep and, even more, goats are considered more thermotolerant than cows [19,43–45], they experience negative effects of high temperatures as well. These include a variety of metabolic and immunologic changes leading to reduced feed intake, decreased milk and meat production and quality, metabolic and immunologic impairment, pest and disease outbreaks, and increased animal mortality [19,26,89,283,287–289]. Several studies demonstrate that highly productive animals are more susceptible to heat stress and that, on the other hand, local and more rustic populations are more resilient to harsh climates, thanks to several morphological and genetic adaptations to the environment in which they developed [20,24,26,287,290,291]. However, these local breeds are often reared in extensive systems, leaving them vulnerable to changes in their environment, and farmers may not have the resources to implement sufficient technology to protect them from excessive heat or other adversities [24–26].

Small ruminant farming holds a significant place within Italy's agricultural landscape, boasting a rich historical legacy dating back centuries [292,293]. Despite enduring fluctuations in headcounts, there has been a clear trend of reduction in overall small ruminant farm numbers over the past century, with the exception observed between 1970 and 1990 [53,59]. Presently, while the population appears relatively stable, with approximately six million sheep and one million goats, a conspicuous decrease in the number of farms is notable, particularly in small-scale, family-operated farms [55,294]. The traditional extensive or semi-extensive farms, despite still being the predominant type for sheep and goats, face challenges such as the abandonment of less profitable activities, shrinking grazing lands, and the wool market crisis [48,68,69,295–297]. However, this decline has been partially compensated by the emergence of large, modernized farms, notably in the dairy sheep sector. A substantial portion of the small ruminant population, exceeding two-thirds, is indeed concentrated within a mere five percent of such farms, mainly located in Sardinia [55,295].

Currently, the small ruminant sector constitutes approximately 1.1% of Italy's total agricultural output. The vast majority of small ruminants are raised for milk or mixed production purposes, positioning Italy as the third-largest producer of sheep and goat milk and cheeses in Europe [295,297]. Notably, a significant proportion of milk is utilized in the production of designated origin products, such as the Pecorino cheese, which enjoys both domestic and international demand, notably in the United States [58,297,298]. Additionally, small ruminant meat, predominantly sourced from lamb, holds seasonal significance, with consumption peaking during Easter and Christmas periods [296].

Despite constituting a modest fraction of the Italian agricultural economy, the social, cultural, and environmental role of small ruminant farming is pivotal, particularly in maintaining and safeguarding unproductive territories. While on a national scale, sheep and goats represent approximately 10% of the total ruminant population in Italy, they are the predominant or exclusive type of livestock in certain marginal, unproductive, and inaccessible areas. In these territories, they serve as vital components of land management strategies, facilitating ecosystem preservation and biodiversity conservation [59,292,295,299,300]. Traditional practices such as transhumance—seasonal migration of flocks between mountainous and lowland pastures—are recognized as intangible cultural heritage by UNESCO [64]. Furthermore, Italy stands as a reservoir of small ruminant biodiversity: with 64 sheep breeds and 41 goat breeds officially recognized (www.assonapa.it), the country ranks third globally in terms of goat breed diversity and sixth for sheep breeds (www.fao.org/dad-is). These breeds originated due to geographic barriers, which hindered communication and the exchange of animals between farms, typically situated in the valleys, whereas in more recent times they have been recognized and selectively bred [303]. This rich genetic heritage underscores the importance of small ruminant farming in preserving and promoting genetic diversity within livestock populations, thereby contributing to the resilience and adaptability of agricultural systems in the face of evolving environmental challenges. Therefore, the purpose of the present study is to describe the distribution of Italian small ruminant breeds and assess their exposure to the variation in summer environmental conditions occurred over a 70-year period, with the final aim of assisting in the management of the populations most threatened by the climate change.

3.1.3 Materials and methods

3.1.3.1 Environmental data

Italian 2m-dewpoint temperature (d2m), 2m-temperature (t2m), and total precipitation (tp) from 1st January 1950 to 30th September 2022 were obtained from “ERA-5-Land hourly data from 1950 to present” dataset [304]. Using these parameters, we calculated hourly relative humidity ($RH = e^{\frac{17.625*d2m}{243.04+d2m}} / e^{\frac{17.625*t2m}{243.04+t2m}}$, according to Lawrence (2005) [305]), and temperature-humidity index ($THI = 0.8 * t2m + RH * (t2m + 14.4) + 46.4$).

The average daily temperature, THI, and total precipitation recorded during summer (hereafter called summer-T, summer-THI, and summer-TP, respectively), i.e., from 1st May to 30th September, were calculated.

We relied on the Climatological EURO-CORDEX definition of HW (“a period of at least three consecutive days on which the daily maximal temperature exceeds the 99th percentile of the daily maximal temperatures of the May to September season of the control period”)

[306]. However, given the wide use of the THI as a measure of heat stress in livestock, we substituted the daily maximal temperature with the daily maximal THI. Moreover, we chose the 30-year period from 1950 to 1979 as the control period. Therefore, we defined a heat wave as a period of at least three days, between May and September, with a maximum daily THI exceeding the 99th percentile of the reference period (1950–1979) at the same geographic coordinates. For each location, we calculated the mean THI occurring during HW days (HW-THI) and the total number of days falling in a HW (nDays) in all years from 1950 to 2022.

R packages and ESRI ArcGis Pro 3.0.2 were used for computation and graphical representation of data.

The selection of these variables is driven by their dual significance: they not only exert a substantial impact on livestock production but also stand out as key climatic variables undergoing rapid change. Heat stress, exacerbated by rising temperatures and THI, stands out as a primary concern, directly impacting animals by reducing feed intake, impairing reproductive and productive performance, and compromising animals' immune system. Furthermore, extreme weather events, such as heat waves, can drastically increase animal morbidity and mortality. Indirect consequences of both temperature and precipitation changes are also to be considered, including shifts in crop and forage quantity and quality, diminished water availability, and alterations in the distribution of pests, parasites, and thus vector-borne diseases [19,20,26,288,289,307].

3.1.3.2 Small ruminant geolocalization data

The geolocalization of all the registered currently (31st December 2022) operational farms were provided the Italian Sheep and Goat Breeders Association (Asso.Na.Pa.) for 41 goat and 47 sheep populations (Table 3.1.1 and Table 3.1.2). These data were paired with the environmental information described above.

Table 3.1.1 Number of farms and mean ± standard deviation of Summer-T, Summer-THI, and nDays during the first and last 30 years of the analysed period for each goat breed.

		Summer-T		Summer-THI		nDays	
Goat breed	N. farms	1950–1985	1986–2022	1950–1985	1986–2022	1950–1985	1986–2022
ALPINA	7	14.93 ± 4.32	16.28 ± 4.31	58.06 ± 6.48	59.99 ± 6.28	1.16 ± 2.54	3.76 ± 5.35
ARGENTATA DELL'ETNA	103	20.17 ± 1.19	20.90 ± 1.24	66.01 ± 1.72	66.98 ± 1.80	0.43 ± 1.23	2.24 ± 4.44
BIANCA MONTICELLANA	14	18.91 ± 1.26	19.94 ± 1.36	64.28 ± 1.88	65.71 ± 2.00	0.96 ± 2.29	4.07 ± 5.85
BIONDA ADAMELLO	79	13.48 ± 3.00	14.68 ± 3.02	56.04 ± 4.70	57.89 ± 4.64	0.96 ± 2.46	3.35 ± 4.70
CAMOSCIATA DELLE ALPI	230	16.02 ± 3.91	17.24 ± 3.90	59.82 ± 6.01	61.57 ± 5.87	0.95 ± 2.24	3.88 ± 5.21
CAMPOBASSO GRIGIA MOLISANA	3	18.31 ± 0.92	19.27 ± 0.99	63.06 ± 1.21	64.38 ± 1.29	0.63 ± 1.59	2.75 ± 3.41
CAPESTRINA	19	18.93 ± 1.27	19.99 ± 1.36	64.23 ± 1.90	65.68 ± 2.00	0.98 ± 2.25	3.95 ± 5.73
CILENTANA FULVA	13	19.21 ± 1.45	20.14 ± 1.48	64.62 ± 2.28	65.94 ± 2.35	0.68 ± 1.73	3.73 ± 5.03
CILENTANA GRIGIA	11	19.24 ± 1.17	20.12 ± 1.14	64.58 ± 1.81	65.85 ± 1.83	0.70 ± 1.72	3.29 ± 4.46
CILENTANA NERA	32	19.27 ± 1.11	20.14 ± 1.10	64.64 ± 1.70	65.90 ± 1.73	0.66 ± 1.64	3.38 ± 4.63
CIOCIARA GRIGIA	12	18.56 ± 1.31	19.60 ± 1.40	63.69 ± 2.02	65.12 ± 2.14	1.00 ± 2.27	3.90 ± 5.73
DELL'ASPROMONTE	102	20.98 ± 1.37	21.70 ± 1.42	67.08 ± 1.96	68.08 ± 2.05	0.46 ± 1.31	2.87 ± 5.10
DI BENEVENTO VALFORTORINA	6	18.63 ± 0.89	19.62 ± 0.86	63.42 ± 1.10	64.74 ± 1.08	0.83 ± 1.97	2.75 ± 3.97
DI MONTECRISTO	1	18.24 ± 0.85	19.35 ± 0.90	62.85 ± 1.09	64.31 ± 1.14	0.80 ± 1.81	2.79 ± 5.06
DI POTENZA	45	19.16 ± 1.67	19.94 ± 1.66	63.71 ± 2.16	64.76 ± 2.16	0.51 ± 1.18	3.18 ± 5.01
FRISA VALTELLINESE	48	10.06 ± 3.77	11.38 ± 3.81	50.56 ± 5.94	52.64 ± 5.87	0.87 ± 2.20	3.29 ± 4.59
FULVA DEI MONTI PICENTINI	1	19.07 ± 0.83	19.67 ± 0.76	63.96 ± 0.95	64.82 ± 0.92	0.49 ± 1.25	1.92 ± 4.08
GARFAGNANA	16	17.65 ± 1.82	18.70 ± 1.85	62.50 ± 2.73	64.02 ± 2.73	0.77 ± 2.02	3.19 ± 5.12
GARGANICA	32	19.93 ± 1.55	20.87 ± 1.61	65.15 ± 2.12	66.32 ± 2.17	0.47 ± 1.27	3.21 ± 4.60
GIRGENTANA	23	21.19 ± 1.18	22.00 ± 1.15	67.14 ± 1.59	68.16 ± 1.56	0.54 ± 1.46	2.49 ± 4.27
GRIGIA VALLE LANZO FIURINA	34	14.09 ± 3.43	15.35 ± 3.55	56.98 ± 5.32	58.86 ± 5.37	1.17 ± 2.50	3.56 ± 5.27
JONICA	3	19.85 ± 1.88	20.71 ± 1.83	64.86 ± 2.66	65.95 ± 2.56	0.57 ± 1.29	2.94 ± 4.44
LARIANA O DI LIVO	34	13.64 ± 0.73	14.96 ± 0.87	56.35 ± 1.15	58.37 ± 1.28	1.16 ± 2.48	4.11 ± 5.25

MALTESE	33	18.30 ± 2.71	19.47 ± 2.68	63.23 ± 3.93	64.84 ± 3.76	0.88 ± 2.23	3.55 ± 5.16
MESSINESE	107	20.18 ± 1.17	20.92 ± 1.20	66.04 ± 1.68	67.02 ± 1.76	0.44 ± 1.26	2.26 ± 4.47
NAPOLETANA	3	20.44 ± 0.85	21.47 ± 0.89	66.68 ± 1.12	68.13 ± 1.16	1.19 ± 1.91	5.57 ± 6.53
NICASTRESE	72	20.16 ± 1.52	20.79 ± 1.58	65.94 ± 2.21	66.88 ± 2.30	0.47 ± 1.24	2.82 ± 4.96
OROBICA O DI VAL GEROLA	98	13.11 ± 2.07	14.38 ± 2.13	55.50 ± 3.29	57.48 ± 3.30	1.04 ± 2.51	3.82 ± 4.93
PEZZATA MOCHENA	35	14.49 ± 3.99	15.53 ± 3.89	57.56 ± 6.00	59.15 ± 5.80	0.85 ± 2.19	3.09 ± 4.31
POMELLATA	5	19.04 ± 1.49	20.05 ± 1.52	64.38 ± 2.38	65.69 ± 2.47	0.99 ± 2.59	3.16 ± 5.14
ROCCAVERANO	31	17.05 ± 2.02	18.29 ± 2.06	61.54 ± 3.09	63.35 ± 3.07	1.20 ± 2.58	4.05 ± 5.43
ROSSA MEDITERRANEA	17	20.01 ± 1.60	20.81 ± 1.58	65.49 ± 2.46	66.55 ± 2.44	0.42 ± 1.20	2.46 ± 4.40
RUSTICA DI CALABRIA	113	19.91 ± 1.61	20.54 ± 1.66	65.30 ± 2.30	66.22 ± 2.40	0.47 ± 1.21	2.70 ± 4.85
SAANEN	152	16.81 ± 3.76	18.03 ± 3.74	60.96 ± 5.65	62.68 ± 5.49	0.92 ± 2.28	3.65 ± 5.14
SARDA (C)	146	20.44 ± 1.32	21.51 ± 1.37	66.00 ± 1.82	67.29 ± 1.86	0.81 ± 2.38	2.34 ± 4.09
SARDA PRIMITIVA	66	19.90 ± 1.24	20.89 ± 1.30	65.60 ± 1.81	66.88 ± 1.91	0.87 ± 2.59	3.11 ± 5.14
SCREZIATA	1	17.63 ± 0.85	18.11 ± 0.76	61.82 ± 1.01	62.52 ± 0.95	0.31 ± 1.08	1.97 ± 4.03
SEMPIONE	3	8.66 ± 0.89	10.11 ± 0.95	48.31 ± 1.45	50.64 ± 1.50	1.01 ± 2.55	3.19 ± 4.03
VALDOSTANA	9	14.05 ± 4.94	15.27 ± 4.71	56.73 ± 7.55	58.59 ± 7.13	1.09 ± 2.40	3.76 ± 5.34
VALLESANA	20	11.93 ± 4.09	13.21 ± 4.10	53.55 ± 6.45	55.54 ± 6.35	1.11 ± 2.52	3.19 ± 4.56
VERZASCHESE	9	14.51 ± 1.60	15.87 ± 1.71	57.70 ± 2.49	59.74 ± 2.57	1.10 ± 2.44	4.27 ± 5.57

Table 3.1.2 Number of farms and mean ± standard deviation of Summer-T, Summer-THI, and nDays during the first and last 30 years of the analysed period for each sheep breed.

Sheep breed	N. farms	Summer-T		Summer-THI		nDays	
		1950–1985	1986–2022	1950–1985	1986–2022	1950–1985	1986–2022
ALPAGOTA	47	14.62 ± 2.53	15.73 ± 2.65	57.83 ± 3.92	59.50 ± 3.97	0.81 ± 2.24	4.43 ± 5.06
ALTAMURANA	10	20.65 ± 1.18	21.59 ± 1.13	66.26 ± 1.55	67.50 ± 1.52	0.45 ± 1.25	3.09 ± 4.96
APPENNINICA	134	18.85 ± 1.55	19.95 ± 1.64	63.96 ± 2.28	65.43 ± 2.37	0.87 ± 1.92	3.26 ± 4.93
BAGNOLESE	119	18.75 ± 1.29	19.72 ± 1.33	63.74 ± 1.91	65.07 ± 1.97	0.75 ± 1.86	3.31 ± 4.75
BARBARESCA	17	20.05 ± 1.59	20.89 ± 1.62	65.83 ± 2.21	66.95 ± 2.23	0.57 ± 1.53	2.65 ± 4.39
BERGAMASCA	52	17.76 ± 2.65	18.82 ± 2.74	62.34 ± 3.88	63.79 ± 3.93	0.80 ± 1.90	2.94 ± 4.11
BIELLESE	14	17.98 ± 2.28	19.27 ± 2.32	62.75 ± 3.31	64.53 ± 3.27	0.98 ± 2.24	3.79 ± 5.39
BRIANZOLA	24	17.19 ± 1.48	18.63 ± 1.64	61.84 ± 2.21	63.84 ± 2.29	1.20 ± 2.50	4.87 ± 5.91
BRIGASCA	4	15.32 ± 2.52	16.38 ± 2.54	59.11 ± 4.09	60.78 ± 4.11	0.80 ± 2.09	2.90 ± 4.85
BROGNE	40	17.68 ± 1.95	18.96 ± 2.10	62.51 ± 2.85	64.27 ± 2.96	0.99 ± 2.32	4.80 ± 5.55
COMISANA	60	20.27 ± 2.25	21.10 ± 2.24	65.70 ± 3.20	66.81 ± 3.19	0.61 ± 1.52	2.73 ± 4.58
CORNELLA BIANCA	4	18.08 ± 1.69	19.32 ± 1.78	62.79 ± 2.34	64.48 ± 2.39	0.88 ± 2.14	3.30 ± 4.80
CORNIGLIO	14	17.49 ± 2.30	18.73 ± 2.44	61.96 ± 3.30	63.64 ± 3.38	1.01 ± 2.25	3.65 ± 4.90
DELL'AMIATA	57	18.97 ± 1.05	20.08 ± 1.10	64.06 ± 1.50	65.53 ± 1.56	0.89 ± 1.84	3.52 ± 5.52
DELLE LANGHE	31	17.60 ± 0.97	18.83 ± 0.97	62.35 ± 1.39	64.11 ± 1.37	1.23 ± 2.58	3.94 ± 5.42
FABRIANESE	33	18.44 ± 1.50	19.52 ± 1.62	63.23 ± 2.17	64.67 ± 2.29	0.80 ± 1.81	2.98 ± 4.02
FRABOSANA	45	15.05 ± 2.66	16.29 ± 2.74	58.44 ± 4.10	60.31 ± 4.14	1.18 ± 2.54	3.35 ± 4.96
GARESSINA	1	20.20 ± 0.74	21.26 ± 0.79	66.31 ± 0.96	67.78 ± 1.07	0.89 ± 2.18	4.32 ± 5.58
GARFAGNINA BIANCA	28	18.21 ± 2.07	19.24 ± 2.11	63.35 ± 3.14	64.84 ± 3.16	0.88 ± 2.13	3.79 ± 5.65
GENTILE DI PUGLIA	36	20.36 ± 1.95	21.27 ± 1.98	65.68 ± 2.69	66.86 ± 2.71	0.47 ± 1.30	2.81 ± 4.47
ISTRIANA-CARSOLINA	6	17.95 ± 1.14	19.12 ± 1.19	63.13 ± 1.73	64.77 ± 1.74	1.07 ± 1.89	5.52 ± 5.98
LAMON	23	14.28 ± 2.07	15.30 ± 2.14	57.34 ± 3.26	58.92 ± 3.29	0.84 ± 2.28	3.92 ± 4.66
LATICAUDA	60	18.96 ± 1.16	19.95 ± 1.14	63.83 ± 1.57	65.14 ± 1.57	0.82 ± 1.97	2.62 ± 3.64

MASSESE	85	18.69 ± 1.72	19.77 ± 1.77	63.96 ± 2.57	65.47 ± 2.61	0.84 ± 2.06	3.63 ± 5.43
MERINIZZATA ITALIANA	130	18.19 ± 1.93	19.00 ± 1.95	62.50 ± 2.59	63.60 ± 2.61	0.52 ± 1.35	2.82 ± 4.39
MOSCIA LECCESE	17	19.34 ± 4.38	20.33 ± 4.23	64.55 ± 6.45	65.95 ± 6.13	0.61 ± 1.71	3.63 ± 5.64
NERA DI ARBUS	98	20.36 ± 1.31	21.50 ± 1.37	65.92 ± 1.84	67.31 ± 1.90	0.68 ± 2.09	1.78 ± 3.61
NOTICIANA	1	22.62 ± 0.59	23.33 ± 0.67	70.01 ± 0.75	70.96 ± 0.88	0.66 ± 1.59	3.95 ± 5.82
PECORA CIUTA	17	10.32 ± 3.15	11.64 ± 3.15	50.99 ± 5.02	53.09 ± 4.94	0.95 ± 2.43	3.20 ± 4.55
PECORA DI CORTENO	15	11.45 ± 2.14	12.86 ± 2.19	52.75 ± 3.30	54.95 ± 3.21	0.97 ± 2.64	3.03 ± 4.29
PINZIRITA	20	21.87 ± 1.05	22.68 ± 1.07	68.65 ± 1.55	69.68 ± 1.57	0.68 ± 1.66	3.50 ± 6.06
PLEZZANA	4	15.37 ± 1.27	16.52 ± 1.31	59.02 ± 1.95	60.73 ± 1.96	0.99 ± 2.14	4.40 ± 5.14
POMARANCINA	34	19.58 ± 0.92	20.66 ± 0.95	65.22 ± 1.36	66.70 ± 1.42	0.73 ± 1.83	3.55 ± 5.47
ROSSET	74	9.67 ± 1.92	11.07 ± 1.92	49.96 ± 3.18	52.18 ± 3.10	1.19 ± 2.58	3.37 ± 4.70
SAMBUCANA	59	14.16 ± 3.19	15.42 ± 3.27	57.00 ± 4.99	58.92 ± 5.01	1.11 ± 2.51	3.10 ± 4.62
SARDA (O)	648	20.18 ± 1.30	21.32 ± 1.34	65.64 ± 1.87	67.05 ± 1.89	0.78 ± 2.24	2.21 ± 4.04
SAVOIARDA	6	16.20 ± 3.00	17.57 ± 3.16	60.16 ± 4.53	62.09 ± 4.61	1.11 ± 2.45	4.04 ± 6.00
SCHWARZNASENSCHAF	23	13.78 ± 3.54	15.04 ± 3.66	56.45 ± 5.49	58.33 ± 5.52	0.96 ± 2.35	3.45 ± 4.62
SCIARA-MOSCIA CALABRESE	5	19.45 ± 1.26	20.08 ± 1.32	64.89 ± 1.81	65.85 ± 1.91	0.37 ± 1.05	2.69 ± 4.65
SOPRAVISSANA	56	18.33 ± 2.00	19.39 ± 2.14	63.13 ± 3.03	64.55 ± 3.18	0.86 ± 1.93	3.18 ± 5.04
TACOLA	102	15.02 ± 3.24	16.35 ± 3.31	58.38 ± 4.99	60.33 ± 4.95	1.12 ± 2.45	3.71 ± 5.37
TRIMETICCIA DI SEGEZIA	1	17.92 ± 0.93	18.82 ± 0.87	62.21 ± 1.13	63.43 ± 1.08	0.43 ± 1.24	2.34 ± 3.35
TURCHESSA	5	19.91 ± 0.98	20.97 ± 1.00	65.56 ± 1.37	67.00 ± 1.42	0.98 ± 2.06	3.67 ± 4.70
VALLE DEL BELICE	248	21.47 ± 1.23	22.29 ± 1.23	67.43 ± 1.88	68.44 ± 1.91	0.63 ± 1.55	2.48 ± 4.49
VICENTINA-FOZA	13	17.05 ± 2.44	18.24 ± 2.63	61.57 ± 3.68	63.25 ± 3.82	0.87 ± 2.25	4.84 ± 5.53
VILLNOESSER SCHAF-FIEMMESE	33	12.34 ± 2.68	13.42 ± 2.75	54.32 ± 4.24	56.00 ± 4.30	0.91 ± 2.26	2.95 ± 4.03
ZERASCA	20	16.86 ± 0.96	17.99 ± 1.01	61.35 ± 1.45	62.98 ± 1.50	1.05 ± 2.36	3.49 ± 4.85

In this manuscript, we have chosen to utilize Asso.Na.Pa. data due to their reliability in accurately documenting breed locations. It is important to note that, according to European and Italian law (D.Lgs 11/05/2018 n.52 and Regulation (EU) 2016/1012), a breeding animal is considered purebred only if registered with a breeding book overseen by an authorized breed society; in Italy, Asso.Na.Pa. stands as the sole authorized entity managing sheep and goat breeding books. Additionally, farms breeding local breeds are incentivized to register to receive subsidies related to autochthonous population conservation. A first census of Italian small ruminant populations, which also led to the recognition of several of them, was carried out between the 70s and the 80s and published in 1983 [303]. Although precise geolocation data for past periods are unavailable, dedicated literature describes the distribution of numerous populations [48,303,308,309]. From these sources, it is evident that for the majority of breeds, changes in distribution over time have been minimal. Also, it is noteworthy that most breeds are named after the geographic area where they originated, and the current distribution indicates that they are still being raised in the same regions.

3.1.3.3 Statistical analysis

For each location, a linear regression was fitted between the year and summer-T, summer-THI, summer-TP, HW-THI, or nDays, in order to calculate the mean yearly change of the parameter.

Moreover, using JMP® Version 16 (SAS Institute Inc., Cary, NC, 1989–2023), two different linear regression models were fitted to these parameters, in order to explore the differences among Italian regions and sheep or goat populations, respectively:

$$Y = m + year + region + year * region + e$$

$$Y = m + year + breed + year * breed + e$$

In these models, Y is the climatic parameter, m is the mean, and e is the random residual. The breed-related model was performed for sheep and goat separately and was weighted for the number of subjects of a particular breed that were present in the farm.

3.1.4 Results

3.1.4.1 Climatic data

As shown in Figure 3.1.1, average Italian Summer-T and Summer-THI had a similar trend, with an initial decrease followed by an evident increase after 1980; 2003 and 2022 presented the hottest summers. On the other hand, average yearly Summer-TP did not show a clear trend in the considered period. The number of HW days (nDays) exhibited an obvious increase in the last 25 years; it is worth noting that the years 1983, 2003, and 2015 were characterized by an abnormally high number of HW days when compared to the period. Lastly, the mean THI occurred during HW events appeared to be very variable, but with a growing trend after 2000.

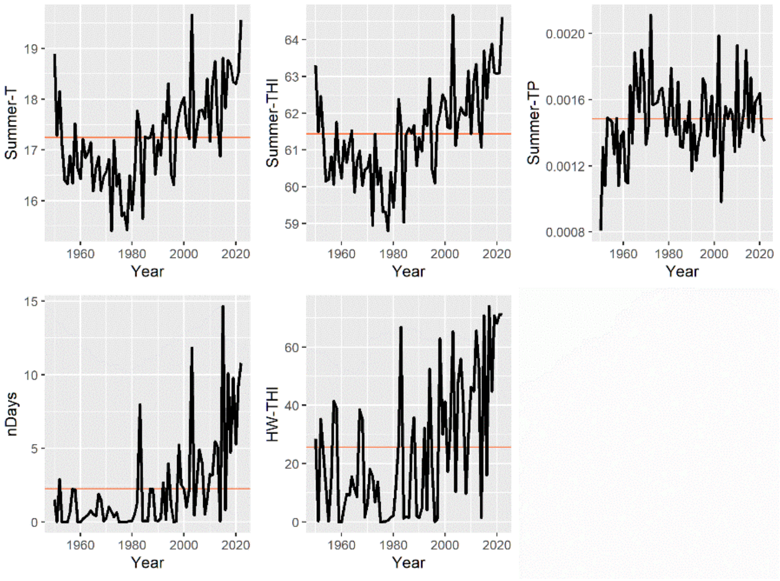

Figure 3.1.1 Lineplots of yearly Italian mean temperature, THI, and total precipitation during summer (Summer-T, Summer-THI, and Summer-TP, respectively), mean number of heat wave days (nDays) and average THI during heat waves (HW-THI) from 1950 to 2022. The horizontal red lines indicate the mean value when the whole studied period is considered.

As reported in Figure 3.1.2, the greatest increase in Summer-T and Summer-THI occurred in the same regions, namely central-northern Italy, with special emphasis on the Po Valley and the Alpine Arch, even though the latter corresponds to the region with the lowest mean Summer-T and THI, as expected. The linear regression model (Supplementary Table S3.1.1) taking into account the effect of the region and the year*region interaction confirmed that Apulia, Sicily, and Calabria present the highest mean Summer-T and THI values, but also the lowest yearly increase, whereas the opposite is true for Lombardy and Valle d'Aosta. Overall, a negative correlation between the region's mean value and slope was observed.

Average Summer-TP was characterised by a south-to-north gradient, with the lowest values in Sicily and Sardinia and the highest in Friuli-Venezia-Giulia and Trentino Alto Adige. Its variation over the studied period showed a variable distribution, with the highest slopes in the northern regions (Valle d'Aosta and Trentino) and the lowest ones in central Italy (Toscana and Lazio); however, an increase in total precipitation was also observed along the southern Apennines Area, particularly in Calabria.

It is evident that the number of HW days (nDays) has dramatically increased in the second half of the studied period. Overall, the regions with the highest mean nDays corresponded to those with the highest yearly increase, and vice versa. Particularly, Lombardy and Friuli-Venezia-Giulia appeared to be the regions at higher risk of experiencing an HW, whereas Sicily and Sardinia, despite presenting mean higher Summer-THI as mentioned before, experienced fewer HW events. An evident increase in nDays was observed along the Tyrrhenian coasts. As expected, the HWs were overall characterized by higher THI in southern Italy (Apulia and Sicily), but the highest increase in HW intensity was observed in the northern regions, especially Valle d'Aosta and Trentino Alto Adige, as well as the coasts.

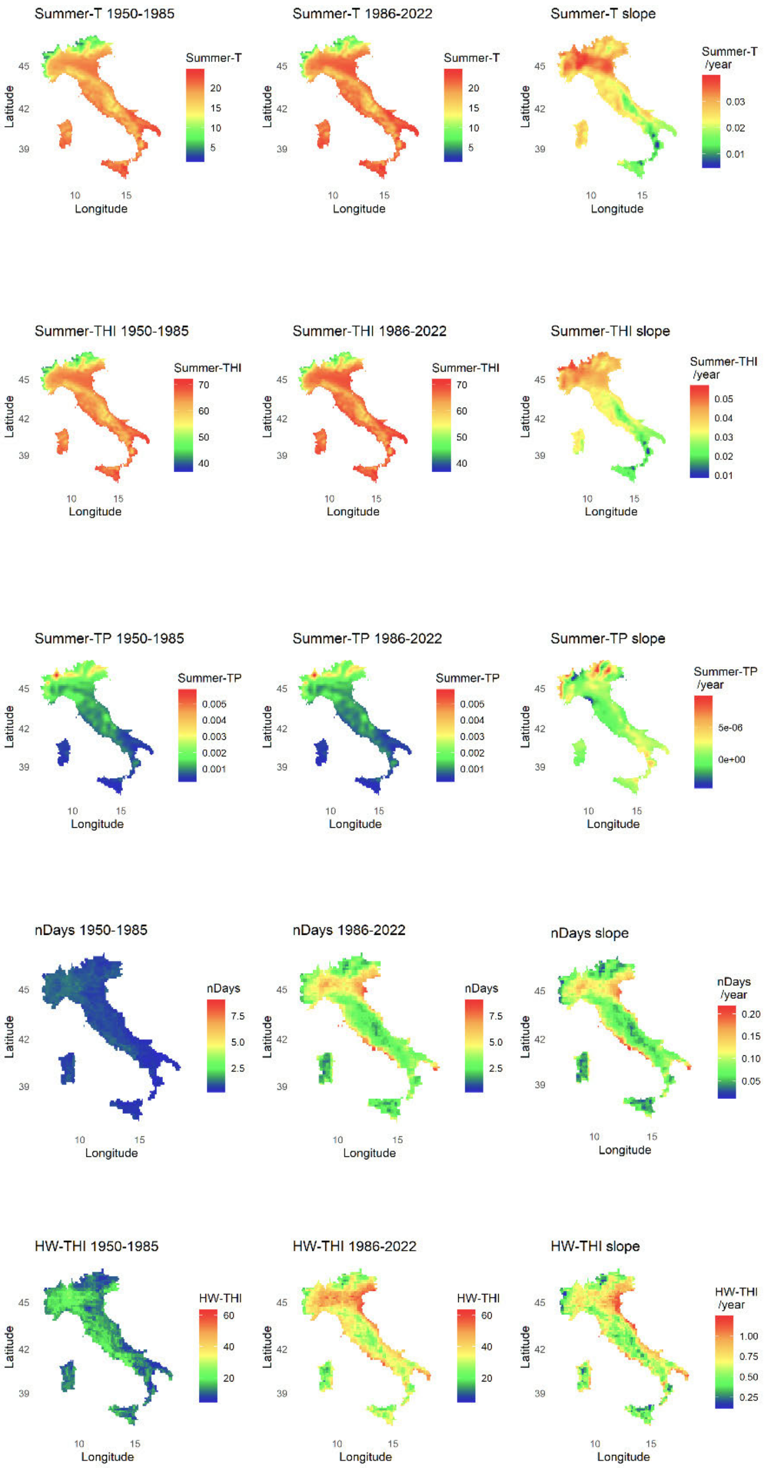

Figure 3.1.2 Comparison of mean temperature, THI, and total precipitation during summer (Summer-T, Summer-THI, and Summer-TP, respectively), mean number of heat wave days (nDays) and average THI during heat waves (HW-THI) in 1950–1985 and 1986–2022, and their slope along this period.

3.1.4.2 Italian sheep and goat distribution and model

All the analysed goat and sheep farms have been plotted in Figure 3.1.3. It should be noted that the present study only considered farms that were registered to Asso.Na.Pa. and resulted still in activity at the end of 2022. All the analysed breeds originated in Italy, with the exception of the Saanen goat, which has Swiss origins. However, it was included due to its widespread breeding in Italy, thus constituting an important component of the Italian goat breeding landscape.

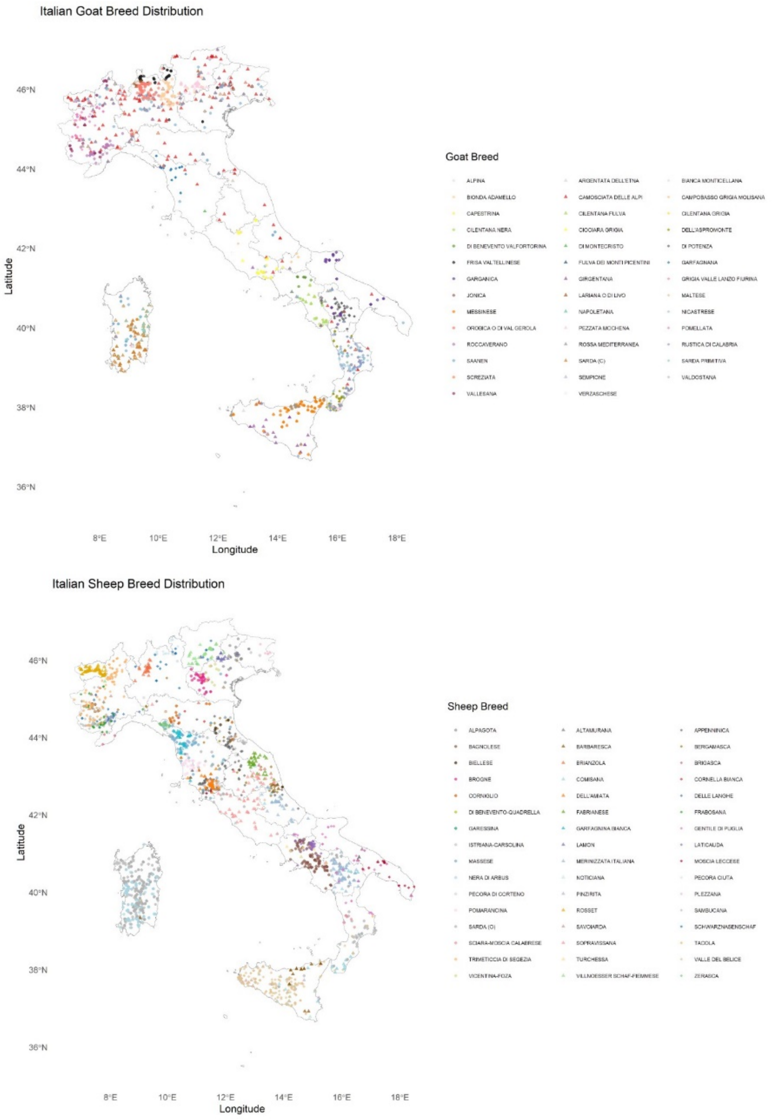

Figure 3.1.3 Distribution of Italian goat and sheep farms by breed.

With a total of 1,349 farms and 124,524 animals, goat breeding is highly represented in the northern part of Italy and, to a lesser extent, in the South—particularly in Calabria and Basilicata—and the two main islands, i.e., Sicily and Sardinia. A different distribution was observed for the 315,204 sheep, reared in 2,363 farms that are more evenly distributed along the Italian peninsula, they being especially numerous in the central-southern regions, in the very northern Italy, and in the two isles.

A model was performed to analyse the climate changes related to the different sheep and goat breeds, according to the locations of the farms they are reared in. Table 3.1.1 and Table 3.1.2 report the mean values of the three main parameters (summer-T, summer-THI, and nDays) in the first and last analysed 30 years for goat and sheep breeds, respectively, whereas the results of the models can be found in Supplementary Table S3.1.2.

Girgentana, Dell'Aspromonte, Napoletana, and Sarda were the goat breeds with the highest mean summer-T and summer-THI (breed least square means ranged from 21.26 to 21.60 °C and from 66.94 to 67.92 THI points, respectively); with regard to sheep, Noticiana, Moscia Leccese and Valle del Belice had the highest values (21.97–22.99 °C and 68.06–70.50 THI points). The lowest mean values were observed for Sempione, Frisa Valtellinese, Valdostana, and Vallesana goats (9.26–12.43°C and 49.29 to 54.29 THI points), and for Rosset, Pecora Ciuta, and Pecora di Corteno sheep (10.00–12.24 °C and 50.49–53.98 THI points). As expected, the animals reared in the northern regions usually experienced lower summer-T and THI. However, the opposite was found for slope, meaning that the breeds that have never been used to, and thus selected for, high temperature and THI, are going to live in regions becoming hotter and hotter if this trend persists. Particularly, the highest slopes were associated with Sempione, Lariana o Di Livo, Frisa Valtellinese, and Orobica goat for both summer-T and summer-THI (+0.030 to +0.03 °C/year and +0.047 to +0.053 THI points/year); among the sheep breeds, Savoiarda, Brianzola, and Pecora di Corteno (+0.032 to +0.034 °C/year and +0.047 to +0.050 THI points/year) had the highest slope for summer-T, and Pecora di Corteno, Rosset, and Pecora Ciuta for summer-THI. On the other hand, southern breeds live in more stable (i.e., having a lower slope) regions; this was particularly true for Screziata, Fulva dei Monti Picentini, and Nicastrese goats—notably, all Calabrian breeds— (+0.009 to +0.012 °C/year and +0.014 to +0.020 THI points/year) and Sciara-Moscia Calabrese and Barbaresca sheep (+0.010 to +0.016 °C/year and +0.0180 to +0.022 THI points/year). Some of the breeds with the most divergent characteristics were plotted in Figure 3.1.4 (only summer THI was considered, given the similarity with summer-T maps and results).

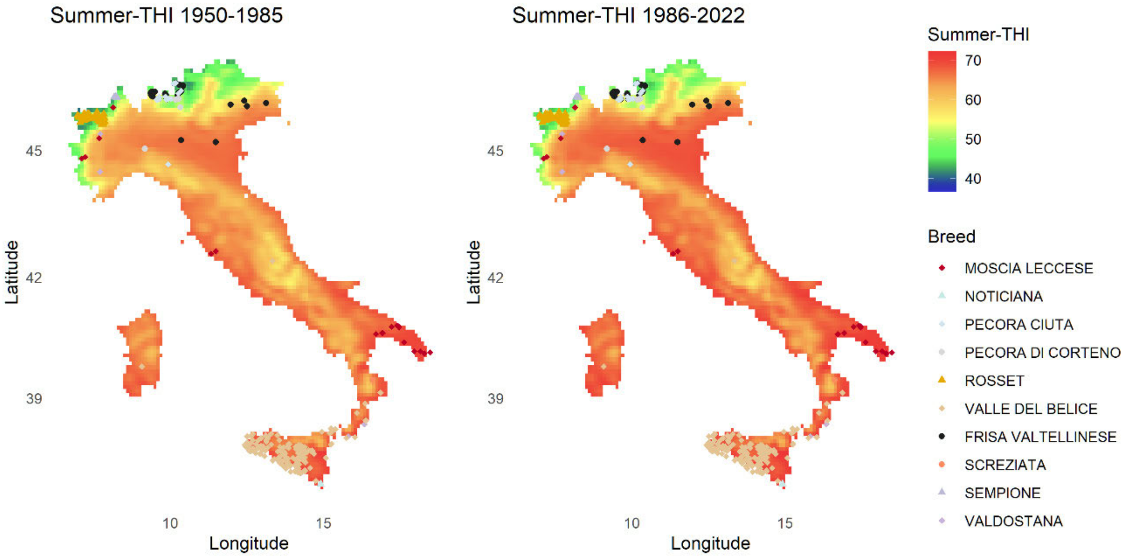

Figure 3.1.4 Representation of sheep and goat breeds with divergent values for summer-THI mean and slope. Southern breeds such as Moscia Leccese and Noticiana sheep and Valle del Belice and Screziata goats presented high mean summer-THI but low slopes, whereas the opposite was found for Pecora Ciuta, Pecora di Corteno, and Rosset sheep and Frisa Valtellinese, Valdostana, and Sempione goats.

Overall, northern breeds had the highest mean summer-TP values—such as Sempione, Orobica, and Vallesana goats (0.0025–0.0033 mm), and Lamon, Brianzola, and Tacola sheep (0.0026–0.0034 mm)—, whereas breeds reared in southern Italy and the isles, including Girgentana, Sarda, and Dell'Aspromonte goats (0.0004–0.0005 mm), and Noticiana, Valle del Belice, and Nera di Arbus sheep (0.0003–0.0005 mm), live in a drier environment. However, the differences in terms of summer-TP slope did not present a clear pattern as observed for summer-T and THI. Indeed, breeds from Calabria (Nicastrese goat and Sciara-Moscia Calabrese sheep), Veneto (Lamon and Vicentina Foza sheep), and Lombardy (Bionda dell'Adamello goat) were associated with an increase in precipitations, and others from Tuscany (Garfagnana goat and Garfagnina Bianca and Massese sheep) as well as Lombardy (Lariana o Di Livo and Orobica goat and Brianzola sheep) with decreased precipitations.

Lastly, the variables related to HW were modelised. We observed that Napoletana, Pomellata, and Ciociara Grigia, located in Campania and Lazio respectively, had the highest mean and slope for nDays among goats (2.96 to 3.37 days/year and +0.12 to +0.13 days/year, respectively), whereas the Screziata, Fulva dei Monti Picentini, and Girgentana (located in Calabria, Veneto, and Sicily, respectively) the lowest values (1.18 to 1.37 days/year and +0.04 to +0.05 days/year). However, it should be noted that some northern breeds, such as Lariana or Di Livo, Orobica, and Camosciata delle Alpi (one of the main and most specialised dairy breeds) had rather high slopes (+0.09 to 0.10 days/year). Consistently with the overall summer values, the highest mean HW-THI were found for Napoletana (76.35 THI points) and other southern breeds such as Dell'Aspromonte, Argentata dell'Etna, and Sarda goats (75.55–75.96 THI points), and the lowest for northern breeds including Sempione, Frisa Valtellinese, Valdostana, and Vallesana (60.90–65.73 THI points). Di Potenza goats had the highest slope (+0.022 THI points/year), and Screziata had the lowest one (+0.004 THI points/year).

With regard to sheep, northern breeds—e.g., Istriana-Carsolina and Brogne—were characterized by both high nDays mean and slope values (2.95–3.33 days/year and +0.11 to +0.12 days/year), whereas southern breeds—e.g., Moscia Leccese and Nera di Arbus—by both low nDays mean and slope values (1.18–1.23 days/year and +0.05 to +0.06 days/year). High mean HW-THI were associated with southern breeds (Noticiana, Moscia Leccese, and Pinzirita, ranging from 76.51 to 77.90 THI points), and low means to northern breeds (Rosset, Ciuta, and Fiemmese, ranging from 63.068 to 65.574 THI points), as expected. However, the highest slope was found for Gentile di Puglia breed, followed by Bergamasca (a sheep diffusely bred for meat production) and Biellese (+0.024 to +0.026 THI points/year), whereas the lowest slopes were found for Alpagota, Lamon, and Fiemmese breeds (–0.001 to +0.012 THI points/year). Some of the breeds with the most "extreme" nDays and HW-THI characteristics were represented in Figure 3.1.5 and Figure 3.1.6 , respectively.

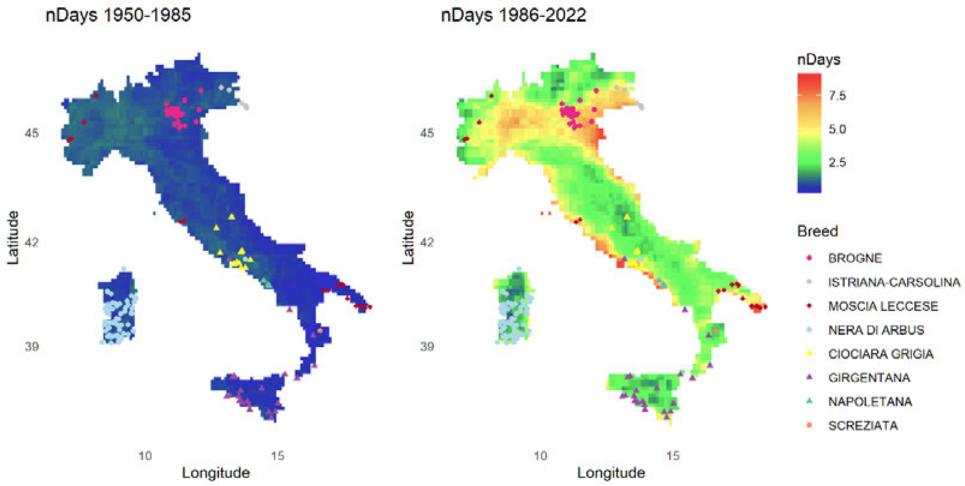

Figure 3.1.5 Representation of sheep and goat breeds with divergent values for nDays mean and slope. Brogne and Istriana-Carsolina sheep as well as Napoletana and Ciociara Grigia goats had both high mean and slope, whereas the opposite was true for Nera di Arbus and Moscia Leccese sheep and Girgentana and Screziata goats.

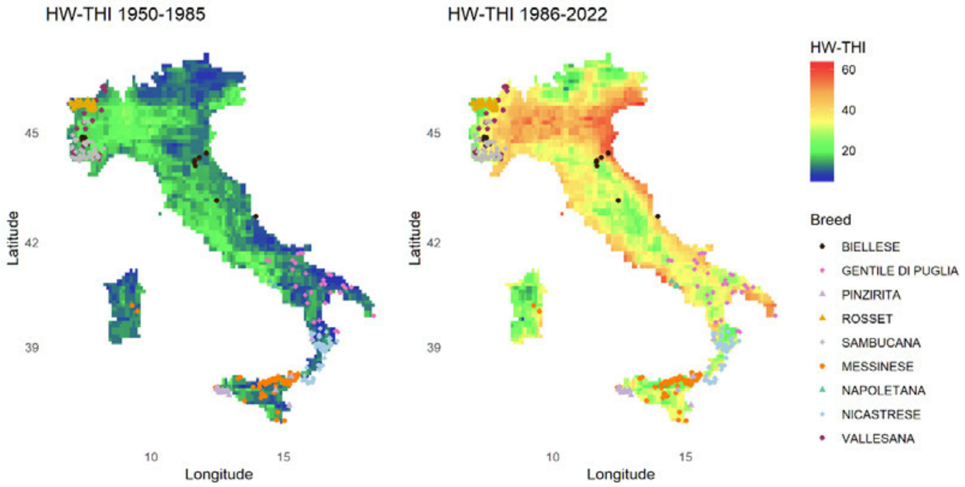

Figure 3.1.6 Representation of sheep and goat breeds with divergent values for HW-THI mean and slope. High mean and slope values were found for Biellese and Gentile di Puglia sheep as well as Napoletana and Nicastrese goats; the opposite was found for Rosset sheep and Vallesana goats. Moreover, Pinzirita sheep and Messinese goats had a high HW-THI mean but a low slope, whereas Sambucana sheep had a low mean but a high slope.

Sarda sheep, Camosciata delle Alpi, and Saanen goats are the most prevalent breeds in Italy. All of them are specialized in milk production and commonly reared in intensive or semi-intensive farming systems, reducing the impact of environmental variables. The Sarda sheep constitutes the largest ovine population, with 166,730 registered animals and 648 farms, with nearly 90% located in Sardinia. Climatic data analysis indicates that this population predominantly inhabits areas characterized by high but stable summer-T and THI, along with low summer-TP and nDays. Moreover, there is a negative slope for nDays (−0.06 days/year) over the studied period (Figure 3.1.7). Camosciata delle Alpi ranks as the most numerous goat breed in Italy, with 13,203 heads distributed across 203 farms, with over 70% of these goats bred in Northern Italian regions, particularly Lombardy. Saanen goat breed counts 12636 registered heads across 152 farms, primarily found in Sardinia and Northern Italy. Among both the Saanen and Camosciata breeds, small farms (with fewer than 50 animals) make up more than half of the total farms, while around 28% and 16%, respectively, house over 100 animals. However, it is noteworthy that 18% of Saanen goats are concentrated in the 2% of farms with over 500 heads. Often, Saanen and Camosciata delle Alpi are reared together with other breeds (two-thirds and half of the farms, respectively). Analysis reveals an increase in summer-T, summer-THI, and nDays for both breeds during the study period (Figure 3.1.7).

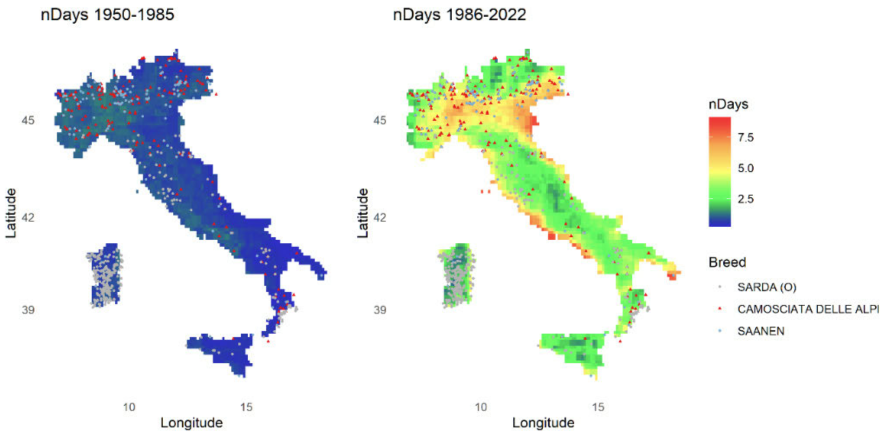

Figure 3.1.7 Representation of the three main sheep (Sarda) and goat (Camosciata delle Alpi and Saanen) breeds reared in intensive or semi-intensive farming systems in Italy and comparison between the mean number of heat wave days (nDays) in 1950–1985 vs 1986–2022.

3.1.5 Discussion

This study represents, at best of our knowledge, the first effort in comprehensively analysing the distribution of all registered and active farms of Italian sheep and goat local breeds and examining the climate changes that these populations have been subjected to in the past seven decades. Our findings is underlined by the Food and Agriculture Organization (FAO), which underscores the importance of assessing the distribution of local breeds, considered invaluable resources for confronting future climate changes, and the potential risks they face [26].

Our data reveal notable disparities in the distribution of goat and sheep farms across Italy. Goats are predominantly concentrated in the northern regions, with a limited presence in the South, specifically Calabria and Basilicata, as well as the islands of Sicily and Sardinia. In contrast, sheep farms are more evenly distributed throughout the Italian peninsula, with higher concentrations in central-southern regions, northern Italy, and the islands. Consistently with what reported by the Italian National Institute of Statistics [294] and the National Database of Animals and Holdings (BDN) [55], Sardinia counts the majority of both farms and animals—about half of sheep and a quarter of goats—, followed by Sicily, whereas goats only are also high represented in Lombardy and Calabria. Our data also underscore the presence of several breeds with extremely restricted breeding ranges; specifically, 10 sheep and 12 goat populations were raised in fewer than ten registered farms. Conversely, our dataset also encompasses some widely distributed populations, including the Sarda sheep, as well as the Saanen and Camosciata delle Alpi goats. All of them are reared for milk and cheese production and mainly rared in intensive or semi-intensive farms.

The climate data presented in this study depict a concerning trend of increasing summer temperatures, temperature humidity index (THI), and heat wave (HW) frequency and intensity, particularly over the last three decades; most notably, we propose a definition of heatwaves that incorporates THI, a widely recognized index for assessing livestock heat stress [310,311]. Our findings are in accordance with the literature about climate changes occurring and foreseen in Europe and Italy [312–316]. The regions most affected by these changes are situated in central-northern Italy, including the Po Valley and the Alpine Arch, thus interesting breeds such as Rosset and Brogne sheep, and Lariana and Frisa Valtellinese goats. This poses a challenge because livestock breeds traditionally raised in these regions have not been selected for, and thus are not adapted to, hot climates. Furthermore, the often small population size of most of the highly impacted breeds exacerbates the situation, calling for potential interventions and changes in farm structures to ensure their survival and productivity. Conversely, the relative stability observed in southern Italy, which has always been characterized by hotter and drier temperatures, positions Sicilian, Calabrian, and Sardinian breeds—e.g., Girgentana and Nicastrese goats and Nera di Arbus sheep—, especially if reared in extensive or semi-extensive systems, as excellent case studies for climatic adaptation and possible resources for future selection and breeding efforts to enhance resilience in the face of ongoing climate changes. In this regard, further evaluation of the effects of heat stress on different Italian sheep and goat populations (as in [317,318]), as well as the establishment of specific reference thermotolerance ranges (as in [319]), might prove beneficial.

In conclusions, this paper provides an overview of the distribution and population size of Italian small ruminants, offering valuable insights into the conditions and changes they have been subjected to over the past seven decades in terms of summer temperature, THI, and HWs. These data are instrumental for devising precise monitoring and intervention strategies for breeds that may face future vulnerabilities. By combining this information with other environmental risks, inbreeding-related concerns, and population size, it would be possible to develop comprehensive strategies to protect these invaluable genetic resources. Moreover, the data presented herein offer a foundation for designing experiments aimed at investigating the environmental adaptability of these animals, by assisting in the selection of the breeds that, having thrived and evolved in diverse environmental conditions, are the most suitable for being compared.

3.1.6 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S3.1.1 Results of the region-related linear regression model.

Supplementary Table S3.1.2 Results of the breed-related linear regression models. Sheep and goats were modelled in two different models. Farms were weighted according to the number of animals of a specific breed they rear.

3.2 MAPPING RISKS AND LANDSCAPES: CONSERVATION INSIGHTS FOR ITALIAN SMALL RUMINANT POPULATIONS

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3.2.1 Abstract

Local livestock breeds are valuable genetic resources that support ecosystem services, cultural identity, and the sustainability of animal husbandry. However, these populations face increasing threats from intensive farming, land abandonment, and environmental risks. This study aimed to assess Italian small ruminant populations' exposure to environmental threats and distribution across different landscapes. Therefore, we integrated geolocation data of 3,712 active registered farms of 41 goat and 47 sheep breeds with publicly available data on environmental hazards, including seismic activity, landslides, floods, and projected climate change, alongside landscape characterization maps of Italy. Additionally, we evaluated extinction risks based on effective population size (Ne) and calculated biodiversity indices at the provincial level to describe small ruminant biodiversity in Italy.

Our findings indicate that over a quarter of Italian small ruminant populations are at short-term risk of extinction, while nearly half face long-term threats. The two most pressing environmental concerns include seismic risk, particularly for breeds in southern Italy, and climate change projections indicating shifts to warmer and drier conditions, especially for Alpine breeds traditionally adapted to colder environments. Landscape characterization revealed that sheep farming is predominantly associated with agricultural landscapes, whereas goat farms are more frequently linked to woodlands and mountainous areas. Each breed exhibits specific environmental adaptations, underscoring their role in sustainable land management. Biodiversity analyses highlighted significant regional disparities, with high diversity in provinces such as Cuneo and Frosinone, whereas others exhibit limited breed representation.

Understanding the spatial distribution of local breeds, their exposure to environmental risks, and their interactions with landscapes is essential for developing targeted conservation strategies. Integrating ecological, genetic, and spatial data allows for effective prioritization of

conservation efforts to safeguard genetic diversity, support rural communities, and promote sustainable livestock farming.

3.2.2 Introduction

Small ruminants are widely distributed worldwide due to their exceptional adaptability to a variety of climatic and managerial conditions and to their ability to provide valuable products even in harsh environments with minimal inputs [226,320].

Italy, known for its rich agricultural heritage and diverse landscapes, has a significant small ruminant sector, accounting for approximately 10% of the total ruminant population in the country. This makes Italy one of the leading producers of sheep and goat milk, as well as renowned cheeses, in Europe. By contrast, sheep and goat meat play a minor role in Italian production, although their consumption remains strongly tied to traditional holiday celebrations such as Christmas and Easter [295,296,321]. While the sector has been moving toward intensification and the increasing prominence of specialized breeds [322], local animal genetic resources—comprising 64 sheep breeds and 41 goat breeds—continue to play a crucial role in maintaining biodiversity and supporting sustainable agricultural systems in the Italian farming panorama [292,323].

The interconnection between livestock and landscapes is profound, particularly for local breeds, which are often reared in extensive and traditional systems. These breeds are uniquely adapted to their environments, forming a strong interconnection with the landscape they inhabit [20,26,31,74]. The effects of grazing on landscapes, however, remain a topic of debate. On one side, husbandry practices can have detrimental consequences, especially when linked to overstocking, overgrazing, and unsustainable land management. These negative impacts include excessive defoliation, land degradation, soil erosion, and water quality deterioration, all of which threaten ecosystem stability and biodiversity [61,62]. However, well-managed livestock grazing has been widely demonstrated to exert substantial positive effects on ecosystems. Sustainable grazing and browsing enhance plant and animal biodiversity, improve land cover, reduce the risks of firebreaks and avalanches, help control weeds and invasive species, and promote habitat connectivity [31,32]. These ecosystem services are particularly significant in remote and marginal areas, which are often at risk of abandonment if not for the presence of local livestock populations, especially sheep and goats [34,324].

Local breeds provide much more than ecosystem services. They are essential to the livelihoods of rural communities, serving as a primary source of income, while also being deeply embedded in cultural traditions. In many cases, these breeds have become integral parts of the landscapes themselves, reflecting a harmonious interplay between people, animals, and the environment [31]. If, on the one hand, this strong bond between animals and their territory has allowed local populations to adapt to harsh environmental conditions, on the other hand, it also makes them vulnerable to climate change. Rising temperatures have been extensively documented to have direct and indirect negative effects on livestock production, reproduction, and survival [19,26,325]. However, climate change is not only causing gradual temperature increases but also leading to a rise in the frequency and intensity of extreme weather events, which further exacerbate the challenges faced by livestock and their ecosystems [75,326].

Italy, in particular, is highly susceptible to geological, hydrological, and hydraulic instability, and climate change is aggravating these issues. The probability of extreme weather-related risks in Italy has increased by 9% over the past two decades [78]. The country is facing a significant rise in both droughts and episodes of heavy rainfall [327]. The hydrogeological characteristics of the Italian landscape, combined with climate change and other non-climatic factors such as increasing urbanization, are worsening the situation. Floods, landslides, and forest fires are common, and their risk is escalating [328–332]. On top of this, Italy is one of the Mediterranean countries with the highest seismic risk. The most affected areas are concentrated along the Apennine mountain range, extending to Calabria and eastern Sicily, with severe consequences in terms of both human lives and economic losses [333]. These natural disasters impose significant costs, both in terms of human casualties and financial damages. The livestock sector is no exception: earthquakes, hurricanes, landslides, and floods can destroy hundreds of stables and kill thousands of animals, leading to long-term repercussions such as resource shortages and the spread of diseases [334–336]. Furthermore, such disasters pose a serious risk to biodiversity. This is particularly critical for local breeds, which are often small in population size and geographically concentrated [142], as in the case of Italian local sheep and goat populations [321].

In light of these considerations, this study aims to provide an overview of the environmental risks posed by extreme weather events to Italian sheep and goat populations, enabling a better understanding of the specific exposure of each breed and the development of more effective management plans. Furthermore, it analyses the landscape and territorial characteristics of the regions where these populations are reared, with the aim of fostering management strategies that valorize Italian livestock and environmental resources by promoting the sustainable coexistence of livestock farming, biodiversity conservation, and ecosystem health.

3.2.3 Materials and methods

3.2.3.1 Farm data

The present study included data of all the farms registered to the Italian Sheep and Goat Breeders Association (Asso.Na.Pa.) and operational as of 31st December 2022. Asso.Na.Pa. is the sole authorized organization in charge of managing sheep and goat herd books in Italy and keeps a record of reliable and accurate geolocation and management data for Italian sheep and goat farms and breeds. Using these data, the effective population size (Ne) was calculated for all the breeds using Wright's formula based on sex ratio [144].

The dataset included 1,349 goat farms, rearing a total of 124,524 animals across 41 populations. Concerning sheep, 2,363 farms were investigated, hosting 315,204 heads belonging to 47 populations (Figure 3.2.1). All the breeds studied were Italian local populations, except for the Saanen goat, which originated in Switzerland but is widely bred in Italy. For the purposes of this study, Camosciata delle Alpi and Saanen goats, along with Sarda sheep, will be considered specialized breeds, as they undergo genetic selection and are typically raised in intensive or semi-intensive production systems. A more detailed description of these data is provided in Bionda *et al.* (2024) [321] and Supplementary Table S3.2.1.

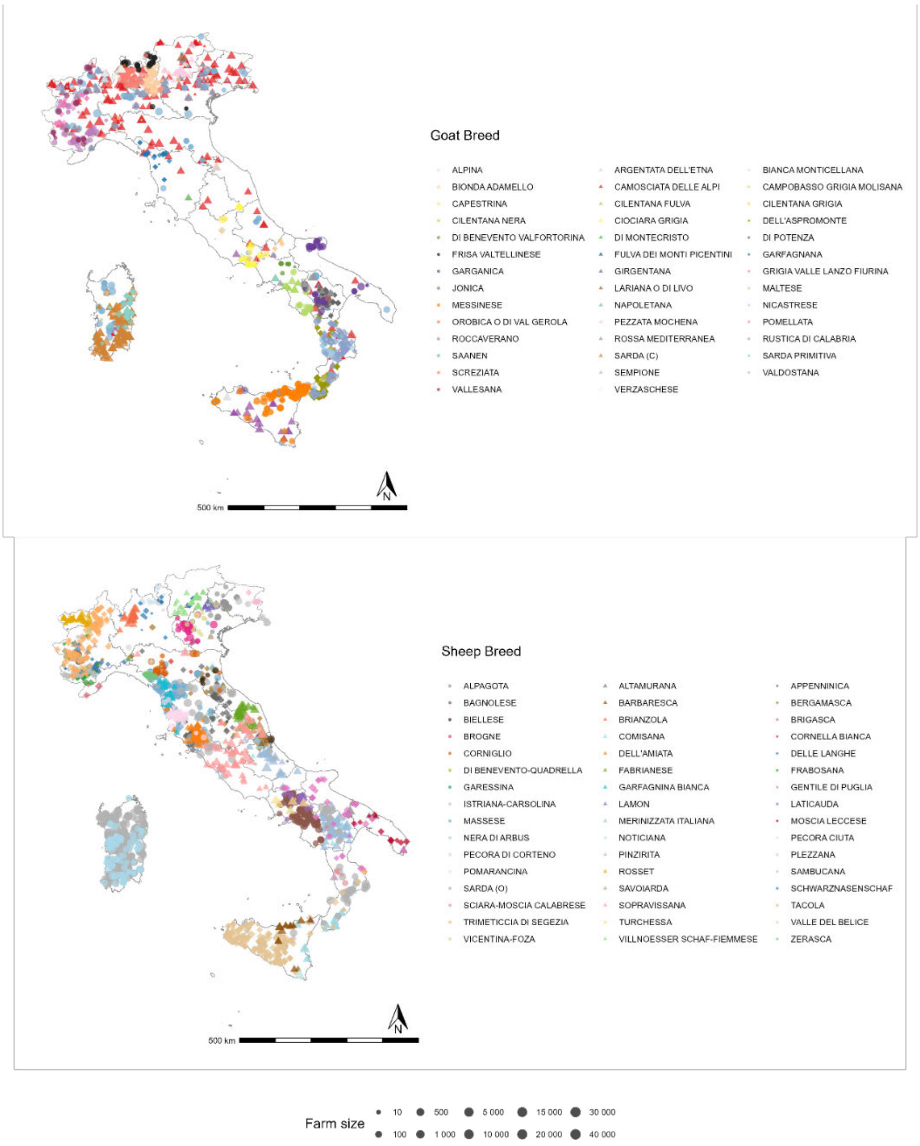

Figure 3.2.1 Distribution and size of Italian registered goat and sheep farms.

After retrieving the GPS coordinates for each farm, a circular buffer with a 10-km diameter around their exact location was calculated with ArcGIS Pro Desktop 3.0.2's *Buffer* tool to account for their physical extension and the fact that typically sheep and goats graze in the areas around the farm. This same extension has already been used in other studies, such as Bertolini *et al.* (2018) [244].

3.2.3.2 Environmental data

3.2.3.2.1 Natural hazards

Maps (in .shp file format) and data related to flood and landslide risks were made publicly available by the Italian Institute for Environmental Protection and Research (ISPRA) [330,332]. The classification of landslide hazard zones (attention zone – AA, moderate hazard – P1, medium hazard – P2, high hazard – P3, and very high hazard – P4) was based on four categories, considering both the probability of occurrence and the magnitude of the landslide events, which were evaluated using events' speed and geometric severity by adapting the procedure developed by the Swiss Federal Office for the Environment (Supplementary Figure S3.2.1A). The flood hazard classification follows the definitions outlined in D.Lgs. 49/2010, identifying three scenarios: low hazard (P1, low probability of flood events or extreme scenarios), medium hazard (P2, recurrence interval between 100 and 200 years), and high hazard (P3, recurrence interval between 20 and 50 years) (Supplementary Figure S3.2.1B).

The classification of seismic zones (updated in April 2023) was retrieved from data published by the Italian Civil Protection Department (Supplementary Figure S3.2.1C). Data were provided as a table listing all Italian municipalities with their respective classification. Using this table, we created a polygon shapefile representing seismic risk. Seismic classification in Italy is defined by four main zones based on the territory's acceleration on rigid ground (ag) with a probability of exceeding equal to 10% in 50 years: High seismicity (Zone 1, ag > 0.25g), Medium-high seismicity (Zone 2, 0.15g < ag ≤ 0.25g), Medium-low seismicity (Zone 3, 0.05g < ag ≤ 0.15g), and Low seismicity (Zone 4, ag ≤ 0.05g).

Data on present (1980–2016) and projected future (2071–2100) Köppen-Geiger climate classifications (Supplementary Figure S3.2.2, definitions in Supplementary Table S3.2.5) were obtained as raster files at a 1-km resolution from Beck *et al.* (2018) [337]. This classification system defines five main climate classes, further subdivided into 30 subtypes based on seasonal temperature and precipitation patterns. Future climate projections were derived using the anomaly method, incorporating 32 climate models under the RCP8.5 scenario [337]. The climate classes present, or expected to be present, in Italy are:

- Dry – Semi-arid steppe – hot (BSh): Low annual precipitation, hot summers.
- Dry – Semi-arid steppe – cold (BSk): Low annual precipitation, cold winters.
- Dry – Arid desert – cold (BWk): Very low precipitation, cold winters.
- Temperate – No dry season – hot summer (Cfa): Hot and humid summers, cool-to-mild winters.
- Temperate – No dry season – warm summer (Cfb): Cool summers and mild winters, relatively narrow annual temperature range and few extremes of temperature.
- Temperate – Dry summer – hot summer (Csa): Hot and dry summers, mild and wet winters.
- Temperate – Dry summer – warm summer (Csb): Warm and dry summers, mild and wet winters.
- Continental – No dry season – warm summer (Dfb): Four distinct seasons with large seasonal temperature differences, having cold winters and warm-to-hot summers.
- Continental – No dry season – cold summer (Dfc): Long and cold winters, short warm-to-cool summers.

- Continental – Dry summer – warm summer (Dsb): Extreme seasonal changes with cold or very cold and wet winters and warm and dry summers.
- Continental – Dry summer – cold summer (Dsc): Cool and dry summers, long and cold winters.
- Polar – Ice cap (EF): Perennial ice cover, extremely cold year-round.

A more detailed definition for all the classes is provided in Supplementary Table S3.2.5.

3.2.3.2.2 Landscape characterization

Italian socio-ecological landscape characterization data were retrieved from ISPRA, including physiographic types, ecosystems, land cover, land use, and naturalistic-cultural value. The physiographic types map (.shp format, 1:250,000 scale) [338] identifies landscape units with homogeneous litho-morphological and land cover characteristics, as well as a specific geographic connotation (Supplementary Figure S3.2.3A). Therefore, characterizing the physiographic context of small ruminant breeding ranges can help in understanding how litho-morphological and land-cover conditions shaped and shape adaptation and management strategies; indeed, historically, small ruminants have been mainly raised in marginal areas where other activities were not feasible. The Authors of this map grouped these landscape units into seven main categories: low plain, hilly, tabular hills, mountainous, tabular mountains, depressed landscapes in mountainous areas, and landscapes characterized by singularity. The complete description of all the categories can be found in Annex 1 of APAT (2003) [339].

The 2018 map of Italian ecosystems (Supplementary Figure S3.2.3B) and 2021 maps of land cover and land use (raster files with a 10x10 m resolution) (Supplementary Figures S3C and S3D, respectively), were retrieved from the ISPRA website [340]. These maps, reported to have been generated by combining data from the Copernicus Land Monitoring Service (2018) with the Italian National Map of Soil Take (2021) from ISPRA [341], provide distinct but interconnected information. According to Directive 2007/2/EC, land cover refers to the "physical and biological cover of the earth's surface, including artificial surfaces, agricultural areas, forests, (semi-)natural areas, wetlands, and water bodies." Following the definition of the same Directive, land use describes the actual biophysical status of the territory "according to its current and future planned functional dimension or socio-economic purpose". A more detailed definition for land use and land cover categories has been translated and adapted from Munafò (2023) [342] and reported in Supplementary Table S3.2.9 and S3.2.8 , respectively. The Ecosystem Map of Italy is based on land cover and potential natural vegetation data and identifies 84 ecosystem types grouped into 11 main categories, which we used for our analyses [343]. In the context of the present study, each of these spatial layers provides a different perspective on the landscape: land cover informs about the actual physical and biological surface—such as vegetation type or bare ground—that livestock are directly exposed to and interact with; land use captures the functional dimension of the territory, reflecting how humans manage or intend to manage the land (e.g., for agriculture, forestry, or settlements), thus offering insights into anthropogenic pressures and livestock farming systems; and the ecosystem classification, by integrating land cover with potential natural vegetation, provides a more holistic ecological framework that reflects the broader biotic and abiotic characteristics of the territory.

ISPRA also provided a shapefile on the naturalistic-cultural value (NCv) of the Italian territory, which was calculated for each physiographic unit (Supplementary Figure S3.2.4A). This index integrates a cultural score given by the presence of cultural and environmental goods, such as protected areas and parks, and the number of PDO and PGI enogastronomic products, with a naturalistic evaluation based on the landscape type and variability and the anthropic impact [344]. Assessing NCv in breeding ranges can provide information about the influence of small ruminant breeding on the development of landscapes of high environmental and cultural significance: indeed, many PDO and PGI products, included in the cultural evaluation, originate from local breeds and represent a tangible link between biodiversity, gastronomy, and cultural identity. In addition, in areas with particularly high NCv, careful planning is needed to ensure harmonious coexistence between breeding activities and the preservation of natural ecosystems.

Additionally, we included data from the Red List of Ecosystems in Italy [345], classified according to the International Union for Conservation of Nature (IUCN) criteria for ecosystem collapse risk assessment (Supplementary Figure S3.2.4B). These criteria consider reductions in geographic distribution, restricted distribution, degradation of the abiotic environment, and disruptions in biotic processes or interactions. A comprehensive description of all ecosystems (from the aforementioned Map of Italian Ecosystems) and their risk status can be found here in Annex C3 of Blasi *et al.* (2023) [345]. Relating breed distribution to ecosystems at different collapse risk levels allows evaluation of the reciprocal relationship between livestock and their habitats. Breeds can be vulnerable to habitat degradation, but they can also influence ecosystem conditions—negatively through overgrazing or habitat alteration, or positively by providing ecosystem services such as vegetation management, biodiversity maintenance, and fire prevention.

3.2.3.3 Livestock biodiversity indices

To assess biodiversity in terms of Italian local populations of sheep and goats, we utilized farm geolocation and size data at the provincial level to compute various biodiversity indices. Although typically used for wild species, these indices can also be well-suited to describe livestock distribution. Specifically, we calculated the Shannon Diversity Index (H'), the Inverse Simpson Index (1/D), and the Gini-Simpson Index (1 - D) [346–348] using the vegan package in R [349].

The Shannon's Diversity Index (H') quantifies both breed diversity and evenness, while the Gini-Simpson Index, which ranges from 0 to 1, represents the probability that two randomly selected individuals belong to different breeds. The Inverse Simpson Index (1/D) provides an estimate of the "effective number of breeds" within a given area, meaning the number of equally abundant breeds that would generate the same diversity level as observed.

Additionally, we derived Pielou's Evenness Index (EH) from the Shannon's Diversity Index, calculated as the ratio between H' and log(Richness), where Richness corresponds to the total number of recognized breeds in Italy. This index, which ranges from 0 to 1, quantifies the uniformity of breed distribution across farms [350].

To evaluate whether differences in these indices could be related to the size of the provinces, we also calculated corrected index values by dividing each by the logarithm of the respective province area. In addition, the Spearman correlation between the indices and the total province area was assessed using the *cor.test* function from the stats package.

3.2.3.4 Data analysis

For each farm, we calculated the percentage of its buffer area covered by different hazard or landscape classes. When environmental data were in shapefile format (landslide hazard, flood hazard, seismic risk, physiographic types, and naturalistic-cultural values), we used ArcGIS Pro's *Tabulate intersection* tool, whereas for raster maps we applied the *exact_extract* function from the exactextractr package [351] in R (version 4.4.1 for all the analyses performed in this study). Data were then summarized by breed and species.

Figures representing the environmental maps were generated using sf and ggspatial packages for shapefile maps [352,353] and the raster and tidyterra packages for raster files [354,355].

A principal component analysis (PCA) was performed with summary indices for provinces and breeds using *prcomp* function and plotted using a modified version of *ggbiplot* function from ggbiplot R package [356]. In the context of this study, PCA was used to explore patterns of variation among breeds and provinces based on multiple environmental risk and demographic indicators. This method is particularly effective for summarizing and visualizing complex datasets, such as the one that includes both geographically explicit variables—such as seismic and hydrogeological risk, climate change projections, and ecosystem vulnerability—and non-geographical variables, such as Ne and biodiversity indices. PCA helps identify the linear combinations of these variables—interpreted as composite measures of exposure to different risks—that best explain the distribution of breeds or provinces in the multivariate space. This approach not only highlights the variables contributing most to the observed variation, but also allows the identification of breeds or areas facing simultaneous threats, which may not be apparent when considering each risk factor in isolation, and/or that, sharing similar risk profiles, might benefit from similarly designed interventions.

For both goat and sheep breeds, the following variables were included: effective population size (Ne), the percentage of area with high or very high naturalistic-cultural value (NCv), the percentage of area with ecosystem risk classified as vulnerable or higher, the percentage of area in seismic zones 1 or 2, the percentage of area with landslide hazard classified as P3 or P4, the percentage of area with medium or high flood hazard, and the percentage of farms predicted to experience a shift in Köppen-Geiger climate classification. The Sarda sheep breed was excluded due to its exceptionally high Ne value. In the PCA, all variables were expressed as percentages except for Ne, which was measured as an absolute count. To prevent the different numerical range of Ne from disproportionately influencing the principal components, we applied a log transformation and then scaled Ne. Percentage-based variables were left unscaled because they all had a possible range of 0–1, making them directly comparable without the need for further scaling. Moreover, standardizing them would have artificially increased the weight of variables with a lower observed range, whereas, by maintaining their original scale, we preserved the relative impact of each risk factor while ensuring a meaningful interpretation of environmental risk and landscape characterization.

At the province level, the PCA included Gini-Simpson index for Italian domestic small ruminant populations, the percentage of the province classified as having high or very high

NCv, the proportion of the province in seismic zones 1 or 2, the percentage of land with medium to high flood hazard, the percentage of the territory classified as P3 or P4 for landslide risk, the proportion of ecosystems considered vulnerable or at higher risk, and the percentage of the province expected to experience a shift in Köppen-Geiger climate classification. Since the possible range of all the variables was 0-1, they were not scaled. Conducting analyses at the provincial level—alongside those focused on breeds—allows us to extend our considerations beyond the current distribution of small ruminant farms. This broader territorial perspective facilitates the identification of areas where other species might be affected or where shifts in livestock distribution may occur in the near future. Moreover, it aligns with the scale at which many policy decisions are made, as interventions are often planned and implemented at the level of provinces or regions rather than for specific breeds alone.

The same datasets used for the PCA were also analyzed using hierarchical clustering to identify groups of breeds or provinces with similar characteristics. We applied the *hclust* function from the stats package, using the "ward.D2" method. The optimal number of clusters was determined with the silhouette method, as implemented in *fviz_nbclust* from the factoextra package [357], complemented by a visual inspection of the dendrogram. In addition, a heatmap showing the mean values of each variable for each group was generated using pheatmap [358].

3.2.4 Results

3.2.4.1 Effective population size

The computation of Ne revealed that among goat breeds (median = 123, IQR = 29-500), 15 (36%) have a value below 50, indicating a short-term risk of extinction, while 16 (38%) fall within the 50–500 range, suggesting a long-term risk of extinction. For three goat populations, the estimated Ne was 0: the Alpina (an ancient population that likely gave rise to most of the breeds reared in the Alpine region), the Fulva dei Monti Picentini (originating from Campania, once considered extinct and recently rediscovered), and the Screziata (a dairy breed from the Campania region, believed to be related to the Napoletana breed and African goats, named for its characteristic speckled coat) [48]. The situation for sheep populations (median = 226, IQR = 117-656) is only slightly better, with 9 (18%) breeds having a Ne below 50 and 25 (51%) falling between 100 and 500. For three populations, Ne was estimated at 0: the Garessina (a Piedmontese population subjected to extensive crossbreeding with both Italian and Merino breeds), the Pinzirita (an ancient Sicilian breed possibly of Asian origin, adapted to harsh mountainous environments), and the Trimeticcia di Segezia (a synthetic meat breed recently developed through crosses of Gentile di Puglia × Ile de France × Württemberg) [48]. Ne estimation for all the breeds is reported in Supplementary Table S3.2.1.

3.2.4.2 Natural hazards

3.2.4.2.1 Seismic risk

A significant portion of small ruminant farms is located in areas with high seismic risk (seismic zones 1 or 2), affecting 45% of goat and 40% of sheep farms. However, risk exposure varies by breed (Figure 3.2.2A-B and Supplementary Table S3.2.2). Nineteen goat breeds (e.g., Dell'Aspromonte, Di Benevento, Nicastrese) and 18 sheep breeds (e.g., Trimeticcia di Segezia, Sciara-Moscia Calabrese, Laticauda) have more than 75% of their breeding range in high-risk seismic areas. Most of these breeds are concentrated in Calabria. In contrast, Sarda and Lariana goats, along with Nera di Arbus and Delle Langhe sheep, are located in zones with minimal seismic risk.

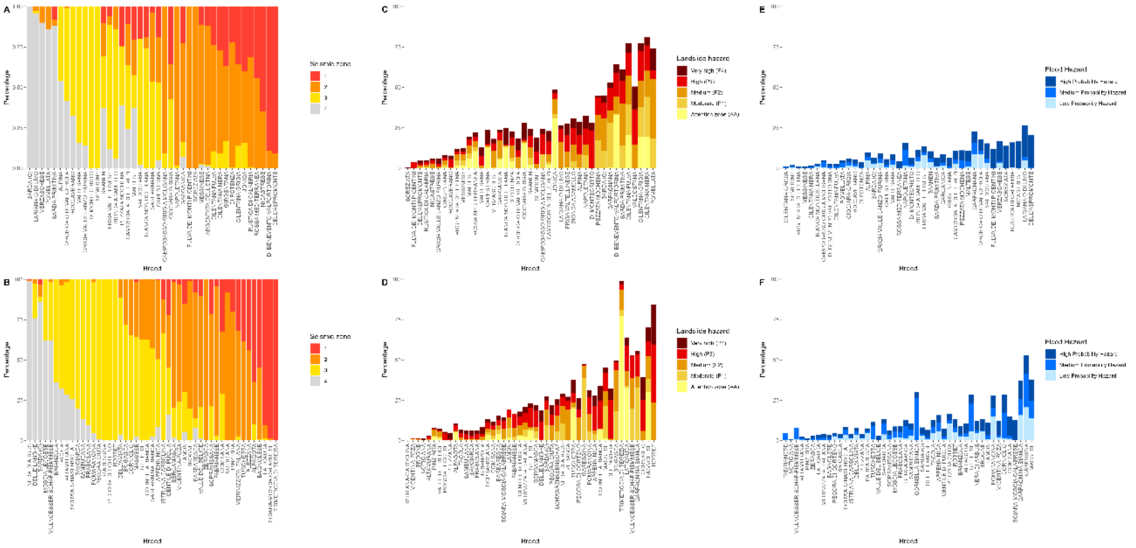

Figure 3.2.2 Percentage of small ruminant farm area located in different seismic zones (A and B for goat and sheep populations, respectively), landslide hazard areas (C and D for goat and sheep populations, respectively), and flood hazard areas (E and F for goat and sheep populations, respectively). Breeds have been ordered according to their weighted hazard score.

3.2.4.2.2 Hydrogeological hazard

The analysis of landslide hazard revealed that seven goat breeds (Figure 3.2.2C), including Pomellata, Cilentana, and Valdostana, and four sheep breeds (Figure 3.2.2D), such as Rosset, Bagnolese, Corniglio, and Garfagnina Bianca, have over one-third of their breeding range in moderate to high landslide risk zones (P2-P4 areas). Values for all zones and breeds are reported in Supplementary Table S3.2.3.

Flooding is a relatively low concern, especially for goat farms, as few farms are located in high-risk flood zones. Specifically, only nine farms had more than half of their buffer area located within high-probability hazard zones, while 41 farms—all raising sheep of different breeds—were located within high- or medium-probability hazard zones. However, when considering the entire breeding range, some breeds face higher risk levels, including Dell'Aspromonte and Lariana goats, as well as Massese, Garessina, and Garfagnina Bianca sheep, with nearly 50% of their territory at risk of flooding (Figure 3.2.2E-F and Supplementary Table S3.2.4).

3.2.4.2.3 Present and projected future climate classification

Currently, 47±32% of goat farms' area covers Csa Koppen-Geiger climate class, followed by Csb (14±17%) and Dfb (12±18%). Sheep farms are similarly distributed, with 57±44% in Csa and 13±30% in Cfa (Supplementary Table S3.2.5).

Projected climate changes indicate that 45% of sheep farms and 36% of goat farms will shift to warmer and/or drier climate zones (Figure 3.2.3). Almost all the farms currently in the coldest climate classes are expected to change their class (72% if considering Cf- and Df- classes, 97% if considering only Df- classes). For example, we observed that Dfb and Dfc farms will shift to Cfa and Dfb classes, Cfb farms will shift to Csa or Cfa, and Csb farms will transition to Csa. Moreover, many farms (17%) currently in Csa will move into Bsk or Bsh classes.

For 18 goat and 27 sheep breeds, over half of their farms are likely to undergo climate classification changes, including, for example, all Verzaschese goat and Pecora di Corteno farms, which for the most will shift from Dfb to Cfb class.

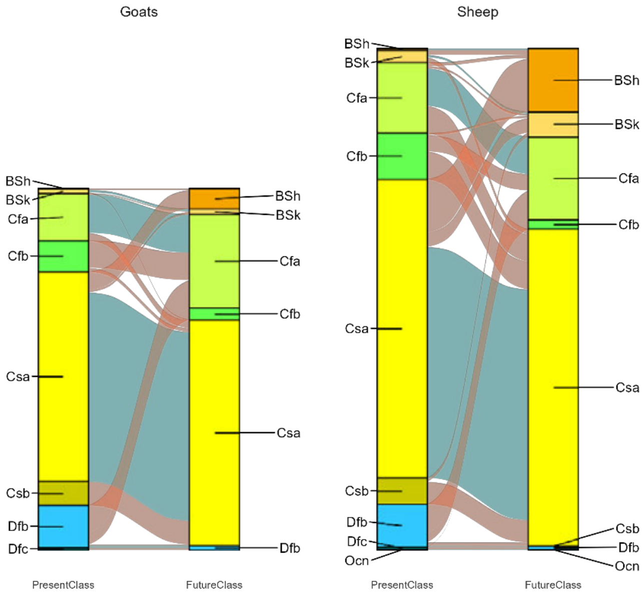

Figure 3.2.3 Alluvial plot of expected switching in Köpper-Geiger climate class for goat and sheep farms.

3.2.4.3 Landscape characterization

3.2.4.3.1 Ecosystems

Small ruminant farms in Italy are primarily situated in Agricultural areas and Forest and woodland ecosystems. However, there are clear differences between sheep and goat farms (Figure 3.2.4A and Figure 3.2.4B, respectively, and Supplementary Table S3.2.6). Agricultural areas are more prevalent among sheep farms (39±17%) compared to goat farms (30±15%), while Forest and woodland dominate in goat farming (42±16% vs. 37±18% in sheep). Both species also rely on Pastures and semi-natural/natural meadows, which comprise approximately 11% of the farmed area.

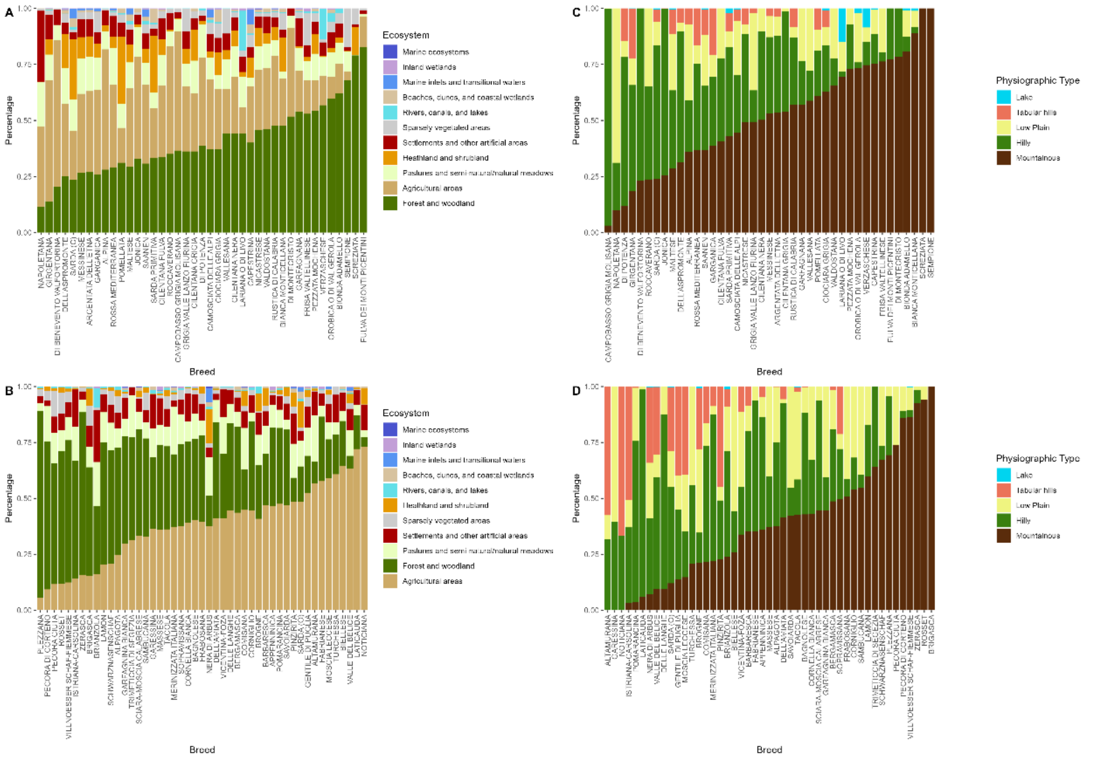

Figure 3.2.4 Percentage of small ruminant farm area covered by different ecosystems (A and B for goat and sheep populations, respectively) and main categories of physiographic type (C and D for goat and sheep populations, respectively). Breeds have been ordered according to the percentage of the most represented category of the species; categories have been ordered according to their percentage at the species level.

Regional variations further distinguish these ecosystems. Agricultural areas are most significant in sheep farms located in central (42±24%), southern Italy (47±24%), and the Islands (54±24%), whereas Forest and woodland ecosystem plays a crucial role in northern Italy and, for goats, also in central Italy. Heathland and shrubland are particularly relevant in Sardinian and Sicilian farms (11% for sheep, 18% for goats) and are notably represented in the Pomellata goat breed.

The farms rearing the two specialized goat breeds, Camosciata delle Alpi and Saanen, present two distinct patterns of ecosystem characterization: farms in southern Italy and the Islands are predominantly in Agricultural areas, while those in northern and central Italy are mainly in Forest and woodland regions. The Sarda sheep, predominantly located in Sardinia, is primarily associated with Agricultural areas across its distribution.

3.2.4.3.2 Physiographic Types

Mountainous terrain is the dominant physiographic type for small ruminant farms, especially for goats (52±24% vs. 38±27% for sheep). Hilly areas also support a substantial portion of both sheep (31±22%) and goat farms (30±22%).

Among breeds (Figure 3.2.4C-D and Supplementary Table S3.2.7), 21 out of 41 goat breeds and 13 out of 47 sheep breeds are found in regions that are at least 50% mountainous. Notable exceptions among goats include Campobasso Grigia Molisana, predominantly located in hilly regions, and Napoletana, which is mainly found in low plains. For sheep, the Altamurana, Noticiana, and Istriana-Carsolina breeding ranges also cover tabular hills.

3.2.4.3.3 Land cover and land use

Analysis of land cover data revealed that both sheep and goat farms are mostly found in areas covered by broad-leaved trees (37±13% and 42±13%, respectively) (Supplementary Figure

S3.2.5A-B and Supplementary Table S3.2.8). For sheep farms, periodic herbaceous vegetation is the second most common land cover type (23±13%), whereas goat farms are more often associated with permanent herbaceous vegetation (16±5%). A notable exception is observed in north-eastern goat farms, where needle-leaved tree cover dominates (38±27%). This is particularly evident for Frisa Valtellinese, Sempione, and Pezzata Mochena goat breeds, as well as some sheep breeds such as the Pecora di Corteno and the Fiemmese.

Forestry is the predominant land use for Italian small ruminant farms, accounting for 39±23% of goat and 29±24% of sheep farms' extension (Supplementary Figure S3.2.5C and S3.2.5D, respectively, and Supplementary Table S3.2.9). Arable land is also significant for sheep, particularly in central-southern Italy and the islands. Several goat breeds, including Pomellata, Sarda Primitiva, and Sarda, are associated with non-economic land uses, whereas some sheep breeds, such as Pinzirita and Moscia Leccese, are found on permanent crops or other agricultural land uses.

3.2.4.3.4 Naturalistic-cultural value (NCv)

On average, goat farms are located in regions with higher NCv than sheep farms. Specifically, 23±19% of goat farms are in high or very high NCv areas, compared to 11±13% for sheep farms.

Marked regional differences emerge, with the highest NCv values recorded for goat farms in the north-east, followed by those in southern Italy and the Islands. Conversely, Sardinian and Sicilian sheep farms have the lowest NCv. Among breeds, Brigasca and Pecora di Corteno sheep, along with Screziata and Cilentana goats, exhibit the highest NCv, whereas Noticiana and Valle del Belice sheep, as well as Campobasso Grigia Molisana and Alpina goats, have the lowest values (Supplementary Figure S3.2.6A-B and Supplementary Table S3.2.10).

3.2.4.3.5 Ecosystem collapse risk

Most of the farms (52±21% sheep, 40±19% goats) are on agricultural lands not considered at risk. However, about 42 and 55% of sheep and goat farm territory, respectively, extends on near threatened or more vulnerable regions.

Among the farms in most endangered locations, we found the north-eastern goat farms (about 70% in nearly threatened or vulnerable ecosystems), whereas goat farms located in Sicily and Sardinia are for 19±14% in endangered ecosystems and 32±22% in vulnerable ecosystems. Among sheep, northern farms are more often found in nearly threatened and vulnerable areas. At a breed level, particular attention should be paid to the Screziata, Pomellata, and Lariana goats as well as Plezzana sheep, which are largely present in endangered and vulnerable areas (Supplementary Figure S3.2.6C-D and Supplementary Table S3.2.11).

Among specialized breeds, over 70% of Sarda sheep farms are in agricultural lands. However, it is to be noted that the two north-western Sarda farms are located for 26±9% of their extension in endangered areas and that the numerous Sarda farms on the islands are for 11±14% on endangered ecosystems and 18±16% on vulnerable ecosystems. Both the Saanen and the Camosciata delle Alpi breeds show variable risk levels, with approximately 40-50% of farms in at least nearly threatened ecosystems, except in Sicily and Sardinia, where 79% of farms are on agricultural lands.

3.2.4.4 Provincial biodiversity indices

Biodiversity indices describing small ruminant breed diversity were calculated for each Italian province (Figure 3.2.5). The highest Shannon Diversity Index (H’) values were observed in Cuneo (1.86), Frosinone (1.76), and Ravenna (1.69), which also exhibit the highest Gini-Simpson Index (1-D = 0.81, 0.81, and 0.79, respectively) and Inverse Simpson Index (1/D = 5.27, 5.21, and 4.80, respectively). Notably, Cuneo harbors 16 breeds, especially represented by Delle Langhe, Sambucana, and Frabosana sheep, whereas both Frosinone and Ravenna host eight breeds. However, the highest species richness was recorded in Turin (17 breeds), although the population is largely dominated by Frabosana (20%) and Tacola sheep (56%), with only a limited number of breeds such as Alpina, Bionda dell'Adamello, and Maltese goats.

Conversely, Chieti, Imperia, Modena, Pesaro-Urbino, Piacenza, Rovigo, and Venice each support only one breed. Excluding these provinces, the lowest diversity values were found in L'Aquila (H' = 0.06, 1-D = 0.02, 1/D = 1.02) and Trapani (H' = 0.07, 1-D = 0.02, 1/D = 1.02). Both provinces maintain four breeds, yet their populations are overwhelmingly dominated by a single breed: Merinizzata Italiana sheep in L'Aquila and Valle del Belice sheep in Trapani.

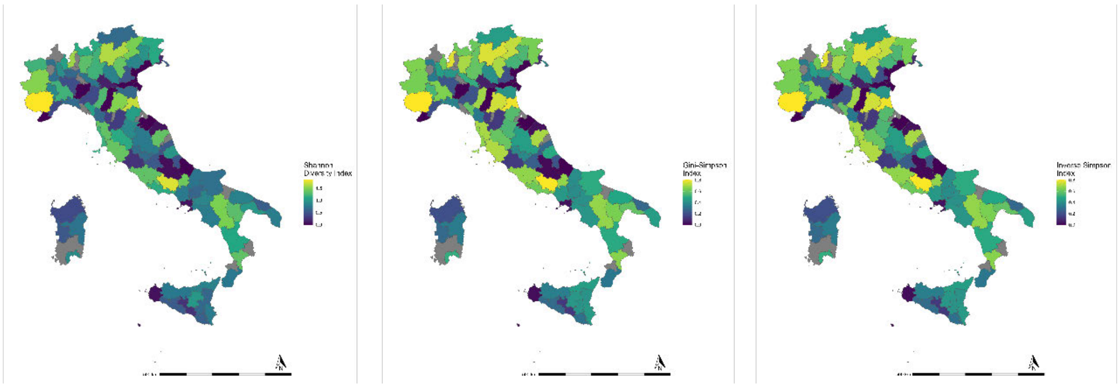

Figure 3.2.5 Shannon Diversity Index, Gini-Simpson Index, and Inverse Simpson Index indicating domestic small ruminant biodiversity at the province level in Italy. Higher values indicate greater biodiversity.

We also assessed whether these indices were correlated with province area using Spearman correlation. We found a correlation of 0.23 for both 1/D and 1–D (p = 0.0239), and 0.26 for H' (p = 0.0102). After correcting the indices using the logarithm of area, all provinces previously described maintained their relative rankings across all indices, with the exception of the adjusted 1–D index. In this case, Ravenna, Como (originally in fourth position based on unadjusted values), and Frosinone showed higher values, while Cuneo followed in fourth position.

At a broader geographic scale (Table 3.2.1), southern Italy exhibits the highest small ruminant biodiversity (H' = 2.65, 1-D = 0.91, 1/D = 10.57), with 45 populations raised in the region. In contrast, the lowest diversity indices were found in the islands (H' = 1.39, 1-D = 0.59, 1/D = 2.43), despite hosting the largest total number of registered small ruminants (over 14,500 individuals). This low biodiversity can be attributed to both the lowest breed richness (21 breeds) and the predominance of Sarda sheep, which account for 62% of the total population.

Table 3.2.1 Livestock biodiversity indices calculated at the macroregional level in Italy for sheep and goat populations.

Macroregion	Shannon Diversity Index	Gini-Simpson Index	Inverse Simpson Index	Pielou's Evenness Index	Richness
Northwestern Italy	2.56	0.89	8.71	0.57	29
Northeastern Italy	2.49	0.88	8.49	0.56	29
Central Italy	2.30	0.86	7.31	0.51	31
Southern Italy	2.65	0.91	10.57	0.59	45
Islands	1.39	0.59	2.43	0.31	21

3.2.4.5 Principal component and clustering analyses

3.2.4.5.1 By breed

A PCA describing sheep and goat breeds was performed including variables related to their effective population size (Ne), as well as the naturalistic-cultural value, environmental and ecosystemic risks, and projected climate change of their breeding range. In the PCA for goat breeds, the percentage of the breeding range located in seismic zones 1 or 2 is the primary driver of PC1 (eigenvalue = 0.87), which explains 44% of total variance, followed by the percentage of farms expected to experience a climate classification shift (-0.44). PC2 (explained variance = 23%) primarily reflects climate change (0.78) but also accounts for seismic risk (0.41) and Ne (-0.42), which has the greatest influence on PC3 (-0.85), which explains 14% of variance. Given the high correlation with geographically-explicit variables, as expected, in the PC1-PC2 plot

(Figure 3.2.6A), breeds appear clustered by geographic location: Southern Italian and Sicilian breeds, which are highly exposed to seismic risk, are positioned on the right; Central Italian and Sardinian breeds are at the bottom; and Northern breeds, mainly affected by future climate change, are on the left. Of particular interest are the breeds located in the top-right quadrant, such as the Screziata, the Fulva dei Monti Picentini, and the Campobasso Grigia Molisana, as these populations have a low Ne and inhabit areas exposed to both seismic risk and climate change. Further insights can be gained from the PC1-PC3 plot (Figure 3.2.6B): breeds with a very small effective population size are positioned at the top, with those more affected by climate change than seismic risk on the left, and vice versa on the right.

Hierarchical clustering analysis indicated three as the optimal number of clusters; however, since four groups were clearly distinguishable in the dendrogram, we analysed them in more detail (Figure 3.2.6C-D). The grouping largely reflected geographical distribution and partially overlapped with the PCA results. Group 1 mainly comprises breeds from Sardinia and central Italy, characterized by moderate-to-low environmental risk—except for the moderate-to-high landslide hazard—and located in ecosystems with high collapse risk but low NCv. Group 2, which includes most of the southern and Sicilian breeds, is associated with highly valued areas and high Ne, yet faced high seismic risk. Group 3 contains the vast majority of northern breeds—excluding Alpina and Valdostana—as well as the Di Montecristo population; this group is mainly characterized by the highest flood hazard, high exposure to climate change, and low seismic risk, with other parameters at average levels. Finally, Group 4 includes four breeds from southern Italy plus the Girgentana goat, all of which are exposed to extinction risk due to low Ne, climate change, and seismic hazard.

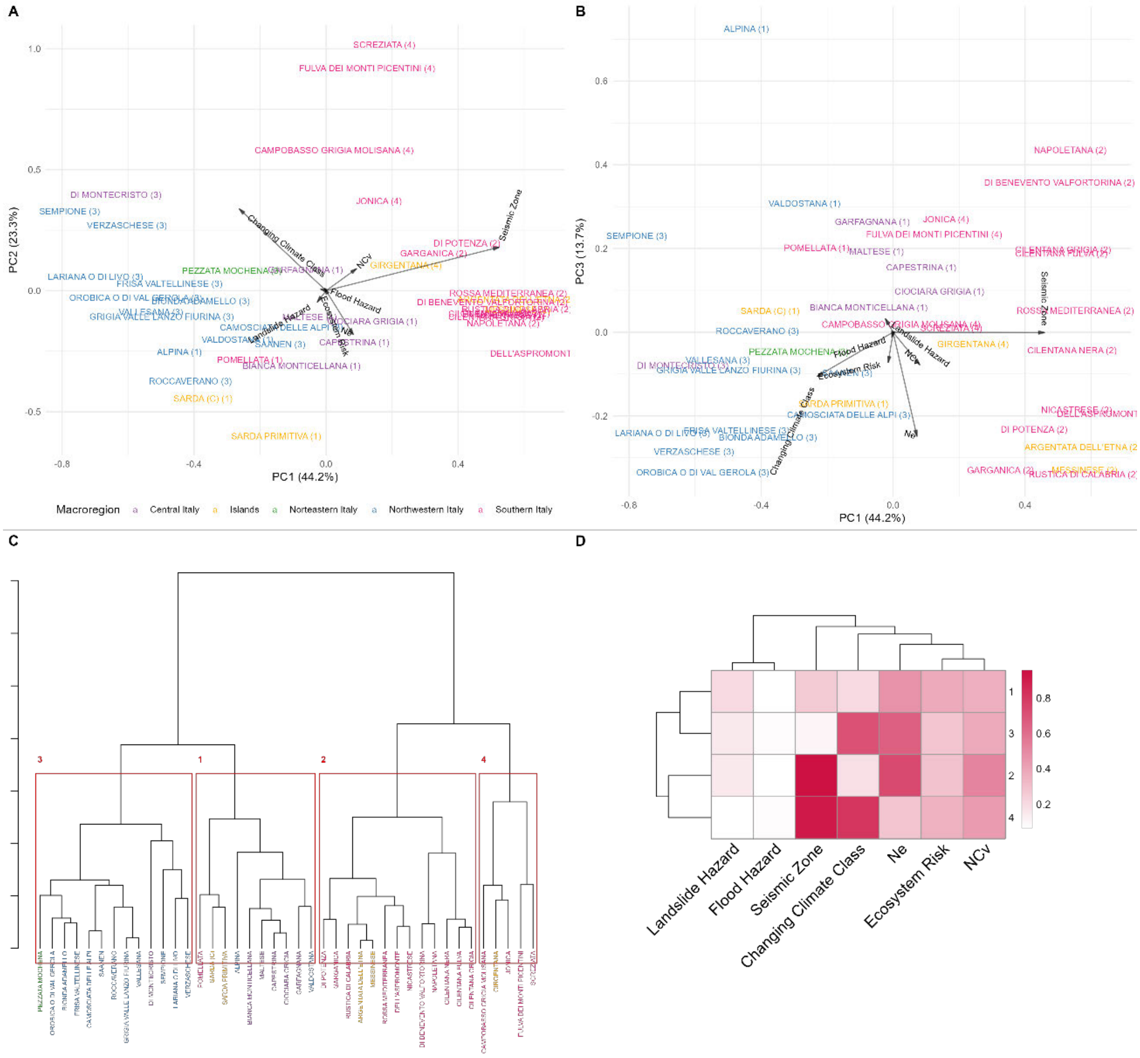

Figure 3.2.6 Principal component analysis (PCA; panels A–B) and hierarchical clustering (panels C–D) of goat breeds. Breed names are colored according to their macroregion of origin. In the PCA, the number in parentheses indicates the cluster shown in panels C–D. According to the silhouette method, the optimal number of clusters is three, corresponding to groups 4, 2, and 3+1. In the heatmap (D), the average value of all variables is reported for each analyzed cluster.

For sheep breeds, PC1 (explained variance = 47%) is mainly driven by seismic risk (eigenvalue = -0.95), with high-risk breeds positioned on the left of the plot. PC2 (explained variance = 23%) primarily identifies breeds expected to undergo future shifts in Köppen-Geiger climate classification (-0.89) while also incorporating Ne (-0.36). In the PC1-PC2 plot (Figure 3.2.7A), populations exposed to both risks cluster in the bottom-left quadrant, including, for example, the Plezzana and Noticiana. When analyzing PC1 and PC3 (Figure

3.2.7AB)—where PC3 explains 13% of variance and is mainly associated with Ne (0.91) and, to a lesser extent, expected climate change (-0.39)—breeds in the bottom-left quadrant, such as the Pinzirita, the Trimeticcia di Segezia, and the Noticiana, warrant special attention, they being small in size and exposed to significant seismic risk.

Hierarchical clustering identified two main subgroups of sheep breeds: the first comprises breeds with low Ne, located in areas with low NCv but high risk of ecosystem collapse and exposure to seismic hazards; the second includes breeds inhabiting areas with high NCv, exposed to flood and landslide hazards. However, each of these groups could be further divided, obtaining four clusters corresponding closely to the quadrants of the PC1–PC2 plot. Group 1 comprises breeds that do not show extreme values for any parameter but tend to occur in areas with moderate-to-high seismic risk and exposure to climate change. Group 2, mainly consisting of northern breeds, shows high exposure to landslide hazard and climate change, and occupies breeding ranges with high NCv but low ecosystem collapse risk. Group 3 includes a small number of breeds that stand out for their high exposure to flood hazard. Group 4 contains only three breeds (Pinzirita, Sciara-Moscia Calabrese, and Trimeticcia di Segezia) with extremely low Ne, reared in areas with moderate-to-high seismic risk (Figure 3.2.7C-D).

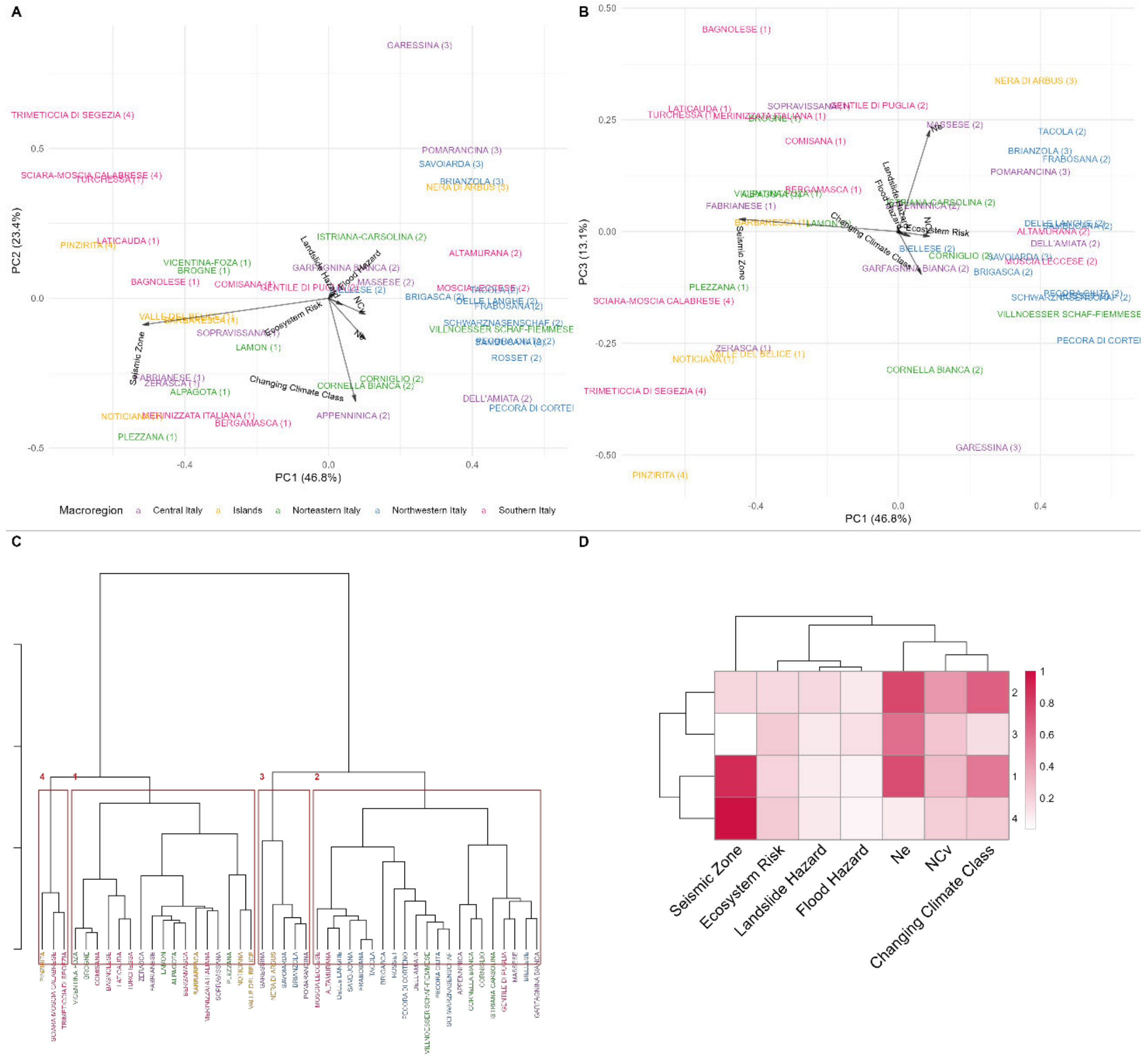

Figure 3.2.7 Principal component analysis (PCA; panels A–B) and hierarchical clustering (panels C–D) of sheep breeds. Breed names are colored according to their macroregion of origin. In the PCA, the number in parentheses indicates the cluster shown in panels C–D. According to the silhouette method, the optimal number of clusters is two, corresponding to groups 1+3 and 4+2. In the heatmap (D), the average value of all variables is reported for each analyzed cluster.

3.2.4.5.2 By province

A PCA was also conducted at the province level using key biodiversity indices, NCv, environmental risk factors, and projected climate change.

PC1, explaining 43% of the total variance, is primarily associated with seismic risk (eigenvalue = -0.97), while PC2, explaining 22% of variance, is mostly driven by the proportion of the territory expected to undergo climate change (-0.94). As a result, provinces in the bottom-left quadrant of the PC1-PC2 plot (Figure 3.2.8A), such as those in Abruzzo, are exposed to both of these risks. PC3 (explained variance = 13%) is mainly influenced by the livestock biodiversity Gini-Simpson index and NCv (eigenvalues: 0.86 and 0.43, respectively), highlighting provinces with high small ruminant biodiversity and elevated NCv values in the upper section of the PC1-PC3 plot (Figure 3.2.8B). Notably, provinces located in the top-left quadrant of this plot warrant particular attention, as they serve as important biodiversity reservoirs while also facing significant earthquake risk.

When applied to provinces, hierarchical clustering distinguished four main groups, consistent with the PC1–PC2 quadrants (Figure 3.2.8C-D). Group 1 comprises provinces with low flood and landslide hazard but high seismic risk and strong exposure to climate change, combined with moderate-to-high NCv and small ruminant biodiversity. Group 2, predominantly in northern provinces, faces the highest exposure to flood and landslide hazard as well as ecosystem collapse risk, and is also highly subjected to climate change; these provinces also show high livestock biodiversity indices and moderate-to-high NCv, warranting particular attention for conservation. Group 3 is mainly composed of central-southern provinces with average values for most variables but high seismic risk. Lastly, Group 4 includes provinces with the lowest NCv and Gini–Simpson index, and only moderate flood hazard exposure.

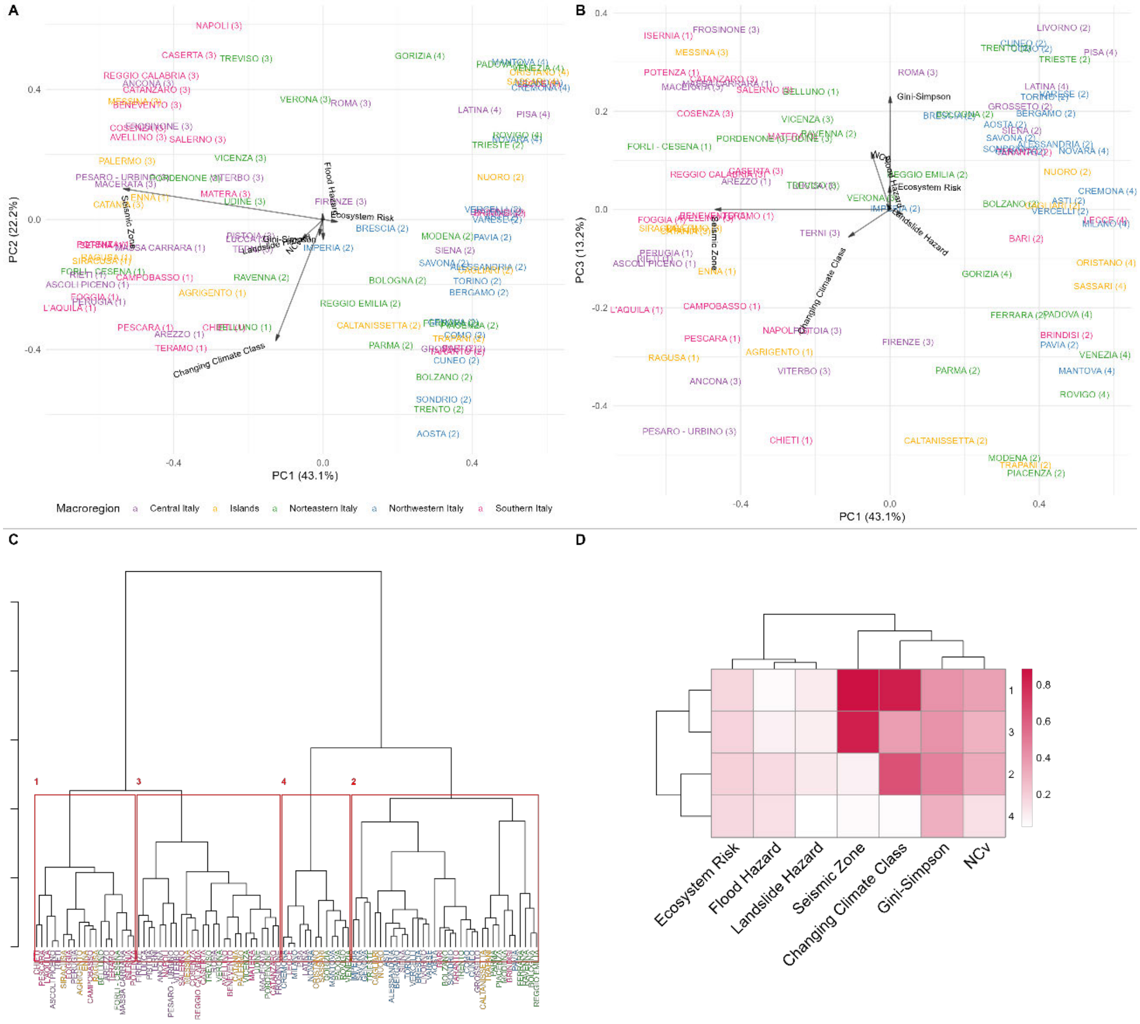

Figure 3.2.8 Principal component analysis (PCA; panels A–B) and hierarchical clustering (panels C–D) at the province level. Province names are colored according to their macroregion of origin. In the PCA, the number in parentheses indicates the cluster shown in panels C–D. In the heatmap (D), the average value of all variables is reported for each analyzed cluster.

3.2.5 Discussion

Understanding the distribution of local livestock populations and the environmental conditions in which they are raised, including the specific threats they face, is a fundamental step in developing targeted management and conservation strategies [26]. Our results show that many Italian small ruminant populations are exposed to multiple and overlapping threats: a significant number have small census and effective population sizes, placing them at high risk of extinction due to reduced genetic variability. This vulnerability is often compounded by their location in areas subject to high environmental risk or projected to undergo substantial climatic shifts. Local populations are deeply connected to the landscapes they inhabit, often marginal, ecologically fragile, or culturally distinctive areas shaped by traditional livestock farming [30,35]. Conservation planning must therefore consider not only the genetic viability of these populations, but also the preservation of the environmental characteristics of the landscapes in which they have evolved. To this end, we characterized the landscape types associated with small ruminant farming across Italy, shedding light on the complex relationships between livestock, land use, and ecosystem function. Integrating genetic, ecological, and spatial information allows for a more comprehensive understanding of which populations are most at risk and highlights the interdependence between livestock biodiversity, landscape conservation, and rural sustainability.

Taking a closer look at these aspects, one widely used indicator of a population's long-term viability is the effective population size (Ne). When Ne falls below critical thresholds, genetic drift and inbreeding accumulate, reducing adaptive potential and increasing susceptibility to diseases, reproductive issues, and environmental changes, ultimately heightening the risk of extinction [41,359]. According to the commonly adopted 50/500 rule [141], over a quarter of Italian small ruminant breeds, including over a third of goat breeds, are at short-term risk of extinction (Ne lower than 50), with six presenting Ne equal to 0, and almost half are at long-term risk (Ne between 50 and 500). These findings highlight the urgent need for systematic genetic monitoring, dedicated genomic studies, and targeted conservation efforts. Strategies such as ex-situ conservation programs (e.g., cryobanks) and sustainable breeding programs should be prioritized to maintain genetic diversity at safe levels. In this context, some national projects have been implemented to advance conservation efforts and conduct genomic characterization of most local sheep and goat populations. Continuous genomic monitoring is essential for developing effective strategies that preserve breed originality while preventing excessive genetic erosion.

Natural hazards further compound these risks, not only by threatening livestock populations but also by damaging infrastructure, thus limiting farmers' ability to sustain their breeding programs [326,334,335]. Recognizing these challenges, the FAO and UNDRR developed the Sendai Framework subindicator C2, which measures direct agricultural losses due to disasters. Analyses across 82 countries show a rising trend in such losses, with floods being the most frequent events, while droughts cause the greatest economic impact [279]. However, as noted by FAO, the current data landscape remains fragmented—datasets often have missing information, lack sectoral specificity, and differ in event classification and data collection standards [279]. For example, a published database on disaster impacts in Italy (2005–2021) includes only qualitative information on damages to production, structures, and infrastructure [360]. Other sources, such as individual event reports, are similarly inconsistent and difficult to compare [279,361,362]. Moreover, current estimates rarely account for indirect effects or cross-sectoral interactions—such as disruptions to feed supply from crop losses, degradation of grazing land, or rural depopulation—which profoundly affect livestock systems [361,363]. Critically, no reliable data are available on losses in animal genetic resources or livestock biodiversity. To address this gap, we integrated breed-specific livestock distribution data with spatial layers of environmental risk, providing a baseline to estimate breed exposure to extreme events and support mitigation planning. Our analyses highlight that a considerable number of small ruminant farms are situated in high seismic risk zones, particularly in southern Italy, exposing breeds exclusively raised in these areas—such as Dell'Aspromonte, Trimeticcia di Segezia, and Laticauda—to potential disruptions. Seismic activity in Italy has already caused significant agricultural losses. For example, after the 2009 L'Aquila earthquake, livestock farms in the region declined by 11% (from over 2,000 farms, mostly small-scale cattle and sheep operations). In 2012, earthquakes in Emilia-Romagna caused even greater economic damage, affecting around 13,000 farms, predominantly involved in cattle and swine production [364]. According to our data, landslides also pose a significant threat, with several goat and sheep breeds having a substantial portion of their distribution area at moderate to high risk. Although flood risk is generally lower, certain breeds, including

Massese and Garfagnina Bianca sheep, face notable exposure. The 2023 floods and landslides in Emilia-Romagna illustrate the potential magnitude of such events, with total damages estimated at €912 million, including €18.5 million from livestock losses alone [365]. These environmental risks necessitate targeted disaster preparedness plans, including the identification of emergency relocation areas and the development of resilient farm structures [326].

Climate change represents an additional threat to livestock populations, both indirectly, by increasing the intensity and frequency of extreme weather events, and directly, by impacting feed production, disease and parasite diffusion, and modifying the environmental conditions to which populations have adapted, often exacerbating the challenges animals must cope with [12,19,26,326]. According to our analyses, climate change projections indicate that many farms will shift to warmer and/or drier climate classes, particularly affecting breeds traditionally adapted to colder environments, such as those in the Alpine region. These shifts may necessitate adjustments in management strategies to ensure breed viability, including selective breeding for heat tolerance, improved water and feeding management practices, and the building of suitable shelters to mitigate heat stress [45,87,322,326].

Beyond environmental risks, the interaction between livestock and surrounding landscapes plays a critical role in biodiversity conservation. Small ruminant populations, especially those reared in extensive or semi-extensive systems, are closely tied to their ecosystems, both influencing and being influenced by them [33,366]. Beyond conservation, genotype by environment interactions (G×E) are also increasingly recognized as major drivers of livestock welfare, resilience, and productivity, which should be accounted for when developing effective and sustainable breeding programs. This underscores the importance of accurately characterizing the full spectrum of environmental drivers that can modulate the expression of genetic potential, influence the detection of G×E, and ultimately determine the success of selection strategies across diverse production contexts [367,368]. Our landscape characterization analysis showed that while agricultural areas dominate sheep farming, goat farms are more frequently associated with woodlands and mountainous terrains, particularly in northern and central Italy. However, what emerged is that each breed is typically reared in, and likely adapted to, specific environmental and landscape conditions, including land cover, ecosystem type, and physiographic features. Local breeds are generally considered adapted to their native environments, but such environments encompass far more than just climatic variables, which are often the sole focus of adaptive studies. In reality, they also include a complex array of biological and abiotic components—such as soil type, vegetation, and habitat structure—that are rarely considered but may play an important role in shaping adaptive traits [276–278]. The variability observed in landscape characteristics among breeds underscores their ability to persist in diverse and sometimes challenging environments, suggesting broader ecological adaptation than typically acknowledged. The distribution of these breeds across regions with different naturalistic-cultural values and varying ecosystem vulnerability further highlights the complexity of the interaction between livestock and their environment. Understanding the spatial distribution of these animals, particularly within vulnerable habitats, can provide insight into the potential impacts of environmental degradation on breeds that rely heavily on natural resources under extensive management systems [93]. In fragile ecosystems, traditional breeds may have developed genetic adaptations that allow them to thrive under harsh climatic conditions and limited resources, contributing to the sustainability of local agroecosystems. On the other hand, knowing that a breed is primarily reared in vulnerable habitats suggests that a disruption or degradation of these ecosystems could have severe consequences for its long-term viability and persistence [77,93,95]. At the same time, livestock presence can influence these landscapes either by supporting biodiversity through traditional and well-managed pastoralism or posing environmental risks when overgrazing or habitat degradation occurs. Understanding these spatial dynamics is crucial, as improper grazing and farm management can disrupt ecosystems, whereas well-managed livestock can contribute positively to biodiversity and ecosystem sustainability [31,32,61,62]. Indicators such as ecosystem collapse risk and naturalistic-cultural value are thus useful not only for understanding breed-environment relationships but also for informing landscape-level decisions. Indeed, finding a balance between animal husbandry and ecosystems can enhance the beneficial impact of livestock on marginal areas, contributing to habitat conservation and soil preservation while supporting the conservation of livestock biodiversity.

In this context, we also analyzed small ruminant biodiversity across Italian provinces, identifying where local populations are more present and/or valorized and where there might be room for improvement. Provinces such as Cuneo, Frosinone, and Ravenna exhibit the highest small ruminant genetic resources, hosting a broad range of breeds that contribute to the overall biodiversity of the national livestock sector. However, other provinces, including Chieti, Imperia, and Piacenza, support only a single breed, demonstrating a marked imbalance in genetic diversity. In some cases, provinces with multiple breeds show low diversity indices due to dominance by a single population, as observed in L'Aquila and Trapani. Such disparities underscore the need for improved registration of local breeds and the official recognition of unregistered populations to ensure their conservation and sustainable use. Many traditional breeds, indeed, remain underrepresented in official databases, limiting their access to conservation funding and breeding programs. While these biodiversity indices are commonly used in ecology to assess multispecies diversity within communities, they are equally well suited for evaluating the distribution and abundance of livestock populations, which should be recognized as a valuable component of local biodiversity. Moreover, integrating our results with biodiversity indices of other livestock and wild species could contribute to comprehensive evaluations of the Italian natural landscape. Such measures would support the development of policies that both safeguard genetic diversity and enhance the sustainable management of rural ecosystems, ensuring that livestock farming continues to be a valuable component of environmental stewardship.

3.2.6 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S3.2.1 Breed demographic data (December 2022) and effective population size (Ne).

Supplementary Table S3.2.2 Percentage of each breed's breeding range under different seismic risk zones.

Supplementary Table S3.2.3 Percentage of each breed's breeding range under different landslide hazard levels..

Supplementary Table S3.2.4 Percentage of each breed's breeding range under different flood hazard levels.

Supplementary Table S3.2.5 Description of Köppen-Geiger climate classes present in Italy and percentage of each breed's breeding range in different present and projected future classes.

Supplementary Table S3.2.6 Percentage of each breed's breeding range in different ecosystems.

Supplementary Table S3.2.7 Percentage of each breed's breeding range in different physiographic types.

Supplementary Table S3.2.8 Description of land cover classes and percentage of each breed's breeding range with different land cover.

Supplementary Table S3.2.9 Description of land use classes and percentage of each breed's breeding range with different land use.

Supplementary Table S3.2.10 Percentage of each breed's breeding range with different naturalistic-cultural values.

Supplementary Table S3.2.11 Percentage of each breed's breeding range under different ecosystem collapse risk.

Supplementary Figure S3.2.1 Italian maps of landslide hazard (A), flood hazard (B), and seismic zones (C).

Supplementary Figure S3.2.2 Present and projected future Köppen-Geiger climate classification of Italy.

Supplementary Figure S3.2.3 Italian maps of physiographic types (A), ecosystems (B), land cover (C), and land use (D).

Supplementary Figure S3.2.4 Italian maps of naturalistic-cultural values (A) and red list ecosystems (B).

Supplementary Figure S3.2.5 Percentage of small ruminant farm area covered by different land cover (A and B for goat and sheep populations, respectively) and land use (C and D for goat and sheep populations, respectively). Breeds have been ordered according to the percentage of the most represented category of the species; categories have been ordered according to their percentage at the species level.

Supplementary Figure S3.2.6 Percentage of small ruminant farm area covered by naturalistic-cultural value (A and B for goat and sheep populations, respectively) and ecosystem risk status (C and D for goat and sheep populations, respectively). Breeds have been ordered according to the weighted score of naturalistic-cultural value or ecosystem vulnerability.

4 BIODIVERSITY AND ADAPTATION OF ITALIAN SMALL RUMINANTS

As discussed in Chapter 1, livestock biodiversity represents a crucial component of agrobiodiversity, particularly in the case of Italian small ruminants, which are strongly tied to local territories, production systems, and cultural traditions. The recognition, characterisation, and conservation of local breeds are fundamental steps in preserving these resources for future generations. According to Sponenberg *et al.* (2018) [11], this process can be described as a threefold mission: discovering populations of zootechnical relevance, securing their continuity through recognition and conservation measures, and sustaining them by promoting their role in contemporary production and rural livelihoods.

In this framework, a first line of research was dedicated to the investigation of the Comune di Sicilia goat, also known as Mascaruna. Although already described in the 19th century by Chicoli (1870) [369], this population remained without official recognition and lacked systematic scientific documentation. Its persistence in the western part of Sicily, its role in local husbandry, and its ability to thrive in harsh marginal environments all pointed to the relevance of studying it in depth. Within the BIOSAVE project ("Use of phenotypic and genomic descriptors for the recovery, definition of genetic originality, and zootechnical management of Sicilian endangered local breeds"), financed by the Sicilian Rural Development Program 2014–2020, the Comune di Sicilia goat was characterised from historical, phenotypic, and genomic perspectives with the ultimate aim of supporting recognition and long-term management.

Historically, breed definitions have relied on morphological traits, production characteristics, and written accounts. Phenotypic assessments remain essential, particularly for establishing external appearance and zootechnical standards [370]. However, traditional approaches may be limited by subjectivity, environmental effects on trait expression, and the difficulty of distinguishing populations closely related and/or displaying similar phenotypes [121,370]. The advent of molecular tools, and in particular SNP genotyping, has made it possible to complement these classical descriptors with objective measures of genetic distinctiveness, internal variability, and demographic history [110,113]. In the case of the Comune di Sicilia goat, the integration of traditional and genomic approaches allowed a comprehensive assessment of its originality in comparison with other Italian breeds (Chapter 4.1).

To further contextualise these findings, the Comune di Sicilia was placed in a broader comparative framework including Mediterranean and African goat populations (Chapter 4.2). Sicily has historically been a crossroads of peoples and agricultural practices, and livestock reflects these exchanges. By situating the Comune di Sicilia within this wider genetic landscape, it was possible to reconstruct aspects of its evolutionary trajectory, to identify ancestral connections, and to investigate signatures of selection associated with adaptation. This step marked a progression from a local focus on breed definition to an exploration of broader historical and adaptive processes, positioning the Comune di Sicilia as part of the Mediterranean's long-standing mosaic of livestock diversity.

The implications of these studies are considerable. The integration of phenotypic and genomic evidence, combined with historical documentation, directly supported the approval for recognition of the Comune di Sicilia goat by Asso.Na.Pa. in 2025. Recognition not only safeguards the population from genetic erosion and uncontrolled crossbreeding but also confers visibility and legitimacy to the work of local breeders who have long ensured its continuity. More broadly, it affirms the value of this goat as both a cultural and genetic resource, well adapted to the Sicilian environment and potentially important under future climatic scenarios. In this sense, the recognition of the Comune di Sicilia goat represents more than an administrative milestone: it exemplifies how scientific research can translate into concrete conservation outcomes, reinforcing the link between biodiversity studies and sustainable rural development.

In parallel, recent national initiatives such as CHEESR (2017–2021) and SHEEP&GOAT (2021–2025), supported by the National Rural Development Plan (PSRN – sub measure 10.2) and coordinated by Asso.Na.Pa. in collaboration with several research and academic institutions, have provided the opportunity to conduct systematic, nationwide investigations of small ruminant biodiversity. These projects combined extensive phenotypic and genomic data, enabling for the first time systematic analyses of sheep and goat breed diversity and structure across the country.

Genomics provides unique advantages in this context. Beyond what can be captured through traditional descriptors, genomic data allow objective quantification of genetic diversity, inbreeding, and relatedness, the reconstruction of demographic histories, and the detection of genomic regions under selection [110,113,120]. This makes it possible to detect both long-term evolutionary processes and more recent changes, providing a robust basis for conservation planning and sustainable management.

From this perspective, livestock biodiversity should not be regarded as a static picture, but as a dynamic process. Breeds continuously evolve under the combined influence of demographic shifts, breeders’ practices, and environmental pressures [371–373]. Having the possibility to compare data across different timepoints offers a unique opportunity to capture these dynamics, to assess the pace and direction of genetic change, and to evaluate how conservation measures are working in practice. Such temporal analyses are particularly relevant in the current context of rapid transformations in agricultural systems and rural landscapes.

Equally important is the environmental dimension. Italy, with its remarkable ecological heterogeneity—from alpine ecosystems to Mediterranean drylands—offers an exceptional natural laboratory for investigating how livestock genomes respond to local conditions. Climate change adds urgency to this perspective: shifts in temperature, precipitation, and the frequency of extreme events are already affecting the ranges, productivity, and management of many breeds. Genomic approaches make it possible to detect candidate regions associated with environmental gradients [179], while more recent tools such as genomic offset allow projections of how well populations may be able to cope with future conditions [199,200]. These approaches provide a quantitative way to identify populations that may be especially vulnerable to ongoing changes and therefore require targeted conservation strategies.

The following chapters (4.3 and 4.4) are situated within this context. They address Italian sheep and goat biodiversity from a genomic perspective, combining the analysis of population structure, demographic trajectories, and extinction risk with investigations into the relationship between genetic variation and environmental change. By integrating temporal comparisons and landscape genomics, these works highlight not only how breeds have evolved in the recent past, but also how they may respond to future challenges under shifting climatic and socio-economic conditions.

Taken together, these perspectives underscore the value of a multidisciplinary approach to livestock biodiversity. Genomic tools provide powerful insights into the hidden structure and adaptive potential of populations, but they gain their full meaning only when complemented with phenotypic characterization, demographic data, and historical documentation. By connecting past, present, and future, and by linking genetic signals to both environmental factors and human practices, it becomes possible to build a truly comprehensive understanding of breed evolution and to design conservation strategies that are both scientifically rigorous and practically effective.

4.1 PHENOTYPIC AND GENOMIC CHARACTERISATION OF THE COMUNE DI SICILIA GOAT: TOWARDS THE CONSERVATION OF AN ENDANGERED LOCAL BREED

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4.1.1 Abstract

The Comune di Sicilia, a local goat breed from Sicily (Italy), is currently undergoing recognition as a distinct breed. This study aims to characterize the population both phenotypically and genomically to advance its recognition process. A total of 78 subjects from two locations were enrolled, and their phenotypic data, including qualitative traits and morphometric measurements of adult animals, were recorded and statistically analyzed. The goats were genotyped using the Illumina 50 k Goat SNPchip, comparing them with 473 goats from 15 Italian breeds. Population structure, phylogenetic relationships, admixture, and genomic inbreeding were analyzed. Additionally, subjects with different morphological traits were compared using F_{ST} and runs of homozygosity, leading to the identification of potential candidate genes associated with anotia and wattle presence in goats. The Comune di Sicilia breed exhibited distinctive genomic and phenotypic features, setting it apart from other breeds in the same region. However, moderate variability, possibly influenced by selection practices, was also observed. To ensure the breed's preservation and prevent excessive inbreeding, a comprehensive approach considering both morphology and genomic background is recommended. This study contributes valuable insights into the genetic peculiarities of the Comune di Sicilia goat, supporting its recognition as a unique and valuable breed.

4.1.2 Introduction

In recent years, the environment was influenced by globalization, urbanization, population growth, global warming, and climate change. Consequently, local breeds were affected as well, and biodiversity is under threat, making it evident that ensuring the sustainability of livestock breeding necessitates the surveillance and preservation of native breeds that are well adapted to the local environment [74,256,374], hence the emergence of the need to preserve, maintain, sustainably utilize, recover, and enhance the components of biological diversity [375]. In this context, local livestock populations play an increasingly important role, as their breeding often sustains the economies of marginal areas that would otherwise be abandoned [292], and represents an interesting alternative for the valorization of typical quality products linked to their place of origin, thus generating an ecologically sustainable livestock economy [376,377]. In particular, in Southern Italy, goat breeding is traditionally practiced with native breeds, well adapted to the environment and able to exploit and enhance it [378]. In Sicily there are about 92,714 [379] goats, including important native breeds (Argentata dell'Etna (3,260, ARG), Girgentana (2,603, GIR), Messinese (8,814, MES), Maltese (988, MAL), and Derivata di Siria (840, DDS)) that are well adapted to marginal areas and are able to produce in the harsh conditions of this region [57,380]. However, in addition to the officially recognized breeds in possession of a registry, there is a small population (around 500 heads) called "Comune di Sicilia" (CCS), bred in the western area of Sicily (Figure 4.1.1). It was first reported by Chicoli (1870) [369], who described both its phenotype and production characteristics in his book "*Riproduzione, Allevamento e Miglioramento degli Animali Domestici in Sicilia*" ("Reproduction, Breeding and Improvement of Domestic Animals in Sicily"). Chicoli described these goats as long- and wire-haired, with a variety of possible coat colors (white, black, brown, and honey), a large head, a rather large size, and very developed udders in females, with a daily production of about three litres of milk.

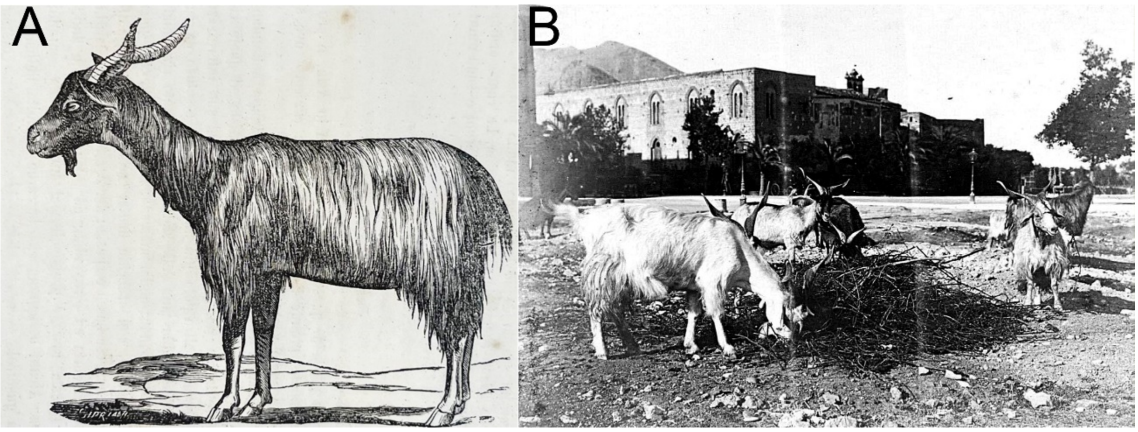

Figure 4.1.1 (A) Figure taken from *"Riproduzione, Allevamento e Miglioramento degli Animali Domestici in Sicilia"* by Chicoli (1870), representing a specimen of the so-called *Capra Comune di Sicilia*. (B) A historical photo of some subjects of this breed (Piazza Francesco Crispi, Palermo, 1927): it was common to see goats roaming the streets of the Sicilian cities, where a local traditional practice involved the "*capraru*" (goat breeder) delivering fresh milk at dawn by milking the goats right at people's doorsteps.

Historically, studies aimed at describing livestock populations primarily relied on phenotypic and historical data. However, recent advancements in molecular tools now offer the opportunity to augment these conventional zootechnical evaluations, providing a more comprehensive and accurate characterization of animal breeds and facilitating an improved recognition process. In this context, the BIOSAVE project, “Use of phenotypic and genomic descriptors for the recovery, definition of genetic originality, and zootechnical management of Sicilian endangered local breeds”, was approved in 2021 and financed by the PSR Sicilia 2014–2020—Sub-measure 10.2b “Support for the conservation of genetic resources in agriculture and forestry”. The overall aim of this project is to ascertain the official recognition and subsequent developmental significance of Sicilian livestock breeds in relation to land, landscape, and sustainability. It also seeks to explore the role of public policies and the multifunctional approach involving research institutions in supporting these local breeds. Hence, based on the Chicoli’s first description of the “Comune di Sicilia” goat [12], and with the support of the BIOSAVE project, the aim of the present study was to define the phenotypic and genomic characteristics of the “Comune di Sicilia” goat, given its presence and historical role in the territory, as an indispensable element for a possible future opening of the appropriate genealogical register.

4.1.3 Materials and methods

This study was performed according to the ethical principles that have their origins in the Italian Veterinarians’ Ethical Code [381] and the Italian and European regulations on animal welfare (Directive 2010/63/EU 2010).

4.1.3.1 Description of the study area and animal management

The study was conducted in western Sicily, in two farms located in Petralia Sottana and Bolognetta, two administrative areas in the province of Palermo representing different agro-ecological areas of Sicily. Petralia Sottana is part of the Madonie Park, with a warm and temperate climate, and is located at an altitude of 1039 m above sea level, specifically between latitude 37°48′0″ N and longitude 14°5′0″ E. This mountainous area is characterized by large extents for grazing with extensive arable crops. The territory of Bolognetta is mainly hilly and characterized by a warm and temperate climate; it is located between latitude 37°57′39″ N, longitude 13°27′78″ E, and altitude 348 m above sea level. In both farms, goats were reared under a semi-extensive farming system, where feeding management is based on grazing spontaneous fodder essences during the day, and during the night, shelter is provided in the stable called “mannara” (from Arabic ‘manzrah’: closed area), which in the local dialect refers to a traditional enclosure where sheep and goats are usually penned at night [382].

4.1.3.2 Phenotypic data collection and statistical analysis

A total of 78 goats (comprising 9 bucks and 69 does) exhibiting the most typical phenotypic characteristics of the Comune di Sicilia (CCS) goat population were carefully selected from the two herds. Specifically, 25 does and 6 bucks were selected from Petralia Sottana farm, and 44 does and 3 bucks from Bolognetta farm.

Morphometric linear measurements were taken only on the 41 adult subjects (4 bucks and 37 does with an age comprised between 18 months and 5 years); specifically, their age was estimated through the evaluation of the dentition [383,384]. Data were scored on eleven morphometric traits following the descriptor list of FAO (2012) and Abd-Allah *et al.* (2019) [370,385] for the phenotypic characterizations of goats.

Accordingly, the following traits were recorded using a graduated stick and a measuring tape and expressed in centimeters (cm):

- Wither height (WH) was measured as the vertical distance from the top of the withers to the ground.
- Croup height (CrH) was measured as the vertical distance from the top of the croup to the ground.
- Chest height (ChH) was measured as the vertical distance from sternum to withers.
- Chest length (ChL) was measured as the distance between the top behind the scapular and the costal arch bounded by the last rib.
- Trunk length (TL) measured as the distance from the point at the top behind the scapular to the base of the tail.
- Croup length (CrL) was measured as the distance between the iliac tuberosity and the ischial tuberosity.
- Chest width (CW) it was measured as the distance between the right retro-scapular area and the left retro-scapular area.
- Hip breadth (HB) was taken as the distance between the two iliac tuberosities.
- Coxofemoral width (CxW) was taken as the distance between the two trochanteric tuberosities.
- Hearth girth (HG) was measured as a circumferential measure taken around the chest just behind the front legs and withers.
- Shin circumference (SC) was measured from the left mid-metacarpus.

Moreover, live body weight (BW) was calculated according to Natsir *et al.* (2010) [386] with the following equation: $BW = 0.0127 \times HG^2 - 0.69 \times HG + 14.7$. According to the latter authors, heart girth measurement was determined to be the best predictor of live BW with a regression coefficient of 0.92. Measurements were recorded in the morning before the animals were released for grazing to avoid the effect of feeding and watering on the goats' size and conformation. All measurements were performed by the same person in order to avoid inter-individual variations as reported by Sheriff *et al.* (2021) [387] and Arandas *et al.* (2017) [383]. All measurements were performed only on adult, healthy, and non-pregnant goats.

Regarding qualitative data, coat color pattern and type, presence or absence of horn, ears, wattles, and beard were also registered for all 78 subjects according to Sponenberg *et al.* (1998) and Henkel *et al.* (2019) [388,389].

Phenothypic data were analyzed with SAS software (release 9.4, SAS Institute Inc., Cary, NC, USA). The univariate procedure of SAS was used to determine the following descriptive statistics for the distribution of the morphometric values ($n = 37$ female goats) obtained: mean, first quartile, median, third quartile, standard deviation, 95% confidence interval of the mean, skewness, kurtosis, and Shapiro–Wilk test to assess if data were normally distributed.

All morphometric data were analyzed with ANOVA mixed models using the GLIMMIX procedure of SAS. The statistical models included the fixed effects of the farm (1: Bolognetta farm; 2: Petralia Sottana farm), horn (yes or no), and wattles (yes or no). Individual goats were included as random effect. In addition, for the ANOVA analysis, trunk length (TL), chest width (CW), and shin circumference (SC) were log-transformed and presented as back-transformed data due to their not-normal distribution. Pair-wise comparisons were performed using the least significant difference test. Statistical significance was declared at $p \leq 0.05$.

4.1.3.3 Genomic analyses

The genomic analyses were performed on 78 CCS goats, consisting of 9 bucks and 69 does. This group comprised all the animals that underwent morphological evaluation as previously described, along with additional subjects selected to ensure a representative sample of the breed. Moreover, special attention was given to limit direct relatedness among the individuals in the cohort. Blood samples were collected and about 3 mL of each sample was placed in a sterile tube containing ethylenediamine tetra-acetic acid (EDTA) and stored in the refrigerator or freezer until analysis. The DNA extraction and genotyping were outsourced and performed using the Goat 60 K SNP BeadChips on an iScan System (Illumina®, USA). Experimental protocol was authorized by the Regional Department of Agriculture, Rural Development and Mediterranean Fisheries—Sicilian Region (Dipartimento Regionale Agricoltura Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea Regione Siciliana) Italy, n. G49J21006760009, prot. 0012062, 14 July 2021.

Table 4.1.1 Datasets used for genomic analyses.

Breed code	Breed name	Region of origin	Initial dataset	Quality check and exclusion of relatives [a]	Breed size reduction [b]
ARG	Argentata dell'Etna	Sicily	48	46	35
ASP	Aspromontana	Calabria	24	24	24
BIA	Bianca Monticellana	Lazio	24	24	24
CAM	Camosciata delle Alpi	Alpine region	30	30	30
CCS	Capra Comune di Sicilia	Sicily	78	72	35
DDS	Derivata di Siria	Sicily	32	25	25
GAR	Garganica	Apulia	40	37	35
GCI	Grigia Ciociara	Campania/Abruzzo	43	40	35
GIR	Girgentana	Sicily	59	56	35
JON	Jonica	Apulia	16	15	15
MAL	Maltese	Malta / Sicily	16	16	16
MES	Messinese	Sicily	24	23	23
MON	Capra di Montefalcone	Molise	24	23	23
NIC	Nicastrese	Calabria	24	24	24
SAR	Sarda	Sardinia	33	32	32

[a] This dataset was used for ROH and selection signature analyses. [b] This dataset was used for population structure analyses.

Genomic data of CCS goats were compared to 437 goats belonging to potentially related breeds coming from data previously published by Cortellari *et al.* (2021) [74] (Table 4.1.1).

PLINK software (version 1.9) [390] was used to screen the genotypes and retain only individuals with a minimum call rate of 95% and SNPs located on autosomes with a minimum call rate of 95% and a minor allele frequency (MAF) of 0.1%. In addition to the previous steps, the genomic data were utilized to examine the relatedness among all subjects, and any directly related animals were excluded from the analysis. Using BITE software (version 1.1) [391], each goat population was reduced in number to a maximum of 35 subjects.

PLINK 1.9 was used to perform a multidimensional scaling analysis (MDS) to visualize the genetic distances among the goat populations included in the study. In-house scripts were used for computing bootstrapped Reynolds distances among breeds [151] and identity-by-state (IBS) distances among single individuals and creating dendrograms based on them. The genetic admixture of all individuals, representing their genetic ancestry, was analyzed using ADMIXTURE 1.3 [161], with the number of genetic clusters (K) ranging from 2 to 16. The best-fitting K was determined by the lowest cross-validation value (c-v). Individual ancestry fractions (Q-values) were also examined.

To elucidate the genetic diversity of the analyzed breeds, expected heterozygosity (He), observed heterozygosity (Ho), and Wright's fixation index (F_{IS}) were calculated using PLINK 1.9. A sliding window approach was used to estimate runs of homozygosity (ROH) in all subjects using the following parameters: ROHquartile = 0.99, minNsnp = 10, maxNsnp = 30, windef = 20, interval = 5, hetallowed = 0, minKblength = 1000, density = 500, maxInternalGap = 500, and maxmiss = 2. The ROH-based inbreeding coefficient (F_{ROH}) was calculated by dividing the total length of ROH in a subject by the total length of the autosomes covered by the SNPs, as described by McQuillan *et al.*, (2008) [133,392]. This parameter was calculated for the total ROH and for five different classes of ROH length to estimate the timing of past breeding events: 1–2 Mb, 2–4 Mb, 4–8 Mb, from 8 to 16 Mb, and > 16 Mb.

The genomic effective population size (Ne) trend, ranging from 13 to 983 generations ago, was estimated using the linkage disequilibrium (LD) method for all the populations using SneP software (version 1.1) [140].

In addition, we investigated the selection signatures associated with specific morphological features found in CCS goats, such as microtia and the presence or absence of wattles and horns using F_{ST} and ROH analyses. Specifically, the following groups were compared: (a) 39 horned vs. 39 polled goats (25 and 21 from Bolognetta and 14 and 17 from Petralia Sottana farm, respectively); (b) 74 goats with ears (46 from Bolognetta and 27 from Petralia Sottana farm) vs. 4 presenting microtia (all from Petralia Sottana farm); and (c) 44 goats with wattles vs. 34 without wattles (18 and 29 from Bolognetta and 16 and 15 from Petralia Sottana farm, respectively). In particular, the SNPs falling in the top 1% F_{ST} values and delta H-score (difference in the proportion of animals in each group presenting a given ROH) were retained and mapped on the ARS1.2. Their associated genes were further investigated.

4.1.4 Results and discussion

4.1.4.1 Phenotypic data

Qualitative phenotypic data were assessed in 78 goats (Table 4.1.2). Most of the population showed medium hair length (79%), while few animals had short hair (21%). The most frequent observed coat color pattern in the study area was badger face with different grades of pheomelanic dilution. Highly diluted pheomelanin (ranging from white to very light tan) was the most represented (about 49%, Figure 4.1.2A), whereas a moderate dilution (Figure 4.1.2B) was found in 29% of the subjects and undiluted pheomelanin (dark red, Figure 4.1.2C) in 22%. Moreover, the black face markings were particularly extended in some animals and covered the whole forehead, forming a mask (Figure 4.1.2 C). The predominance of animals showing a diluted color may be attributed to the breed characteristics itself or the owner's preference for light coat color as it is important for the adaptation of environment; in fact, a light coat reflects 60% of direct solar radiation in comparison to a dark color [82] with a less absorption of heat.

Table 4.1.2 Description of morphological traits of the studied cohort of Comune di Sicilia goats.

		Males (*n* = 9)	Females (*n* = 69)	Total
Horns	Present	22%	42%	40%
	Absent	78%	58%	60%
Wattles	Present	33%	45%	44%
	Absent	67%	55%	56%
Coat color	White badger face	45%	49%	49%
	Tan badger face	45%	28%	29%
	Dark red badger face	10%	23%	22%
Coat length	Short	0%	23%	21%
	Medium	100%	77%	79%
Ears	Present	78%	97%	94%
	Absent (anotia)	22%	3%	6%
Ear length	Short	29%	19%	20%
	Medium	0%	9%	8%
	Long	71%	72%	72%
Ear carriage	Erect	43%	70%	68%
	Semi-erect	43%	21%	23%
	Atonic	14%	9%	9%

Figure 4.1.2 Representative coat colors observed in Comune di Sicilia breed. All the enrolled goats presented a badger face pattern, with different grades of pheomelanin dilution, from white (A) to tan (B) to dark red (C). In some goats, the black facial markings extended to form a mask (C).

Wattles were present in 44% of the analyzed goats. Additionally, 40% of the individuals, both male and female, were horned, in most cases presenting spiral or lyre horns. It is noteworthy that the presence of horns in goats is advantageous for self-defense and thermoregulation [393] and seems to be associated with a better reproductive performance [394]. In fact, being hornless is associated with intersexuality and to a physiological defect known as polled intersex syndrome (PIS), which directly affects the reproduction and other phenotypic traits. However, it is interesting to note that although most of the evaluated heads (especially males) were polled, breeders reported no reproductive problems, and at a visual examination, external genitalia were normally formed. The absence of the auricular pinna (anotia) was observed in a small number of goats.

Table 4.1.3 summarizes the morphological traits of adult female CCS goats, whereas the results for male subjects are reported in Supplementary Table S4.1.1. The measurements of body weight (BW), heart girth (HG), croup height (CrH), chest height (ChH), wither height (WH), chest length (ChL), croup length (CrL), hip breadth (HB), and coxo-femoral width (CxW) were normally distributed according to Shapiro–Wilk test ($p > 0.05$). However, the trunk length (TL), chest width (CW), and shin circumference (SC) showed a not normal distribution ($p < 0.05$): TL presented a left-skewed asymmetrical and slightly leptokurtic distribution; CW was right-skewed; and SC was leptokurtic.

Table 4.1.3 Descriptive statistics of morphological traits in adult females of Capra Comune di Sicilia breed (n = 37).

		Quartiles							p-Value [1]
Traits	Mean	Q1	Median	Q3	SD	95% CI	Skewness	Kurtosis	Shapiro–Wilk
Body weight (BW)	44.7	39.4	44.9	49.3	8.20	42.0–47.47	−0.03	1.18	0.62
Hearth girth (HG)	82.5	79.0	83.0	86.0	5.98	80.6–84.5	−0.59	2.18	0.19
Croup height (CrH)	68.6	67.0	68.5	70.0	3.48	67.6–69.8	0.04	−0.30	0.40
Chest height (ChH)	35.2	34.0	35.0	36.0	2.02	67.5–69.8	0.35	−0.46	0.13
Wither height (WH)	70.1	69.3	69.5	72.5	3.35	69.0–71.2	0.01	−0.82	0.32
Chest length (ChL)	39.9	38.0	40.2	42.0	3.44	38.7–41.0	−0.16	−0.09	0.85
Trunk length (TL)	73.7	71.0	75.0	77.0	4.87	72.1–75.33	−0.84	0.73	<0.05
Croup length (CrL)	26.2	25.5	26.1	27.0	1.42	25.7–26.7	0.17	−0.17	0.31
Chest width (CW)	20.6	19.5	20.5	21.5	1.91	19.9–21.2	0.82	0.23	<0.05
Hip breadth (HB)	19.0	17.5	19.0	20.5	1.68	18.5–19.5	−0.20	−0.91	0.14
Coxo-femoral width (CxW)	17.6	17.2	19.5	21.0	2.87	18.2–20.1	−0.30	−0.19	0.47
Shin circumference (SC)	8.2	8.0	8.0	9.0	0.96	7.9–8.5	−0.33	2.49	<0.05

[1] Shapiro–Wilk test to assess if data are normally distributed. The test compares the scores in the sample to a normally distributed set of scores with the same mean and standard deviation; $p < 0.05$ indicates that variable is not normally distributed.

Comparison of quantitative and qualitative traits between the two farms are summarized in Supplementary Table S4.1.2. There were statistical differences between farms in CrH, WH, CW, CxW (farm, $p < 0.05$), and a tendency for SC (farm, $p = 0.07$), whereas there were not observed statistical differences for other morphological traits. No significant effects on the morphological measurements were instead found between animals with and without horns and with and without wattles.

When comparing the CCS goats' average body traits with those reported in the breed standards of the other native Sicilian goats (Girgentana, GIR; Messinese, MES; and Argentata dell'Etna, ARG) [48], some differences were observed among the breeds. In fact, the GIR goats are the heaviest (on average, GIR = 46 kg, MES = 38 kg, ARG = 38 kg, and CSS = 44.7 kg), with the greatest HG (on average, GIR = 94 cm, MES = 80 cm, ARG = 80 cm, and CSS = 82.5 cm), WH (on average, GIR = 80 cm, MES = 67 cm, ARG = 67 cm, and CSS = 70.1 cm), TL (on average, GIR = 95 cm, MES = 64 cm, ARG = 66 cm, and CSS = 73.7 cm), and CW (on average, GIR = 28 cm, MES = 17 cm, ARG = 18 cm, and CSS = 20.6 cm) compared to the other Sicilian breeds; whereas, regarding the ChH, there was not observed much difference between breeds taken into account for the comparison (on average, GIR = 35 cm, MES = 31 cm, ARG = 32 cm, and CSS = 35.3 cm).

These results, in line with historical references of the breed [369], highlight unique phenotypic traits compared to other Sicilian goat breeds.

The observed morphologic variability might be attributed to lack of a systematic selection program in the breed that would help for setting up specific selection criteria. Moreover, the differences between the two sampled farms might depend on the management system, genetic by environment interaction, the breed characteristic itself, or the presence of strains within the breed. The existed variation is an opportunity for sustainable improvement, conservation, and utilization work that would be designed for this breed.

4.1.4.2 Genomic population structure and inbreeding

Following quality control and the removal of direct relatives, a total of 487 animals and 48,039 SNPs were retained and used for ROH and selection signature investigation. To ensure homogeneity across the 15 Italian breeds, a maximum of 35 animals were considered for each breed, resulting in a final dataset of 411 subjects for population structure analyses (Table 4.1.1).

The results of multidimensional scaling (MDS) analyses are presented in Figure 4.1.3. Regarding the CCS, the majority of subjects clustered together, with only a small subset being less clearly distinguishable from other populations. Overall, the MDS plots showed distinct clustering of almost all breeds, with CAM being the most isolated one. GIR and ASP were also separated from other southern Italian populations, although ASP showed more variability and some overlap with other breeds. Additionally, the second component of the MDS plot separated MAL goats from the other breeds. Interestingly, the third component clearly separated the breeds reared in Southern Italy and the isles from those living in central Italy, which is consistent with the findings of Cortellari *et al.* (2021) [74].

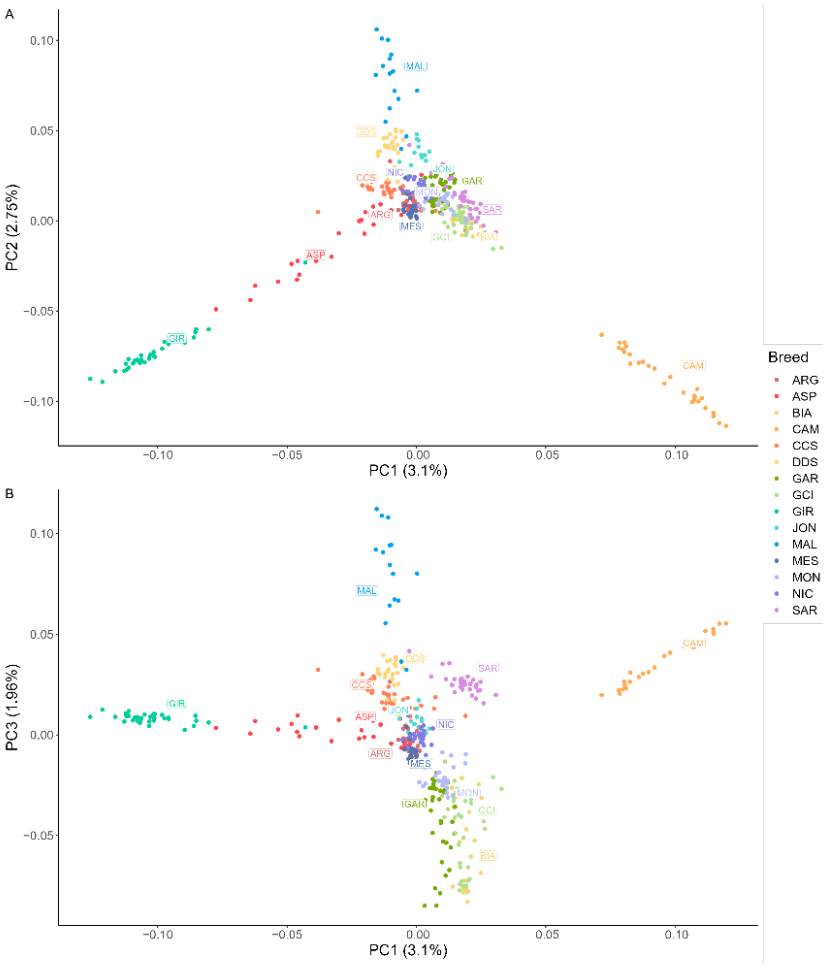

Figure 4.1.3 Representation of the first three principal components of the multidimensional scaling analysis.

The results of the phylogenetic tree analysis based on Reynold distances (Supplementary Figure S4.1.1) are consistent with the MDS analysis, with the central Italian breeds (BIA, GCI, GAR, and MON) clustering together. The CCS is closely related to ASP and GIR, which are also reared in close proximity. In addition, the tree is based on identity-by-state (IBS), and including all individual subjects (Figure 4.1.4) clearly showed that all CCS animals clustered together and were easily distinguishable from other populations. Specifically, the CCS breed was closely related to other Sicilian breeds. Notably, the only non-distinguishable pairs were MES and ARG, and BIA and GCI.

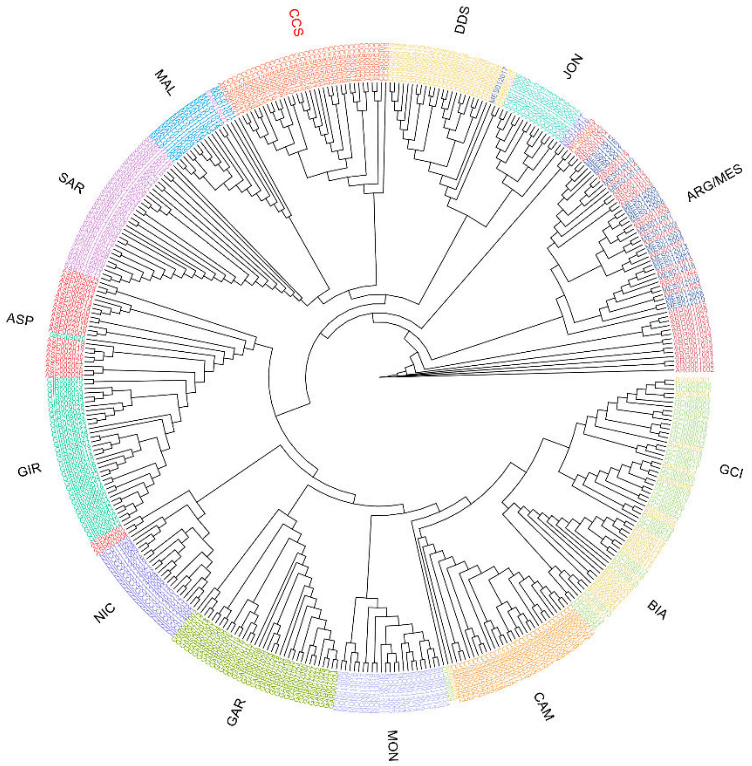

Figure 4.1.4 Dendrogram representing the phylogenic relationship according to identity-by-state distances.

The admixture analysis revealed that K = 11 was the best-fitting number of clusters, as determined by the model's c-v value. Supplementary Figure S4.1.2 reports the c-v values and the admixture plots for all the analyzed K. However, a unique genomic signature for CCS was already apparent at K = 7 (Figure 4.1.5A). At K = 11 (Figure 4.1.5B), CCS exhibited a distinct genetic background, with a Q-score for their own cluster of 59 ± 28%. Specifically, 15 (43%) of the CCS goats had a Q-score over 67%, 13 (37%) between 33 and 67%, and only 7 (20%) under 33%. Interestingly, a difference was observed between the two sampled farms, with the 10 animals from the first farm presenting a mean Q-score of 45 ± 14% and the 25 from the second farm of 80 ± 30%. When two additional clusters were added to the admixture model (K = 13, Figure 4.1.5C), a second CCS-related genomic signature was observed. Notably, this separation was not related to the farm of origin of the goats. Instead, subjects with the highest values for the two CCS-related clusters all came from the second farm, while the most admixed ones were from the first one. The observed findings of increased admixture and greater phenotypic variability at the Petralia Sottana farm can be elucidated by the composition of the sampled animal nucleus. Indeed, this caretaker breeder deliberately acquired prime representative specimens from the surrounding geographical breeding area, aiming to form a herd characterized by minimized inbreeding. However, this obviously led to a greater variability among the animals.

Collectively, these findings provide strong evidence supporting the genomic originality of the CCS breed, marking a significant advancement in its recognition process. However, the evidence of the presence of subjects with a more admixed background highlights the importance of complementing phenotypical evaluations with genomic analyses to select the most suitable breeding animals and optimize their matings.

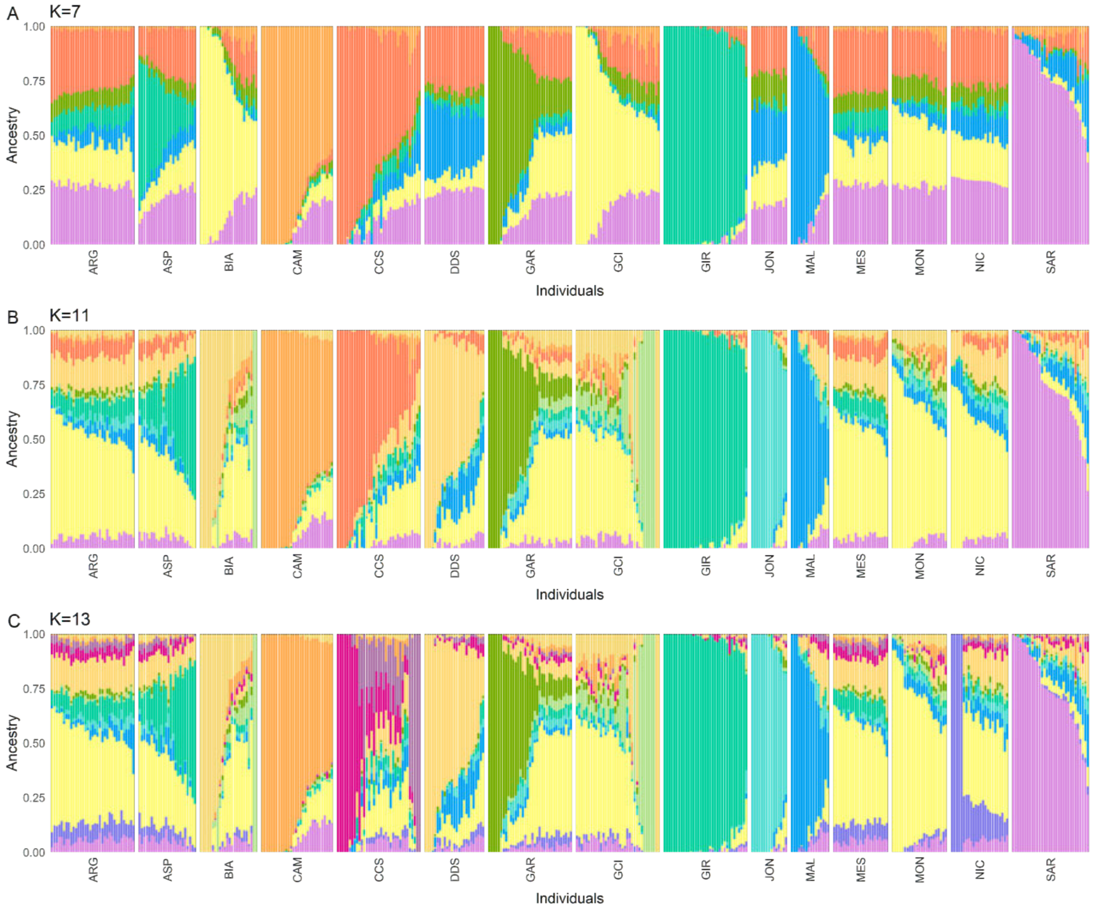

Figure 4.1.5 Admixture models using a number of clusters (K) equal to 7 (A), 11 (B), and 13 (C). The best-fitting model was found to be the one with K = 11. Each color represents a different cluster and each bar a different subject.

Genetic variability and ROH were investigated for all the included breeds (Table 4.1.4 and Figure 4.1.6). CCS breed showed a slightly lower observed heterozygosity (H_o) than expected heterozygosity (H_e), with a F_{IS} equal to 0.012. This is in line with what was observed for most of the other Southern Italian breeds. Genomic inbreeding (F_{ROH}) ranged from 1.5% (MES) to 15% (MAL). CCS, in particular, had a mean F_{ROH} equal to 6.0%, a value near most of the other breeds. Interestingly, more than 50% of the F_{ROH} derived from ROH > 16 MB in NIC, MON, CCS, and ASP breeds, implying recent inbreeding events [395]. These results are consistent with the fact that despite CCS and most of the other studied breeds having ancient origins, they only underwent standardization and selective breeding in relatively recent years. As a consequence, while the breed's current inbreeding value is under control, it remains crucial to monitor it periodically to prevent any excessive reduction in genomic variability.

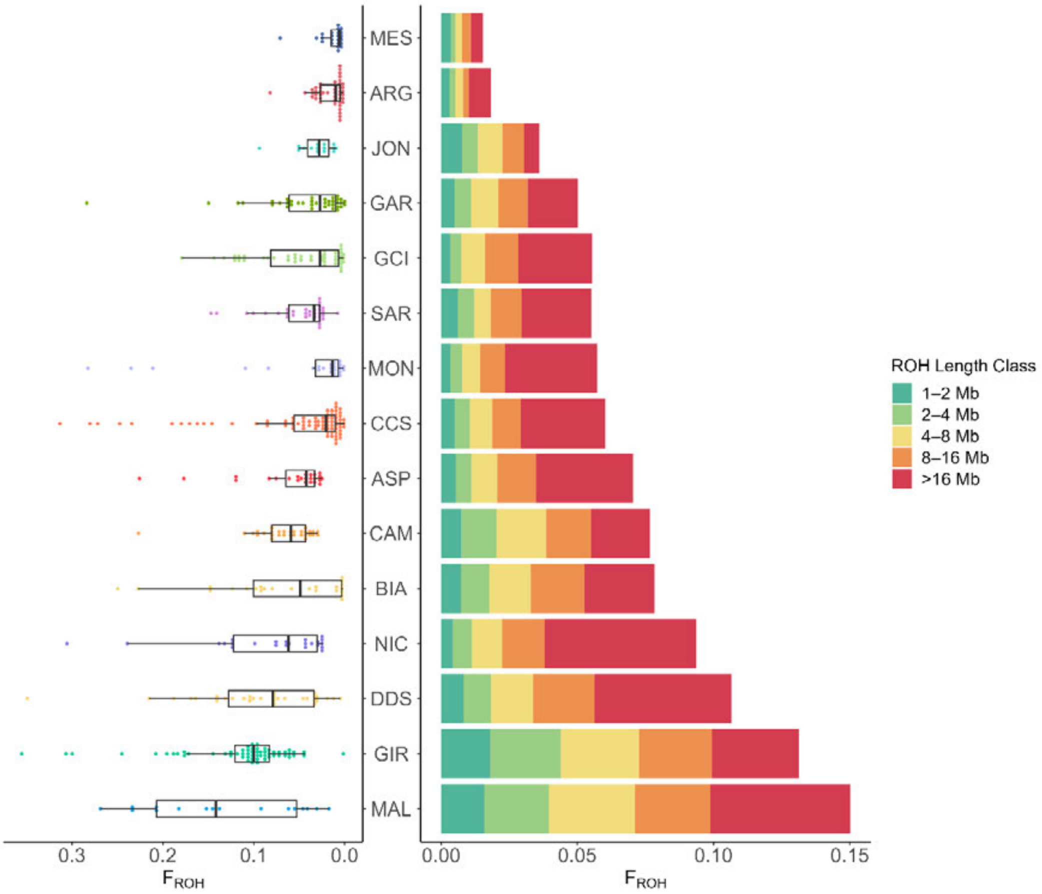

Figure 4.1.6 Boxplot of the total ROH-based inbreeding coefficient (F_{ROH}) and barplot of F_{ROH} by ROH length by breed.

Table 4.1.4 Summary of the parameters related to genomic variability and ROH-based inbreeding coefficient (F_{ROH}).

Breed	H_e	H_o	F_{IS}	Mean number of ROH	ROH total length	ROH mean length	F_{ROH} 1–2 MB	F_{ROH} 2–4 MB	F_{ROH} 4–8 MB	F_{ROH} 8–16 MB	F_{ROH} >16 MB	F_{ROH} total
ARG	0.412	0.413	−0.003	9.826	44.892	4.076	0.003	0.002	0.003	0.002	0.008	0.018
ASP	0.401	0.399	0.004	24.417	173.243	7.602	0.005	0.006	0.009	0.014	0.036	0.070
BIA	0.398	0.390	0.017	34.875	192.638	4.331	0.007	0.010	0.015	0.020	0.026	0.078
CAM	0.392	0.403	−0.024	37.967	188.512	4.806	0.008	0.013	0.018	0.017	0.022	0.077
CCS	0.405	0.400	0.012	22.038	148.080	4.874	0.005	0.006	0.008	0.010	0.031	0.060
DDS	0.396	0.377	0.045	39.000	262.158	5.928	0.008	0.010	0.015	0.022	0.050	0.107
GAR	0.402	0.404	−0.006	22.421	123.347	4.364	0.005	0.006	0.010	0.011	0.018	0.050
GCI	0.408	0.403	0.012	17.953	136.492	7.634	0.003	0.004	0.009	0.012	0.027	0.055
GIR	0.364	0.360	0.010	75.237	323.191	4.107	0.018	0.026	0.029	0.027	0.032	0.131
JON	0.372	0.413	−0.107	24.600	88.705	3.365	0.008	0.006	0.009	0.008	0.006	0.036
MAL	0.368	0.363	0.011	72.688	369.527	4.701	0.016	0.024	0.032	0.028	0.051	0.150
MES	0.404	0.410	−0.015	10.087	37.870	3.319	0.004	0.002	0.002	0.003	0.004	0.015
MON	0.403	0.400	0.005	17.609	141.027	4.994	0.003	0.004	0.007	0.009	0.034	0.057
NIC	0.403	0.393	0.022	26.083	230.473	8.508	0.004	0.007	0.011	0.016	0.056	0.094
SAR	0.407	0.402	0.011	23.438	135.752	6.576	0.006	0.006	0.006	0.011	0.026	0.055

The genomic effective population size (Ne) based on LD was computed for each of the studied breeds. In Figure 4.1.7, Ne values are reported for time intervals ranging from 13 to 50 generations ago. A consistent decreasing pattern in Ne is observed across all the populations under analysis. Among these populations, CCS goats exhibit one of the highest Ne, following behind ARG and SAR breeds, with values closely resembling those of the GCI breed. More precisely, CCS recorded an Ne of 181 at the 13-generation mark.

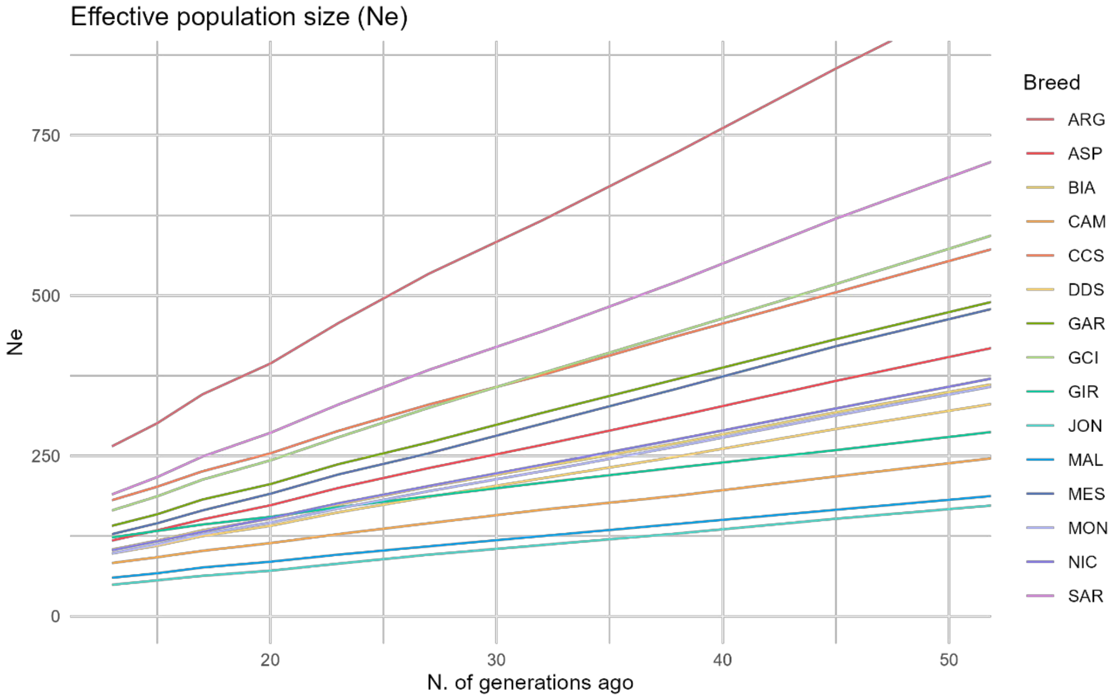

Figure 4.1.7 Genomic effective population size (Ne) trends.

4.1.4.3 Selection signatures

F_{ST} and ROH were used to compare CCS goats with different phenotypic characteristics. For these analyses, all 78 CCSs were included. All the results related to the selection signature analyses, including the complete name of the genes, are reported in Supplementary Table S4.1.3.

Comparing horned ($n = 39$) and polled ($n = 39$), we identified 480 SNPs within the top 1% F_{ST} values (0.12–0.25), which fell within 136 genes. With regard to ROH analysis, 197 SNPs on 184 genes were in the top 1% delta H-score. Three genes—*PCDHAC2*, *NRG2*, and *HBEGF*—were found by both analyses. As previously mentioned, polled animals in our study exhibited normally formed genitalia, and PIS was excluded through PCR analysis. However, to the best of our knowledge, none of the genes identified by our analyses were previously associated with horn development in goats or other species. It is plausible that a breed-specific mutation is responsible for polledness in this particular breed. For example, this was the case in cattle species, where several were identified as causative factors for polledness [396–398]. Thus, it would be essential to conduct further research to elucidate the genetic basis of this trait in goats and explore potential breed-specific genetic variations.

In the comparison of CCS goats with ears ($n = 74$) and with anotia ($n = 4$), 480 SNPs and 143 genes were identified with F_{ST} analysis (top 1% = 0.44–0.87), and 189 SNPs and 178 genes with ROH analysis. Three genes, namely *ATP12A*, *RNF17*, and *CENPJ*, were in common. Interestingly, one of the ROH regions, located on chromosome 7, was found to be a selection signature in La Mancha goats, a breed in which the anotia trait is fixed [260]. This ROH includes 16 genes, among them *HSPA9*, mutations of which are responsible for human Even-Plus syndrome [399]. Individuals affected by this syndrome often present microtia [399]. Notably, several other genes identified through F_{ST} and/or ROH analyses are associated with various syndromes that frequently include microtia or similar ear malformations among their symptoms according to the Human Phenotype Ontology database [400]: *CENPJ* and *RNF17*, both found by both the analyses, to primary autosomal recessive microcephaly 6 and Seckel syndrome; *TCOF1* to Treacher Collins syndrome; *EYA1* to brachio-oto-renal, branchiootic, and oto-facio-cervical syndromes; *SPEN* and *RERE* to 1p36 deletion syndrome and RERE-related neurodevelopmental syndrome; and *ORC6* to Meier–Gorlin syndrome 3 and ear-patella-short stature syndrome. Moreover, *GJB2* and *6* are associated to syndromic or non-syndromic deafness, whereas *SUFU*, which presented extremely high F_{ST} values, is considered to play a pivotal role in mammalian cochlear hair cell differentiation [401]. Despite being derived from an unbalanced sample, these findings represent a significant step in understanding the genetic basis of the anotia trait in goats and undoubtedly warrant further investigation.

F_{ST} analyses on subjects with ($n = 44$) and without wattles ($n = 34$) led to the identification of 480 SNPs on 137 genes in the top 1% (0.08–0.20). Instead, 203 SNPs on 197 genes were comprised in the top 1% of delta H-score in ROH analysis. *FAN1* and *TRPM1* genes were retained in both methods. Among the identified genes, *SLC9A9* and *NEDD4* genes were previously accounted as a potential candidate gene for wattle presence in goat [402]. Interestingly, our analyses identified both the *NEDD4* gene (included in ROH of animals with wattles) and its binding protein *N4BP1* (through F_{ST}), further supporting their potential roles in the development of wattles in goats. Another study, instead, suggested possible association between the same phenotype and *CSMD1* [403], which emerged from our analyses as well. Additionally, two other genes might be relevant: *KIF7*, whose mutation causes acrocallosal syndrome in humans, often presenting preauricular skin tags [404], and *ADAMTSL3*, known to influence the shape of the comb in chickens [405].

4.1.5 Conclusions

Local livestock populations, such as the Comune di Sicilia goat breed, play a crucial role in the preservation of rural communities in harsh and marginal areas, ensuring income for these communities and contributing to the preservation of the territory.

The investigation of the Comune di Sicilia goat revealed that, presently, this population demonstrates both genomics and phenotype distinctiveness, setting it apart from other breeds reared in the same breeding ranges, representing a step forward in its recognition process. However, it is important to acknowledge that some degree of variability exists within the population, likely influenced by breeders' selection preferences and the relatively recent initiation of a formal selection process.

To ensure the recognition and conservation of this population, a combined approach is imperative, taking into account both the morphology and appearance of the animals as well as their genomic background. This comprehensive evaluation will enable the identification and choice of the most suitable individuals, promoting the breed's originality while preventing excessive inbreeding.

Furthermore, the Comune di Sicilia breed presents intriguing phenotypic peculiarities that warrant further investigation from a genomic perspective. Such research can provide valuable insights into the development of these traits within the goat species.

4.1.6 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S4.1.1 Descriptive statistics of morphological traits in adult male "Comune di Sicilia" goats.

Supplementary Table S4.1.2 Comparison of morphological traits between subjects with or without horns, with or without wattles, and between farm 1 (Bolognetta farm) and farm 2 (Petralia farm) in adult female Comune di Sicilia goats.

Supplementary Table S4.1.3 Selection signatures in Comune di Sicilia goats: horned vs. polled, normal ears vs. anotia, and wattle presence vs. wattle absence.

Supplementary Figure S4.1.1 Dendrogram representing the phylogenetic relationship among breeds according to Reynolds distances.

Supplementary Figure S4.1.2 Admixture analysis for a number of clusters (K) ranging from 2 to 15 and representation of cross-validation values (c-v) for all the tested K.

4.2 GENOMIC INSIGHTS ON THE HISTORY AND SELECTION TRAJECTORIES OF THE COMUNE DI SICILIA GOAT

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4.2.1 Abstract

Background: The Capra Comune di Sicilia (CCS), also known as Mascaruna, is a Sicilian local goat population first described in 1870 and is currently the focus of a recovery project aimed at its characterization and formal ethnic recognition. To elucidate the ancestral genetic components and selection trajectories of the CCS population, we genotyped 78 CCS goats using the Goat 60K SNP BeadChip, integrated with genotype data from 1,920 individuals representing 66 goat breeds of Mediterranean and African origin.

Results: CCS exhibited relatively high heterozygosity (0.408) and moderate levels of inbreeding (0.04), an estimated effective population size of 185. Genetic ancestry analysis revealed gene flow from Maltese, Girgentana, Rossa Mediterranea and Saanen populations, alongside evidence of putative Greek ancestry shared with most Mediterranean breeds in our dataset, reflecting Sicily's profound historical and cultural ties with Greece. To better understand the evolutionary trajectories of the CCS population and to explore the potential contribution of Greek goat ancestry, we investigated selection signatures using iHS and ROH analyses. We identified 76 and 31 SNPs intercepting 38 and 12 genes, respectively, under putative selection. Subsequently, we applied XP-nSL and ROH analyses using Greek populations as ancestral references, identifying 21 and 431 SNPs associated with four and 157 genes, respectively, under putative selection. Overall, these selection signature analyses highlighted genes under positive selection related to traits such as milk and meat production, body size and growth, fertility, coat colour, fat deposition, and ear and horn development.

Conclusion: Our findings shed light on the historical and genetic distinctiveness of the CCS population, emphasizing its uniqueness and providing critical insights into its genetic background. This information is essential for supporting informed efforts to formally recognize CCS as a distinct and valuable breed.

4.2.2 Introduction

Animal genetic variability is crucial for addressing future challenges such as climate change and environmental adaptability [1]. According to FAO, livestock biodiversity is threatened, with 7% of the 8,800 identified animal breeds already extinct and 17% at risk of extinction. Globalisation and population growth aggravate this issue by encouraging the replacement of local livestock populations with highly productive breeds. Such practices erode the genetic uniqueness of local livestock, which are often uniquely adapted to the environments they inhabit and deeply connected to traditional practices and products [1,11,406].

The island of Sicily is the southernmost region in Italy and currently harbours around 92,714 goats (*Capra hircus*, [379]) including five local breeds, namely: Messinese, Argentata dell'Etna, Girgentana, Maltese, and Derivata di Siria [74]. These breeds have thrived under the island's harshest climatic conditions, such as long, dry, and warm summers. Among the local goat breeds of Sicily, the 'Comune di Sicilia' (CCS) represents a small population comprising approximately 500 individuals. Breeders often refer to it as *'Mascaruna'* (masked, painted face, in the Sicilian dialect) due to its distinctive black facial markings (Figure 4.2.1A). This goat is exclusively bred in western Sicily and remains unrecognised as an official breed, albeit its longstanding significance in Sicilian agriculture and rural traditions. Initially documented by Chicoli in 1870 [369], this population is distinguished by its remarkable robustness and adaptability to the challenging terrains and the variable climatic conditions of Sicily.

The CCS population is primarily bred for milk production, which was traditionally consumed fresh, with goats being milked door-to-door (Figure 4.2.1B). Today, Comune di Sicilia milk is used for exclusive cheese production by employing advanced dairy processing technologies, and is primarily destined for gourmet catering (Figure 4.2.1C).

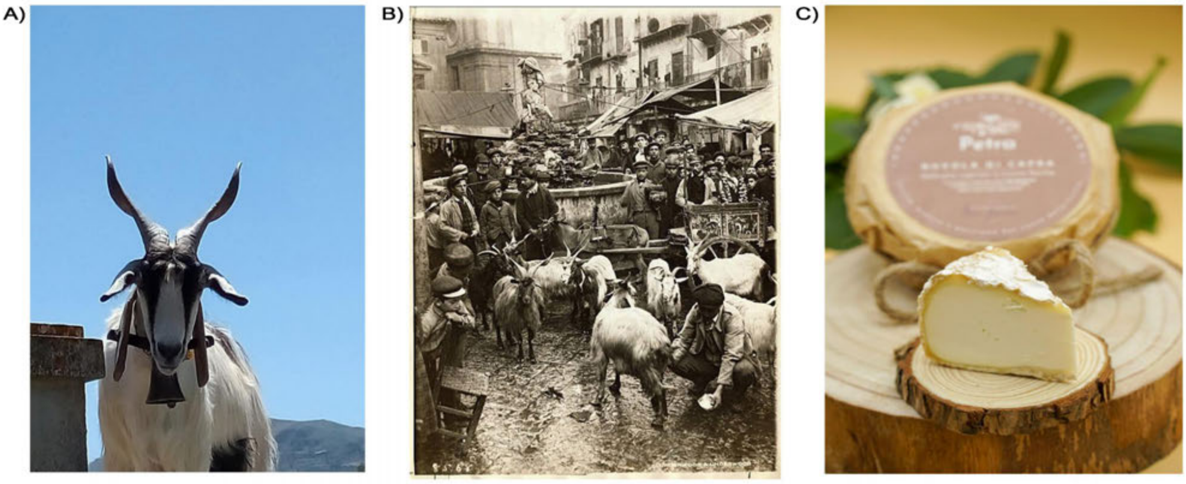

Figure 4.2.1 The Comune di Sicilia Goat. A) Comune di Sicilia goat. B) Palermo 1906 - Door-to-door delivery of Comune di Sicilia goat's milk. C) Typical cheese from Comune di Sicilia milk (courtesy from "Agricola Petra" farm).

A first phenotypic and genomic characterisation of the CCS goat was conducted by Bionda and co-workers [407] as part of a project funded by the Sicilian regional government (BIOSAVE; PSR Sicilia 2014-2020-Sottomisura 10.2b). In that study it was reported that most of the animals exhibited medium hair length, wattles and lyre or spiral horn. The coat colour was generally light, with diluted pheomelanin and characteristic black "badgerface" markings. Along with the presence of horns and wattles, the light coat colour has been suggested as an environmental adaptation to hot climates, potentially reducing solar radiation absorption [82]. When compared with other Sicilian and Italian breeds, the genomic composition of CCS revealed a distinct genetic structure, despite being closely related to other Sicilian breeds [407]. Importantly, the population has not undergone controlled selection and 'guardian breeders' take great care in breeding the goat in purity, avoiding cross-breeding with other populations. Here, we apply genomic tools to offer a novel perspective on the adaptation strategies of CCS, tracing its ancestral genetic components and identifying selection trends.

4.2.3 Materials and methods

4.2.3.1 Biological samples and dataset creations

Genotype data of 78 Comune di Sicilia goats (comprising nine bucks and 69 does) reared in farms located in Sicily, were retrieved from Bionda *et al.* 2023 [407]. For comparison, publicly available genotype data of 66 breeds from Mediterranean area, Turkey, North Africa and South-East Africa were combined with Comune di Sicilia data using PLINK v1.9 [408] ; the merged dataset comprised 1,920 animals (Table 4.2.1). SNPs located on sex chromosomes or with unknown map positions were removed, and SNP positions updated to *C. hircus* reference genome ARS1.2. PLINK v1.9 was used to remove variants and samples with missing call rates exceeding 0.05, SNPs with minor allele frequency (MAF) <0.1, and closely related individuals (first-degree relations).

Table 4.2.1 Datasets used for genomic analyses.

Breed	Acronym	Origin	N (QC)*	H_o (SD)*	F_{ROH} (SD)*	N_e*
Argentata dell'Etna	ARG	Italy	48 (46)[1]	0.418 (0.01)	0.012 (0.02)	944
Capra dell'Aspromonte	ASP	Italy	24 (23)[1]	0.404 (0.03)	0.047 (0.06)	239
Bianca Monticellana	BIA	Italy	24 (22)[1]	0.397 (0.03)	0.067 (0.07)	169
Camosciata delle Alpi	CAM	Italy	30 (23)[1]	0.405 (0.02)	0.076 (0.05)	133
Capestrina	CAP	Italy	24 (21)[1]	0.405 (0.04)	0.049 (0.08)	138
Comune di Sicilia	CCS	Italy	79 (60)	0.408 (0.03)	0.051 (0.07)	185
Derivata di Siria	DDS	Italy	32 (24)[1]	0.384 (0.04)	0.105 (0.08)	113
Facciuta della Valnerina	FAC	Italy	24 (21)[1]	0.398 (0.05)	0.072 (0.10)	157
Fulva del Lazio	FUL	Italy	22 (19)[1]	0.417 (0.01)	0.024 (0.03)	161
Garganica	GAR	Italy	40 (32)[1]	0.408 (0.03)	0.045 (0.06)	191
Grigia Ciociara	GCI	Italy	43 (35)[1]	0.405 (0.03)	0.051 (0.05)	222
Girgentana	GIR	Italy	59 (53)[1]	0.362 (0.03)	0.133 (0.07)	179
Garfagnina	GRF	Italy	28 (23)[1]	0.401 (0.02)	0.058 (0.05)	226
Jonica	JON	Italy	16 (10)[1]	0.416 (0.01)	0.04 (0.03)	295
Maltese	MAL	Italy	16 (16)[1]	0.368 (0.04)	0.141 (0.09)	81
Messinese	MES	Italy	24 (23)[1]	0.415 (0.01)	0.015 (0.02)	345
Capra di Montefalcone	MON	Italy	24 (22)[1]	0.405 (0.04)	0.056 (0.09)	74
Nicastrese	NIC	Italy	24 (20)[1]	0.401 (0.03)	0.062 (0.06)	18
Rossa Mediterranea	RME	Italy	46 (35)[1]	0.414 (0.02)	0.038 (0.03)	231
Saanen	SAA	Italy	44 (37)[1]	0.417 (0.01)	0.047 (0.03)	55
Maltese sampled in Sardinia	SAM	Italy	15 (13)[1]	0.379 (0.04)	0.118 (0.08)	617
Sarda	SAR	Italy	33 (32)[1]	0.407 (0.02)	0.032 (0.04)	798
Capra di Teramo	TER	Italy	43 (28)[1]	0.376 (0.04)	0.122 (0.08)	255
Bermeya	BEY	Spain	24 (23)[2]	0.406 (0.01)	0.02 (0.01)	230
Mallorquina	MAQ	Spain	20 (17)[2]	0.367 (0.05)	0.115 (0.11)	369
Malaguena	MLG	Spain	42 (40)[2]	0.419 (0.01)	0.024 (0.03)	35

Murciano-Granadina	MUG	Spain	20 (20)[2]	0.404 (0.01)	0.043 (0.03)	165
Palmera	PAL	Spain	15 (15)[2]	0.281 (0.01)	0.165 (0.02)	92
Blanca de Rasquera	RAS	Spain	20 (20)[2]	0.38 (0.03)	0.09 (0.07)	151
Pyrenean	PYR	France	27 (23)[2]	0.378 (0.04)	0.101 (0.09)	120
Chios	CHI	Greece	10 (10)[3]	0.423 (0.01)	0.02 (0.02)	72
Crete	CRE	Greece	8 (8)[3]	0.409 (0.01)	0.017 (0.01)	47
Lesbos	LES	Greece	12 (11)[3]	0.388 (0.02)	0.087 (0.04)	277
Peloponnese	PEL	Greece	12 (12)[3]	0.421 (0.01)	0.016 (0.01)	151
Skyros	SKY	Greece	9 (9)[3]	0.404 (0.01)	0.036 (0.03)	175
Carpatian goat	CRP	Romania	14 (14)[2]	0.426 (0.01)	0.014 (0.01)	166
Barki	BRK	Egypt	153 (108)[2]	0.4 (0.02)	0.026 (0.05)	827
Nubian	NBN	Egypt	84 (79)[2]	0.359 (0.02)	0.113 (0.04)	18
Oasis	OSS	Egypt	72 (69)[2]	0.368 (0.04)	0.085 (0.09)	126
Saidi	SID	Egypt	60 (58)[2]	0.385 (0.04)	0.047 (0.09)	60
Ankara	ANK	Turkey	20 (19)[2]	0.399 (0.02)	0.04 (0.04)	125
Kil	KIL	Turkey	25 (22)[2]	0.405 (0.01)	0.026 (0.02)	374
Kilis	KLS	Turkey	40 (36)[2]	0.402 (0.02)	0.025 (0.04)	65
Barcha	BAR	Morocco	4 (4)[2]	-		-
Draa	DRA	Morocco	4 (4)[2]	-		-
Ghazalia	GHA	Morocco	4 (4)[2]	-		-
Moroccan goat	MOR	Morocco	10 (10)[2]	0.382 (0.05)	0.066 (0.13)	139
Noire de l'Atlas	NDA	Morocco	4 (4)[2]	-		-
Nord	NOR	Morocco	4 (4)[2]	-		-
Tunisian	TUN	Tunisia	23 (21)[2]	0.402 (0.02)	0.027 (0.04)	867
Abergelle	ABR	Ethiopia	53 (51)[2]	0.369 (0.01)	0.020 (0.04)	1087
Gumez	GUM	Ethiopia	41 (40)[2]	0.375 (0.01)	0.019 (0.03)	38
Keffa	KEF	Ethiopia	49 (43)[2]	0.355 (0.02)	0.049 (0.07)	33
Woyito Guji	WYG	Ethiopia	46 (40)[2]	0.370 (0.01)	0.017 (0.02)	936
Gishu	GSH	Uganda	3 (2)[2]	-		-
Karamonja	KAR	Uganda	20 (18)[2]	0.373 (0.01)	0.01 (0.01)	1163
Kigezi	KIG	Uganda	5 (3)[2]	-		-
Mubende	MUB	Uganda	23 (19)[2]	0.357 (0.03)	0.045 (0.08)	138
Nganda	NGD	Uganda	11 (11)[2]	0.375 (0.03)	0.017 (0.02)	138
Sebei	SEB	Uganda	24 (22)[2]	0.370 (0.01)	0.011 (0.01)	2082
Galla	GAL	Kenya	23 (23)[2]	0.375 (0.01)	0.008 (0.01)	592
Small East African	SEA	Kenya	31 (31)[2]	0.367 (0.01)	0.021 (0.04)	307
Gogo	GOG	Tanzania	13 (12)[2]	0.37 (0.01)	0.02 (0.03)	100
Maasai	MAA	Tanzania	20 (18)[2]	0.366 (0.01)	0.015 (0.02)	91
Malya	MLY	Tanzania	12 (9)[2]	0.402 (0.03)	0.06 (0.05)	117
Pare White	PRW	Tanzania	22 (19)[2]	0.351 (0.03)	0.05 (0.08)	123
Sonjo	SNJ	Tanzania	22 (18)[2]	0.367 (0.01)	0.012 (0.01)	45

[1] [74]

[2] [71]

[3] [409]

*N and QC: Sample size before (N) and after (QC) quality checks.

*H_o: Observed heterozygosity.

*F_{ROH} : Inbreeding.

*SD: standard deviations (SD).

*N_e : Current effective population size.

4.2.3.2 Genetic diversity and population structure

Genomic diversity indexes were assessed to gain a preliminary overview of the genetic integrity of the populations analysed and to detect potential biases such as reduced variability, inbreeding, or outlier samples, which could compromise the reliability of downstream analyses. Specifically, observed heterozygosity (H_o) was analysed with PLINK v1.9 for breeds with at least five individuals. Runs of homozygosity (ROH) were analysed in all the breeds using PLINK's *--homozyg* flag with the following parameters: a sliding window of 20 SNPs was used, and a region was classified as a ROH if it was at least 1 Mb long and contained at least 20 consecutive homozygous SNPs, to exclude short ROH originated by linkage disequilibrium; regions with a density lower than 1 SNP / 500 kb or a gap of over 500 kb between consecutive SNPs were excluded; no heterozygous SNPs were permitted, whereas up to two missing genotypes per window were allowed to account for potential genotyping errors [410]. The individual genomic inbreeding coefficient (F_{ROH}) was calculated as the ratio of the total length of ROH to the total length of the autosomal genome covered by SNPs [133]. To leverage the relationship between the length of ROHs and the number of generations ago since inbreeding events occurred [395], the F_{ROH} values were partitioned into five ROH length categories: 1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, and >16 Mb.To determine the most recent effective population size (N_e) estimate [146], we combined two methods: SNeP v1.11 [140] and GONE v1.0 [411]. N_e is a parameter widely used for evaluating biodiversity, as it provides a measure of the number of individuals in a Wright-Fisher population, having the same amount of random genetic drift as the real population [146]. SNeP was run, setting the maximum and minimum distance between pairs of SNPs to: 20 Mb and 280 kb, respectively, and using the Sved & Feldman [412] recombination rate modifier. The effective population size of the Sicilian goat populations (Comune di Sicilia, Girgentana, Messinese, Rossa Mediterranea, Argentata dell'Etna, Derivata di Siria) was also estimated through GONE using default parameters.

The ordinal relationships between populations and individuals were investigated through principal component analysis (PCA) as implemented in PLINK v1.9 [408]. To further visualise the evolutionary relationships among populations, we performed a Neighbour-Net analysis using Reynolds' distances, computed with a custom script (available at https://github.com/barbatom/ReynoldsDist) and plotted using SplitsTree v6.3.30 [413]. A global ancestry analysis was conducted using ADMIXTURE v1.3.0 [161]. We first included the whole dataset with K theoretical ancestral populations where K = {2,...,20, 25, 30}. To better detail Comune di Sicilia ancestral components, a subset of breeds sharing at least an ancestral component with Comune di Sicilia was selected (Table 4.2.1). To ensure homogeneity across the subset, a maximum of 22 animals (median population size in our dataset) were randomly selected for each breed. Admixture was then performed at K values from 2 to 24. Cross-validation (CV) values were computed at each K and results visualised through CLUMPAK [414]. This subset was then used for downstream analysis. A supervised admixture analysis focusing on specific ancestry components in Comune di Sicilia sourcing from Maltese, Girgentana, Rossa Mediterranea, Saanen and Greek groups was also performed using ADMIXTURE. The evolutionary relationships and genetic migration events between populations were evaluated with TreeMix v1.13 [158]. Ankara was set as an outgroup and 12 migration events were computed and visualised using the R plotting functions provided within the Treemix suite. The contribution added by each migration vector computed by Treemix was calculated as the f index estimate, which represents the fraction of the variance in the sample covariance matrix (W). A formal test of introgression was performed using the f3 test as implemented in the TreeMix suite.

To better investigate admixture among distantly related breeds, we examined the proportion of haplotypes shared between breeds computing Identity By Descent (IBD) among individuals. We used RefinedIBD v4.1 [415], applying a sliding window of 10 Mb and reporting only IBD segments with a minimum length of 0.2 Mb and LOD score of 3.0, calculated after trimming 0.15 Mb from the end of the shared haplotype. Shared haplotypes for each pair of breeds were calculated as the median length of haplotypes shared among all possible pairs of individuals from the respective breeds. Individual pairs with no shared haplotypes were assigned length = 0. Haplotype sharing with median values above the 95th percentile of all breed pairs was visualised using the *circlize* R package [416].

4.2.3.3 Selection signatures

The Integrated Haplotype Score (iHS) [169] was computed for Comune di Sicilia using selscan v1.2.0a48 [417], with default parameters. iHS describes the decay of haplotype homozygosity for both the ancestral and derived haplotypes extending from a query site. The iHS scores were normalised chromosome-wise using the *scale()* function in R. The FDR and p-value have been estimated using the R *stats* functions *p.adjust()* and *p.norm()*, respectively. SNPs with positive normalised values and FDR <0.05 were considered significant.

Selection signatures were compared within and between Comune di Sicilia and Greek goats using ROH. Here we included Chios, Crete, Lesbos, Peloponnese and Skyros as representative Greek goats, chosen to reflect the diversity of phenotypes within the Greek goat population.

As described by Michailidou *et al.*, 2019 [418], over the millennia of goat exploitation in Greece, many genetic nuclei have evolved, giving rise to distinct goat types associated with their region of origin. Today, the majority of native Greek goats are descended from the Eghoria breed, which is characterized by significant phenotypic variability, comprising approximately 39 different types, primarily distinguished by their geographic origin.

A homozygosity score (H-score), ranging from 0 to 1, was calculated for each SNP as the proportion of individuals within the breed with a ROH that included the SNP. ROH islands were identified as ROH including more than 15 SNPs, after having joined SNPs within regions of 0.25 Mb. The ROH islands with the top 1% H-score SNPs were identified [256]. Additionally, we specifically compared ROH in Comune di Sicilia and in Greek goats (pooled as a single group). The two groups were compared by calculating a ΔH-score for each SNP as the difference between the H-scores of Comune di Sicilia and the Greek group, and the SNPs with the highest 1% ΔH-scores were considered the most divergent between the groups.

For comparison, we applied XP-nSL, a haplotype-based statistics that detects selection by comparing haplotype patterns between two populations [417]. This statistic uses the decay of homozygous haplotype as a function of recombination distance to detect loci under selection [419,420]. Population structure analyses suggested Greek goats as ancestral to Comune di Sicilia. Consequently, we tested five pairwise comparisons contrasting the Comune di Sicilia against five greek goat breeds Chios, Crete, Lesbos, Peloponnese and Skyros. XP-nSL estimates were performed using selscan v1.2.0a48 [417] with default parameters. The XP-nSL scores were first normalised chromosome-wise using the *scale()* function in R. Then, the p-value and FDR were estimated using *p.adjust()* and *p.norm()*, implemented in the R *stats* package, respectively. SNPs with positive normalised values and FDR<0.05 were considered significant when identified in at least two out of the five pairwise comparisons.

4.2.3.4 Candidate genes

Candidate genes were identified in the up- or down-stream flanking regions of the SNPs under putative selection. A flanking region size was determined as the mean distance between two SNPs (26 kb) belonging to the same chromosome and was calculated for all autosomes using a custom script. Protein-coding genes found within these genomic areas were retrieved from the Ensembl Genes 91 database, based on the goat ARS 1.2 reference genome using the BioMart tool [421] and further considered candidate genes. All the genes identified in the genomic areas were processed using the functional annotation tool implemented in DAVID Bioinformatics resources 6.8 [422] to determine enriched functional terms. Additionally, the regions around the SNPs identified in the analysis were intersected, using bedtools [423], with the known quantitative trait loci (QTL) in goat species, publicly available at AnimalQTLdb website [424] (accessed 27/10/2024).

4.2.4 Results

4.2.4.1 Genetic diversity and population structure

After SNP filtering, 47,581 SNPs and 1,681 individuals were retained for subsequent analyses. H_o ranged from 0.281 in the Palmera goat breed to 0.426 in the Carpatian goat. Among Italian breeds, H_o values ranged from 0.362 in Girgentana to 0.418 in Argentata dell'Etna, with the Comune di Sicilia population recording H_o = 0.408 (Table 4.2.1; Supplementary Figure S4.2.1). Inbreeding coefficients (F_{ROH}) varied from 0.004 in the Gishu breed to 0.165 in the Palmera breed. The Italian groups showed a mean F_{ROH} of 0.064, with the Comune di Sicilia recording F_{ROH} = 0.051 (Table 4.2.1; Supplementary Figure S4.2.1).

The N_e estimates computed with SNeP ranged from 18 in Ghazalia, Nubian, Barcha and Nicastrese to 2,082 for the Sebei population. Among Italian goats, the highest N_e value was estimated for Argentata dell'Etna (944; Table 4.2.1). Comune di Sicilia recorded N_e = 185 (Table 4.2.1). We focussed the use of GONE on determining the demographic changes in the Sicilian breeds exclusively; all showed rapid changes in N_e in the last 10 generations (Supplementary Figure S4.2.2). Comune di Sicilia, Messinese, Rossa Mediterranea, Argentata dell'Etna show a decrease of N_e around 10 to 15 generations ago which could coincide with the beginning of the '90s if applying a three years per generations conversion. Similarly, CCS and RME showed similar trajectories, suggesting similar demographic pressures or genetic patterns mainly linked to a decline around 12 generations ago (Supplementary Figure S4.2.2).

The PCA (Figure 4.2.2A and Supplementary Table S4.2.1) clearly clustered individuals according to their sampling areas. The first principal component (PC1; 7.0% of explained variance), discriminated between European and African breeds. Additionally, the separation between goats from North Africa (Egypt, Tunisia and Morocco) and South-East Africa (Uganda, Ethiopia, Tanzania, Kenya) was evident in PC1 and further emphasised by PC2 (1.7% of explained variance). This clustering was confirmed by the Neighbour-Net analysis

(Figure 4.2.2B), which showed European breeds grouping separately from the African breeds, with proximity observed among Mediterranean breeds. The CCS goats were positioned at the base of the branch grouping the Sicilian breeds (Girgentana, Capra dell'Aspromonte, Argentata dell'Etna, Messinese, Nicastrese; Figure 4.2.2B).

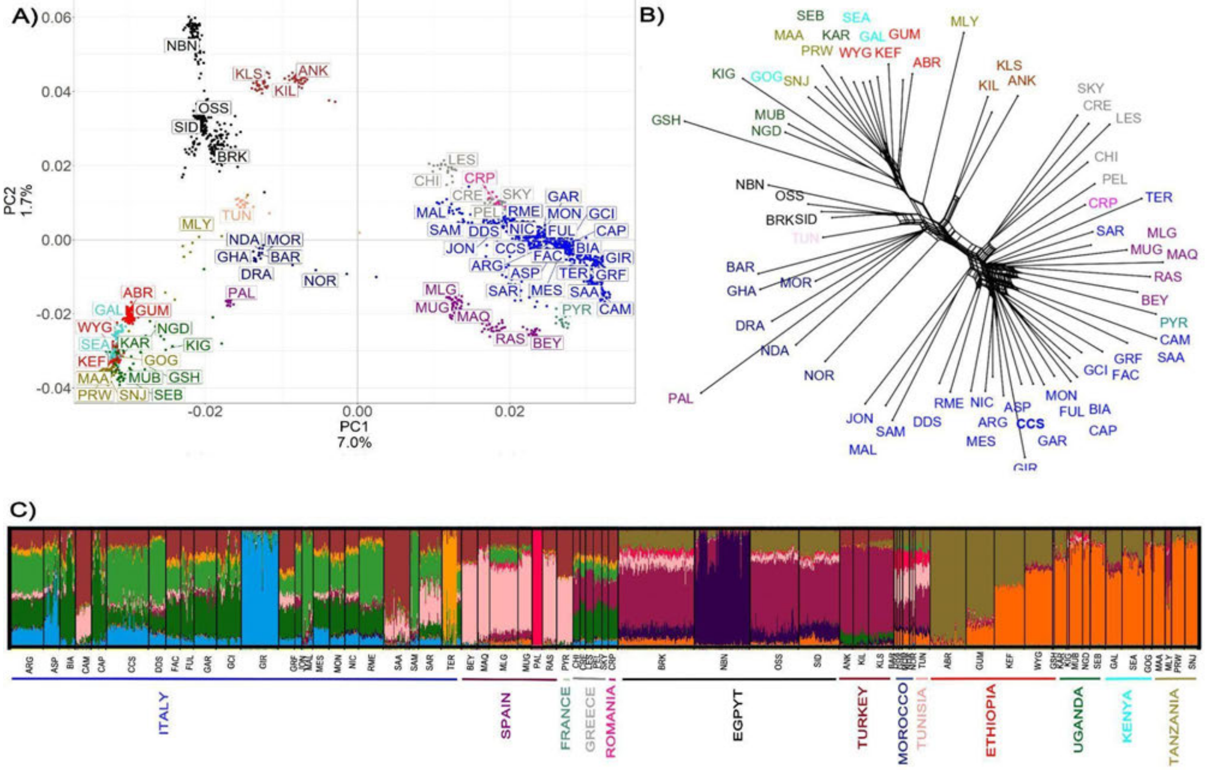

Figure 4.2.2 A) PCA analysis. B) Neighbour-Net to explore the relationship between populations. C) Admixture at K 11. See Table 4.2.1 for breed acronyms. Colours in breeds labels reflect their geographical provenance: Italy (Blue), Spain (dark pink), France (light green), Greece (grey), Romania (fuchsia), Egypt (black), Turkey (dark red), Morocco (blu navy), Tunisia (pink), Ethiopia (red), Uganda (green), Kenya (water green), Tanzania (dark yellow).

In the admixture analysis, the European goat breeds separated from African goats at K = 2 (Supplementary Figure S4.2.3); at K = 3, the European, North Africa and South-East Africa goats split as three different clusters. In the European group, at K = 6 the Girgentana and Palmera acquired private clusters (Supplementary Figure S4.2.3). Starting from K = 12, Comune di Sicilia showed cluster components attributable to introgression sourcing from Maltese, Girgentana, Rossa Mediterranea and Saanen. This putative introgression was confirmed by a supervised admixture analysis (Supplementary Figure S4.2.4).

At K = 14, a combination of ancestral components common between Sicilian and Greek breeds emerged (Supplementary Figure S4.2.3). To better define this cluster combination (that we named: Mediterranean component) we performed an additional admixture analysis using a subset including those breeds sharing any ancestry components with Comune di Sicilia, namely: Mediterranean Italian goat (CCS, MAL, GIR, ARG, MES, ASP, NIC, RME, JON, GAR, GCI, MON, FUL, TER, FAC, GRF, SAR, SAA), three Spain breeds (MAQ, BEY, RAS), the Greek goats (CHI, CRE, LES, PEL, SKY), one Turkish goat (ANK), two Egyptian goats (NBN, OSS) and one South-African goat (PRW). Despite the reduction of the dataset, the Mediterranean component was still not uniquely attributable to any specific population, whilst confirming the ancestry specificity of Comune di Sicilia compared to Sicilian breeds and the possible introgression from other populations (Supplementary Figure S4.2.5).

Treemix analysis provided further detail on the relationship among breeds (Figure 4.2.3). The phylogenetic tree showed the basal position of the Greek goats in relation to the other Mediterranean goats (Figure 4.2.3). The first seven migration edges representing potential events of introgression (gene flow events) accounted for the model significance explained by the f statistic, with the first migration edge having an f value of 0.927. Vectors from 8 to 12 brought only a small increase in f value. Almost all the gene-flow events depicted by Treemix, involved migration events from African to European breeds. One, albeit having a low migration weight, described the putative gene-flow sourcing from Girgentana into Comune di Sicilia. However, when tested through f3 tests, no gene-flow events were statistically supported.

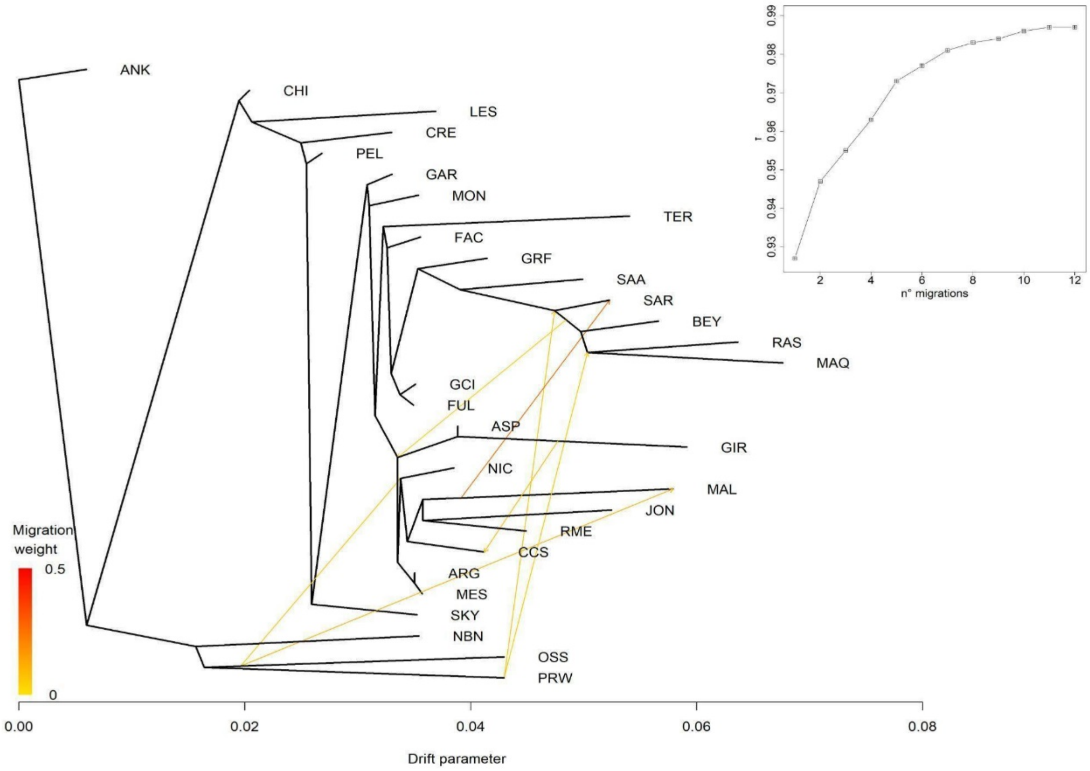

Figure 4.2.3 Treemix and index f representing the variance fraction of the sample covariance matrix. The arrows point towards the receiving group and are coloured according to the percentage of ancestry received from the donor.

IBD haplotype sharing was analysed across all 1,412,040 possible pairs of individuals. From the 2,211 across-breed pairs, we selected the top 5% (54 pairs) with the highest haplotype sharing (Supplementary Figure S4.2.6). Most of the haplotype sharing was observed within the Italian breed group. Significant haplotype sharing was also detected among other breeds from the same geographic region, particularly among Egyptian breeds and between two Ugandan breeds (Kigezi and Mubende). Additional notable sharing was found between the Moroccan Draa and Palmera breeds, between Kigezi and the Tanzanian Malya, and between the Spanish Malagueña and the two Maltese breed samples, Maltese and Maltese sampled in Sardinia. Regarding the Comune di Sicilia, we observed haplotype sharing with several Italian breeds, ranked by decreasing length: Maltese, Maltese sampled in Sardinia, Girgentana, Derivata di Siria, Aspromontana, Rossa Mediterranea, and Jonica (Figure 4.2.4).

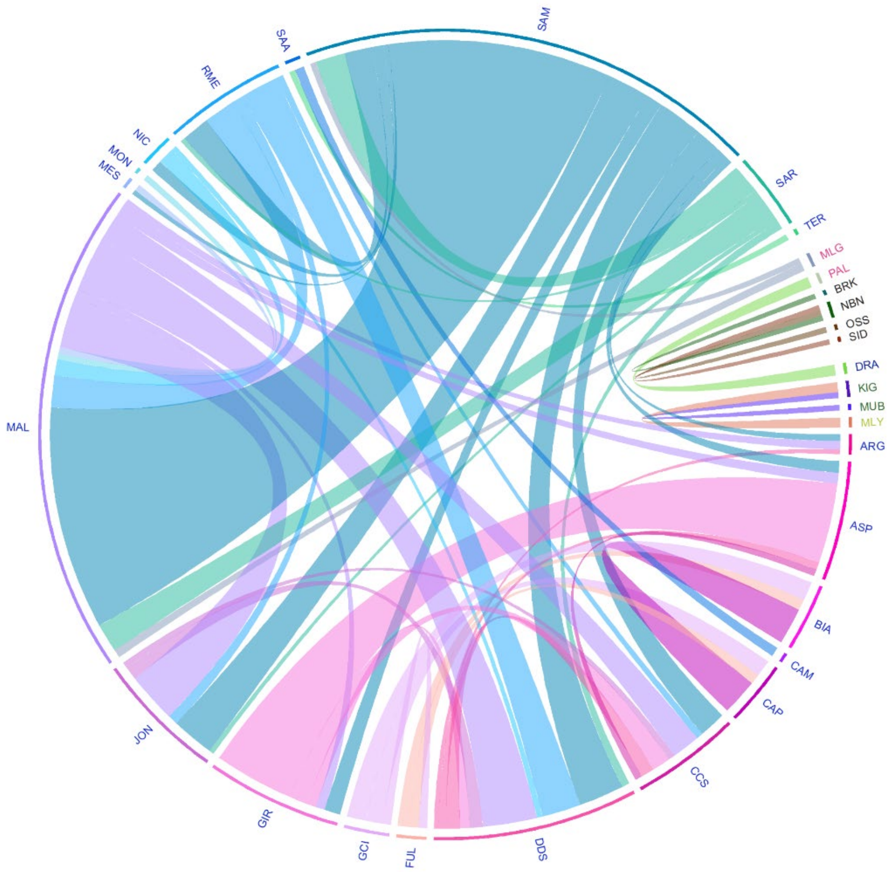

Figure 4.2.4 Significant IBD haplotype sharing (top 5%) among the analysed populations.

Each segment is coloured according to the breed, and the breeds' names are coloured according to their geographic origin. The plot including all the analysed breeds is reported in Supplementary Figure S4.2.6.

4.2.4.2 Selection signatures

After identifying the Greek populations as phylogenetically ancestral to Sicilian breeds, we used multiple approaches to investigate selection signatures within the CCS goat and between it and the Greek populations.

Integrated Haplotype Score (iHS) was conducted within the Comune di Sicilia population. After normalisation, up to 76 SNPs with positive normalised value and FDR <0.05 were identified under putative selection (Supplementary Figure S4.2.7 and Supplementary Table S4.2.2). Within the flanking region of these 76 SNPs we identified 38 genes, reported in the Supplementary Table S4.2.3. Some of them are associated with relevant traits in goats, for instance, a SNP on chr19 is associated with the axis inhibition protein 2 (*AXIN2*) gene [425]; another SNP on chr20 is associated with slit guidance ligand 3 (*SLIT3*) gene [426]; SNP on chr23 associated with bone morphogenetic protein 5 (*BMP5*) gene [427]; finally a SNP on chr3 associated with COP9 signalosome subunit 8 (*COPS8*) [428].

Within the CCS population, 31 ROH islands were the most represented (Table 4.2.2) and intercepted nine genes (Supplementary Table S4.2.4). The same analysis applied to the group of Greek goats identified 39 ROH islands (Table 4.2.2) and intercepted 12 genes (Supplementary Table S4.2.5). Noticeably, there was a partial overlap between seven pairs of ROH islands between CCS and the group of Greek goat populations, located on Chr 6, 7, 12, and 14 (Table 4.2.2).

Table 4.2.2 ROH islands with top 1% H-scores in the Comune di Sicilia goat and Greek goat populations. Start and ending positions are reported in bp.

Chromosome	CCS	Greek goat populations
1		71,555,288 - 72,515,767
		73,271,679 - 75,247,247
		82,425,050 - 82,425,050
4		24,064,901 - 29,175,654
		34,029,382 - 37,506,521
		44,425,867 - 44,713,179
		68,272,675 - 69,493,491
		77,314,196 - 78,604,124
6	33,821,580 - 35,079,672	
		35,476,109 - 36,018,329
	36,691,987 - 38,590,135	38,091,505 - 38,091,505
		38,558,049 - 38,657,309
		40,604,705 - 41,757,660
7	60,280,996 - 60,353,593	57,275,997 - 61,898,852
8	30,403,572 - 31,610,802	
	86,509,148 - 86,740,383	
10		48,722,676 - 49,422,136
		51,797,025 - 52,128,425
12	30,507,715 - 31,682,480	
	34,149,004 - 34,358,090	
	35,500,432 - 37,100,314	
	37,575,687 - 38,537,857	
	40,776,415 - 43,300,094	43,067,469 - 43,300,094
	43,598,089 - 45,351,957	43,598,089 - 44,644,892
	48,444,019 - 51,582,056	49,190,178 - 49,190,178
		50,018,490 - 51,550,631
		57,083,945 - 57,135,484

	59,890,806 - 61,075,465	57,846,679 - 61,321,277
	83,606,274 - 84,286,254	
14	16,847,835 - 17,420,173	16,578,275 - 17,721,484
	50,645,797 - 50,985,021	
		51,039,398 - 51,119,648
15	55,796,071 - 55,929,538	
16		34,002,754 - 35,427,296
		41,005,540 - 46,056,925
		73,818,187 - 74,173,056
17		7,751,582 - 8,947,492
		47,134,487 - 48,539,264
		50,716,197 - 51,705,980
18	47,759 - 1,390,724	
	1,871,235 - 1,871,235	
	14,688,398 - 16,281,856	
	19,640,095 - 22,334,972	
	25,487,914 - 26,002,471	
	28,148,047 - 28,245,263	
		36,016,922 - 38,217,154
20		47,819,874 - 50,481,194
		52,775,546 - 54,536,289
21	17,579,963 - 18,887,870	
	19,192,398 - 22,541,417	
	22,935,500 - 25,298,413	
	25,579,321 - 29,862,147	
22		48,063,417 - 48,690,678
		49,085,004 - 50,302,698
23		46,896,853 - 46,896,853
	14,639,337 - 14,902,624	
	16,985,379 - 19,182,071	
	19,561,000 - 19,671,961	
	20,029,297 - 21,814,503	
24		34,619,454 - 35,405,960
27		2,564,672 - 2,857,933
		3,219,064 - 3,403,973
		3,855,656 - 5,351,731

A total of 532 SNPs were selected as being associated with the top 1% ΔH-score (≥ 0.13) between Comune di Sicilia and the Greek breeds, located on chromosomes 4 (n=8), 6 (n=27), 12 (n=86), 18 (n=81, two separate regions), 21 (n=229), and 23 (n=101) (Supplementary Figure S4.2.8 and Supplementary Table S4.2.6). Within the flanking regions of these SNPs, we could identify 157 protein-coding genes (Supplementary Table S4.2.7). Three of the identified regions also included goat QTL related to haemoglobin concentration and hematocrit (21:19225288-19225292 and 21:19565627-19565631), and to milk and milk fat and protein yield (23:18237572-18237576).

We used the Greek populations as background for determining selection in Comune di Sicilia through pairwise cross-population selection sweep analysis, as implemented in XP-nSL. After normalisation, up to 27 SNPs located on five out of the 29 goat chromosomes were identified under putative selection for the Comune di Sicilia – Chios (CCS-CHI) pair; 20 SNPs, located on three chromosomes (mainly on chromosome 2), were identified for the Comune di Sicilia-Crete (CCS-CRE) pair; 14 SNPs, located on four chromosomes, were identified for the Comune di Sicilia-Peloponnese (CCS-PEL) pair; and finally, 31 SNPs, located on four chromosomes, were identified as under putative selection for the Comune di Sicilia-Skyros (CCS-SKY) comparison (Supplementary Table S4.2.8). All these four pairwise comparisons had significant SNPs on chromosome 2. No significant selection signal was detected in the Comune di Sicilia-Lesbos (CCS-LES) comparison (Figure 4.2.5).

A total of 21 SNPs were identified as being under putative selection at least two of the four pairwise comparisons (Supplementary Table S4.2.9). Among these 21 SNPs, located on Chr1 (n=2), Chr2 (n=14), Chr6 (n=6), Chr8 (n=5) and Chr18 (n=1), those on chromosome 6 and 18 overlapped with the SNPs within the top 1% ΔH-score calculated by comparing ROH in Comune di Sicilia and Greek goats. Within the flanking region of these 21 SNPs we identified four genes (Supplementary Table S4.2.10): the promoter FOXL2 inverse complementary gene (*PFOXic)* on Chr1; two genes identified on Chr6 included one of the family with sequence similarity 184 member B (*FAM184B*), and a DNA-binding transcription factor activity gene (*LCORL)*; lastly, ENSCHIG00000024996 gene on Chr2 had no description in the consulted databases. Noticeably, the *LCORL* gene was also found to be within the most represented ROH in the Greek goat populations, and both *LCORL* and *FAM184B* were identified in the top 1% ΔH-score differentiating CCS and Greek goats.

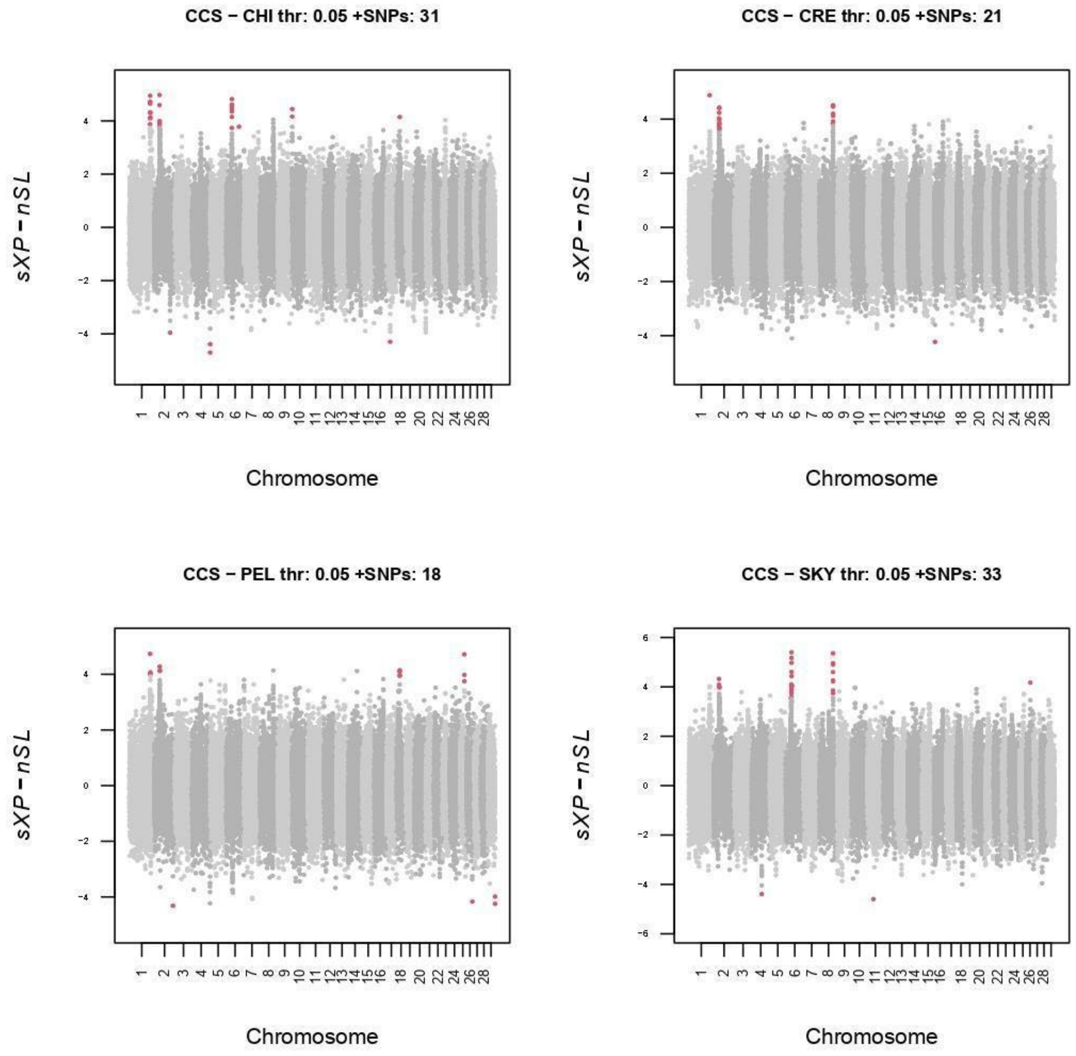

Figure 4.2.5 Manhattan plots summarising the results obtained for each pairwise comparison using XP-nSL statistics. Only pairwise comparisons with significant SNPs identified as being under putative selection (coloured in red) are shown.

4.2.4.3 Consensus genes

Comparing all the results of the selection signatures' investigation, ten genes were found to be identified by multiple tests. Specifically, *FAM184B* and *LCORL* were identified by both XP-nSL and ROH analysis when comparing Greek populations and Comune di Sicilia, with *LCORL* also associated with the most common ROH in Greek goats. The StAR Related Lipid Transfer Domain Containing 5 (*STARD5*), Transmembrane Channel Like 3 (*TMC3*), and Transient Receptor Potential Cation Channel Subfamily M Member 1 (*TRPM1*) were identified both by the iHS analysis in the CCS breed and the ROH comparison (top delta H-score). The *STARD5* gene is known to be associated with lipidic metabolism in humans [429] and *TRPM1* with deafness in domestic animals [430]. Additionally, the Methenyltetrahydrofolate Synthetase (*MTHFS*), Armadillo Like Helical Domain Containing 2 (*ARMH2*), RHO Family Interacting Cell Polarization Regulator 2 (*RIPOR2*), Zinc Finger and SCAN Domain Containing 31 (*ZSCAN31*), and ENSCHIG00000009125 were also associated with the highest H-score when ROH were analysed in the Comune di Sicilia goats.

4.2.5 Discussion

Local livestock populations, such as the Comune di Sicilia goat, represent an important source of genetic variability to contrast the future challenges in animal husbandry [1].

We evaluated the genetic uniqueness and selection history of the Comune di Sicilia within the broader context of Mediterranean goat populations. Population structure analyses revealed that the Comune di Sicilia clustered with other Sicilian breeds but subsequently formed a distinct and separate cluster. Notably, we observed a moderate introgression signal originating from the Girgentana breed, likely reflecting the traditional practice among Sicilian farmers of rearing multiple breeds together to optimize productivity and profits. Notwithstanding, the Comune di Sicilia appears to have a unique genomic signature evident at a rather low K respect to the other Italian and Sicilian goats. Overall, our results confirm previous findings [407] and provide another important evidence that supports the genomic variability and originality of Comune di Sicilia.

To investigate the introgression and ancestral history of Comune di Sicilia, several analyses were performed. First evidence of potential introgression was given by the admixture analysis where the Comune di Sicilia had shared ancestry components with Maltese, Girgentana, Rossa Mediterranea and Saanen goats, despite presenting an otherwise unique ancestry signature. Such introgressions are consistent with their shared geographic origin and the occasional shared enclosure among Comune di Sicilia, Girgentana and Rossa Mediterranea. The presence of Saanen—a specialised dairy breed—components, might be due to the efforts of breeders to improve the Comune di Sicilia's milk production traits. The presence of introgression signals sourcing from Maltese goats might be related with goat translocation from Malta to Sicily at the end of 1800, as mentioned by historical records [369]. It is plausible that these animals represent the ancestors of the Maltese breed, given their distinctive traits, such as "long and pendulous" ears, which are still observed in modern Maltese goats and are also present in the Comune di Sicilia population [407].

Admixture analysis revealed an ancestral component shared by Sicilian, Greek, and other Mediterranean breeds, suggesting a common historical genetic background. Notably, this component was not specifically attributable to any single breed within our dataset, indicating it may represent a deeply rooted ancestral genetic signature, widely conserved across diverse populations. This widespread ancestral pattern may reflect complex demographic events such as gene flow, admixture, or population drift, and highlights the importance of geographical context in the genetic structure. Interestingly, Treemix analysis positioned the Greek populations in a basal position relative to the Sicilian breeds. This result confirms the findings of Michailidou *et al.*, 2019 [418], which reported a close relationship between Eghoria-related and southern Italian breeds, such as Garganica, Rossa Mediterranea and Jonica. Due to the history of Greek colonisation in the XII BC, which encompassed much of southern Italy, including Sicily, it is likely that goat populations ancestral to the current Greek breeds were introduced to Sicily and contributed to the genomic makeup of the Sicilian populations. Hence, we selected the Greek breeds as background reference for selection sweep analyses. Both selection analysis approaches (XP-nSL and ROH-based) identified genes related with fertility and litter size, milk production, ear shape, coat colour, hair, fat deposition, meat production, and body size.

ROH islands in the regions on chromosomes 7, 12, and 18 showed strong signals of homozygosity and intercept genes involved in traits such as reproduction, milk production, and immune response. These findings align with those from Tsartsianidou *et al.* (2025) [431], which reported these same chromosomal regions as responsible for breed-specific adaptation. Several of the ROHs found in the Greek breeds harboured genes involved in pathways previously reported under selection in Eghoria and Skopelos goats, including traits related to milk production and quality, prolificacy, and immune function [418,431]. Likewise, within those ROHs differentiating CCS and Greek breeds, we identified SNPs intercepting genes with similar functional roles. For instance, the ROHs on chromosomes 6 and 12 in CCS goats have been reported in Sicilian [432] and central-southern Italian goats [433], respectively, and were also found to differentiate goats from southern and northern Italy [256]. Among the genes identified in the comparison between CCS and Greek goats by the XP-nSL analysis, we found *PFOXic* that is involved in the development of horns and ovaries in goats and is coexpressed with *FOXL2* and regulated by the Polled Intersex Syndrome region [434]. We hypothesize that *PFOXic* might be related to the presence of polled CCS goats [407]. Other genes such as *FANCA, NTRK3, CPEB1, BLM, MGFGE8*, and *SPP1* were previously associated with fertility and litter size in goats [435–441]. Specifically, *BLM* plays a role in follicle development and granulosa cell proliferation and its variants were significantly correlated to litter size in goats [437]. *MGFGE8* gene is also associated with milk traits, encoding the lactadherin [438], which belongs to the family of milk fat globule membrane proteins that envelope fat globules during milk secretion [442]. This gene is associated with milk fat yield and total solid in goats and seems to play a role in mammary gland development [443]. Other identified genes are associated with milk production, such as *ANPEP* [444] and *POLG* [427]. One of the most intriguing characteristics of the Comune di Sicilia is the variability in ear shape, including the presence of individuals exhibiting anotia. Among the genes identified, some are associated with external ear conformation, such as *ORC6*, responsible for Meier-Gorlin syndrome in humans [445]. Moreover, a region on chromosome 6 including *LCORL*, *MEPE*, *FAM184B*, and *ISBP* genes, was found to be associated with ear size in cattle and, in particular, *IBSP* was a good candidate for this trait, since it could harbour a missense mutation affecting the protein structure and was highly expressed in small-ear cattle breeds [446,447]. A gene related to this trait could also be *BMP5*, identified in Comune di

Sicilia through iHS analysis. This gene is well described to be responsible for the "short-ear" phenotype in mice, which is also associated with skeletal malformations and altered development of some soft tissues as well as with impaired wound and fracture healing [448–452].

Interestingly, we identified the *AP3B2* gene, known for its association with coat colour intensity [453] which might be relevant given that Comune di Sicilia goats typically exhibit a very light, often nearly white, pheomelanin pigment [407]. Furthermore, the Comune di Sicilia typically has medium-long hair, which likely accounts for the identification of genes associated with fiber production. The *POLG* gene, for instance, is implicated in Cashmere hair growth through lncRNA MTC-mediated regulation [454]. Moreover, the *SPP1* gene was found to be differentially expressed in short- and long-haired Cashmere goats [455], and *KLF5* is regulated by circRNA-0100 during the differentiation of secondary hair follicles stem cells [456]. Additionally, *KLF5* is involved in the differentiation of goat subcutaneous adipocytes [457]. Related to the fat deposition, the iHS selection signature analysis in Comune di Sicilia, identified the *COPS8* gene. *COPS8* has been recently reported as a candidate gene to understand brown to white adipose tissue transition during the first two weeks of life in goats [458]. In addition, it was demonstrated the role of *COPS8* to treat obesity in the development of perirenal fat in goats [459].

A gene that would support the possibility of a selection toward meat production and quality improvement is *MRPL46.* Specifically, proteomic analyses in goats showed a differential expression of this protein in Xinjiang goats' *longissimus dorsi* muscle over a 48-hours period after death and could observe an effect on meat tenderness and colour [460].

We identified two genes in both of the comparative analyses known to be associated with body size and growth: *FAM184B* and *LCORL*. Interestingly, *t*hese two genes have been shown to distinguish between goat populations from northern and central-southern Italy [256], which suggest them as specific to the Mediterranean breeds. *FAM184B* was associated to body size and growth-related traits in Merino sheep [461–464], Angus cattle [465], and Xinjiang

Brown Cattle [466], but also to quantitative trait loci (QTL) identified in beef cattle such as average daily gain, feed intake, subcutaneous fat thickness [467], carcass weight [428] and liver and spleen weight [468]. *LCORL* has been recognised to be involved in selection for body size in several livestock species [244,469–472], including goats from different countries [473], cattle [466], European commercial and local pig breeds [474], sheep [475], and equines [476]. In addition *LCORL* is also associated with QTL in cattle such as bone [477], carcass [428], and sperm motility in Holstein bulls [478].

Detailed analysis of the genes identified through iHS analysis and ROH-based selection in Comune di Sicilia revealed the presence of loci associated with fertility, including *CTNNA1*, *SLIT3*, and *BMP5*, as well as a locus related to milk production, *AXIN2*. The *CTNNA1* gene, identified in the ROH-based selection analysis, is associated with litter size in goats [479], and the gene *SLIT3* plays an important role in goat fertility [426]. A recent study reported that *SLIT3* mRNA transcript was found highly expressed in primordial follicle oocytes [480], suggesting its major role in the formation and activation of follicles [481]. The *BMP5* gene, belonging to the BMP subfamily, is involved in goat fertility and fecundity [427]. This gene plays a crucial role in ovarian folliculogenesis by enhancing proliferation of granulosa cells, which is associated with the growing female gamete in the mammalian ovary [482,483]. It is possible that this gene is selected to enhance the fertility of the Comune di Sicilia goat. Notably, this gene is also associated with body size in cattle [484]. Among the genes identified, *AXIN2* was found under selection. Recently, it was reported that this gene was upregulated during the formation of goat mammary epithelial cells via Wnt/β-catenin signalling [425]. It has been reported that Wnt/β-catenin signalling plays a crucial role in the proliferation and acinus-like formation of the goat mammary epithelial cells [425]. The *AXIN2* is also related with muscle growth in cattle [430] and adipogenesis in mice [429,485].

4.2.6 Conclusions

Here we provide a comprehensive genomic investigation of the Comune di Sicilia goat, offering novel insights into its evolutionary history within the Mediterranean context.

Our analyses, while confirming previous findings, highlight the distinct genomic identity of CCS, despite the complex admixture pattern due to historical and cultural exchanges. Importantly, we also identified a deeply rooted Mediterranean genetic component, likely originating from ancient lineages shared throughout the area. The discovery that Sicilian goats share genetic ties with Greek populations underscores the historical and cultural connections between Sicily and Greece, which have shaped the genetic heritage of local goat breeds. Furthermore, the genes under selection in the Comune di Sicilia are strongly associated with traits crucial for the island’s animal husbandry, particularly those linked to animal products critical to the region.

These findings highlight the unique characteristics of this breed and emphasize the importance of its recognition. Although the population is not currently at immediate risk of genetic erosion, implementing monitoring plans is essential to ensure its conservation. To this end, strategies such as controlled mating informed by genotyping can help mitigate high levels of consanguinity.

Our results confirm the distinctiveness of the Comune di Sicilia goat breed and offer a new perspective on its history. This uniqueness reflects the long-standing efforts of local breeders to develop and maintain productive and adaptive traits. Therefore, we advocate for the Comune di Sicilia goat to be recognized and protected not only as a living document of the Mediterranean's livestock history, but also as a valuable and resilient genetic resource, well-adapted to its environment and particularly relevant in the face of current climate challenges.

4.2.7 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S4.2.1 Eigenvalues of the principal components.

Supplementary Table S4.2.2 SNPs under selection pressure on CCS autosomes according to the iHS statistic.

Supplementary Table S4.2.3 Description of the candidate genes according to the iHs statistic for the CCS goat.

Supplementary Table S4.2.4 Description of the candidate genes associated with the top 1% H-score in the CCS according to the ROH-based selection signatures.

Supplementary Table S4.2.5 Description of the candidate genes associated with the top 1% H-score in the Greek population according to the ROH-based selection signatures.

Supplementary Table S4.2.6 SNPs associated with the top 1% ΔH-score (≥ 0.13) between CCS and the Greek breeds according to the ROH-based selection signatures.

Supplementary Table S4.2.7 Description of candidate genes associated with the top 1% ΔH-score (≥ 0.13) between CCS and the Greek breeds according to the ROH-based selection signatures.

Supplementary Table S4.2.8 SNPs under selection pressure (in colour red) on all goat autosomes according to the XP-nSL statistic.

Supplementary Table S4.2.9 The SNPs under putative selection identified in at least two of the four pairwise comparisons according to the XP-nSL statistic.

Supplementary Table S4.2.10 Description of the candidate SNPs identified in at least two out of five pairwise comparisons according to the XP-nSL statistic for the CCS goat.

Supplementary Figure S4.2.1 Distribution of inbreeding values (FROH) for all groups.

Supplementary Figure S4.2.2 Description: Demographic changes in the Sicilian breeds, GONE results.

Supplementary Figure S4.2.3 Admixture plot from K 2 to 30 considering all breeds studied.

Supplementary Figure S4.2.4 Supervised Admixture analysis to check the introgression in CCS from Maltese (MAL), Girgentana (GIR), Rossa Mediterranea (RME), Saanen (SAA) goats and Greece breeds.

Supplementary Figure S4.2.5 Admixture plot (K = 2–24) for goat breeds sharing the ancestral "Mediterranean" component.

Supplementary Figure S4.2.6 Significant IBD haplotype sharing (top 5%) of all the analysed breeds. Each segment is coloured according to the breed, and the breeds' names are coloured according to their geographic origin.

Supplementary Figure S4.2.7 Manhattan plots summarizing results obtained using iHs statistics. SNPs identified as being under putative selection are coloured in red.

Supplementary Figure S4.2.8 Manhattan plot summarizing the results obtained using ROH-based selection signatures. SNPs identified as being under putative selection are coloured in red; positive ΔH-score values indicate SNPs with high H-score in CCS and vice versa.

4.3 GENOMIC INSIGHTS INTO THE RECENT EVOLUTION AND BIODIVERSITY OF ITALIAN SHEEP BREEDS

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4.3.1 Abstract

Italy hosts a remarkable ovine biodiversity shaped by centuries of history, regional traditions, and environmental heterogeneity. This diversity sustains agricultural production as well as ecosystem services and cultural heritage. Yet, many local breeds are undergoing severe demographic decline. To explore these dynamics, we analyzed census data from all registered Italian sheep, which revealed highly variable situations across breeds but confirmed that most are currently at risk of extinction. To complement this picture, we genotyped 34 Italian sheep populations using the Ovine50K BeadChip and compared them with foreign breeds with recognized herd books in Italy. Genomic analyses of diversity (including inbreeding and effective population size), population structure, and genomic background provided insights into the state of genetic variation and relationships among breeds, including patterns of introgression. By comparing these results with data from populations sampled twenty years ago, we assessed temporal changes in diversity, genomic background, and selection signatures. Fst analyses highlighted genomic regions that have undergone the most marked shifts, allowing us to explore associated genes and QTLs. Correlations between Fst and environmental changes across 20 variables further emphasized the role of local adaptation in shaping genomic landscapes. In addition, local ancestry inference in two breeds (Gentile di Puglia and Nera di Arbus) with evidence of recent admixture identified genomic regions influenced by gene flow. Overall, our study illustrates the complex evolutionary dynamics of Italian sheep breeds and underscores the importance of integrating demographic analyses with genomic tools to guide their conservation and sustainable management.

4.3.2 Introduction

Livestock genetic variability plays a pivotal role in ecosystem conservation, land management, and climate change mitigation. In recent decades, breed conservation has gained importance as the biodiversity embedded in domesticated animals is increasingly recognized as essential for maintaining overall biodiversity [3,11,28].

FAO's guidelines for the conservation of animal genetic resources emphasize improving knowledge of breeds worldwide, considering their economic and geographic context, genetic and economic value, and extinction risk, with growing reliance on molecular technologies [12,26,110,486]. Globally, over 7,000 local breeds are reported, but the risk status of about 60% remains unknown; of the breeds with known status, nearly 70% are considered at risk of extinction (DAD-IS, www.fao.org/dad-is, accessed 22/08/2025).

Several factors drive livestock diversity: (i) evolutionary forces, such as mutation, genetic drift, gene flow, and selection; (ii) geographic distribution and adaptation to specific environments and farming systems; (iii) economic and socio-cultural context; and (iv) breeding plans and technologies, including the use of artificial insemination, crossbreeding, and the introduction of exotic breeds to improve performance [12,276]. Government policies and strategies also play a major role, shaped by economic and cultural factors, and can strongly affect livestock diversity and sustainability [371,487]. Thus, breeds are dynamic entities, continuously evolving under diverse forces that drive genetic change [488]. Strengthening the management of animal genetic resources requires long-term investment and strategies that ensure both genetic and socio-economic sustainability of local breeds [372,486].

Italy, thanks to its history, traditions, and environmental variability, harbors exceptional biodiversity in all livestock species [74,489]. The Italian Sheep and Goat Breeders Association (Asso.Na.Pa.), recognized by the Ministry of Agriculture, manages herd books for 73 sheep breeds, 63 of which are considered local (51 under conservation programs). The remaining breeds are of foreign origin but are also included in conservation programs (www.assonapa.it). Italian sheep breeds are particularly important as reservoirs of genetic variability contributing to food security, environmental preservation, and the rural economy, especially in mountainous and hilly areas [321,490]. However, these breeds face challenges including competition with cosmopolitan breeds, inbreeding due to small population sizes, outcrossing to improve productivity, and climate change [321,490].

Most Italian sheep breeds are used for milk and meat production. Milk is primarily destined for cheese-making, including nine PDO Pecorino cheeses and other traditional products. Regarding meat, two PGI labels—*Agnello del Centro Italia* and *Agnello di Sardegna*—enhance the value of sheep production (https://www.qualigeo.eu/). Conversely, wool production remains underexploited due to structural market issues, although regional projects aim to revive its role in the textile sector. Beyond production, sheep—particularly local populations—provide non-material benefits such as ecosystem services and socio-cultural and historical value [33,68,491].

A central activity of Asso.Na.Pa. is monitoring sheep biodiversity across the Italian peninsula and islands. Since its establishment, the association has tracked breed distribution and census trends using pedigree data. Each year, census data on males and females are reported by the Ministry to the National Focal Point of FAO, which updates extinction risk assessments in the Domestic Animal Diversity Information System (DAD-IS; https://www.fao.org/dad-is/en/). Since 2017, this work has been strengthened by two national projects—CHEESR (2017–2021) and SHEEP&GOAT (2021–2025)—funded under the National Rural Development Plan (PSRN, submeasure 10.2) and coordinated by Asso.Na.Pa. These projects have introduced genomic tools for both biodiversity conservation and selection, generating extensive genomic characterization of 34 Italian sheep breeds.

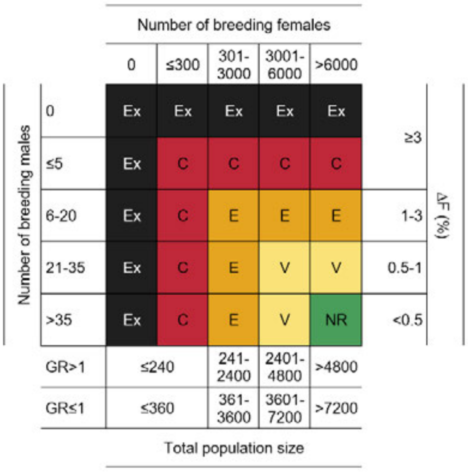
This study investigates the genomic landscape of Italian sheep by examining the relationships among national breeds and their connections with foreign populations, providing an overview of current diversity and population structure. We also explored temporal changes by comparing the same populations sampled twenty years ago, assessing the recent evolution of genetic diversity, genomic background, and selection signatures in the context of each population's history. In addition, we evaluated the role of environmental adaptation in shaping these patterns and analyzed introgression in two breeds to identify genomic regions affected by crossbreeding, offering insights into the dynamic forces driving the biodiversity and evolution of Italian sheep.

4.3.3 Materials and methods

4.3.3.1 Demographic data and analysis

Census data were provided by Asso.Na.Pa. and consisted of the number of registered farms and live animals calculated on December 31st of each year of the period considered (2010-2024).

The percentage change in animal and farm was calculated for each breed as the difference between the values recorded in 2024 and those recorded in 2010, divided by the value recorded in 2010. For each breed and year, a growth rate was calculated as anti-log[[logN2-logN1)/t], where N1 and N2 are the number of animals at two consecutive censuses, and t is the time interval (in years) between the two censuses [36]. Years with 0 registrations were excluded. For each breed, ΔF for year 2024 was calculated as 1/(2*Ne) where Ne is the effective population size, calculated using classic Wright's formula: (4*Nmales*Nfemales)/(Nmales+Nfemales) [36,144]. Breed risk categories were assigned according to the criteria proposed by FAO [36], as shown in Figure 4.3.1, based on the number of breeding females and males, the overall population size, growth rate, and ΔF.



	Number of breeding females					
Number of breeding males	0	≤300	301-3000	3001-6000	>6000	ΔF (%)
0	Ex	Ex	Ex	Ex	Ex	≥3
≤5	Ex	C	C	C	C	
6-20	Ex	C	E	E	E	1-3
21-35	Ex	C	E	V	V	0.5-1
>35	Ex	C	E	V	NR	<0.5
GR>1	≤240		241-2400	2401-4800	>4800	
GR≤1	≤360		361-3600	3601-7200	>7200	
	Total population size					

Figure 4.3.1 Breed risk categories according to FAO. For each breed, the risk category was assigned according to the least favorable parameter. Abbreviations: NR = Not at risk; V = Vulnerable; E = Endangered; C = Critical; Ex = Extinct; GR = Growth rate; ΔF = Inbreeding rate.

4.3.3.2 Sampling

Within the CHEESR and SHEEP&GOAT projects, 7,086 animals from 43 sheep breeds (selected among those with at least three registered farms at the time of sampling) were genotyped using the Illumina OvineSNP50 BeadChip (versions V1, V2, and V3). For each breed, samples were chosen among animals registered to the herd book, generally selecting one male and two unrelated females per farm, with sample size ranging from 12 to 60 animals across 4 to 20 farms. For Massese (MAS) and Comisana (COM), all samples came from the closed nucleus maintained at the Asso.Na.Pa. genetic center (https://www.assonapa.it/centro-genetico), while Sarda (SAR) animals were taken from the AGRIS Sardegna genomic flock and related farms; these data were aimed at genomic evaluations. Additional samples were collected from some breed to genomic analysis of parentage and/or wool quality. For other breeds targeted for biodiversity or wool-quality studies, such as Gentile di Puglia, Delle Langhe, Istriana, Sopravissana, and Merinizzata Italiana, sampling involved five animals per farm from 10 farms, and in some case-studies one male and two unrelated females were collected from 10 farms together with an additional group of related animals from a single farm. Datasets were obtained from pre-existing data based on routine animal recording procedures; moreover, DNA sampling for all individuals was conducted using nasal swabs and no invasive procedures were applied. Thus, in accordance with the 2010/63/EU guide and the adoption of the Law D.L. 04/03/2014, n.26 by the Italian Government, an ethical approval was not required for our study.

Given the differences in the sampling (which also included related animals) and the big sample size, a pre-selection of animals to include in the dataset was performed for COM, MAS, and SAR breeds according to the following criteria: animals were retained only if enrolled in the main section of the herd book and males with positive parentage verification. After this filtering, the resulting dataset comprised 2,193 sheep from 43 different populations.

Table 4.3.1 Composition of the dataset used for genomic analyses and results for each breed.

Breed code	Breed name	Raw N.	N. (N. after breed size balancing)	F_{ROH} (mean ± sd)	He	Ho	Ne (SNeP)	Ne (GONE)
ALT	Altamurana	13	13 (13)	0.067 ± 0.048	0.347	0.358	14	122
APN	Appenninica	71	67 (30)	0.057 ± 0.067	0.365	0.362	35	254
BGN	Bagnolese	44	42 (30)	0.042 ± 0.050	0.376	0.368	38	871
BGS	Bergamasca	20	19 (19)	0.039 ± 0.051	0.353	0.363	20	112
BLS	Biellese	21	21 (21)	0.045 ± 0.048	0.358	0.357	23	206
BRB	Barbaresca	14	14 (14)	0.054 ± 0.083	0.354	0.359	15	143
BRI	Brianzola	24	24 (24)	0.087 ± 0.086	0.351	0.343	24	103
BRO	Brogne	37	36 (30)	0.057 ± 0.054	0.361	0.358	35	290
CIU	Pecora Ciuta	30	30 (30)	0.068 ± 0.098	0.373	0.361	30	110
COM	Comisana	34 (1438)[a]	34 (30)	0.032 ± 0.013	0.356	0.363	34	177
CRG	Corniglio	76	73 (30)	0.054 ± 0.038	0.351	0.353	32	122
DBN	Di Benevento (Quadrella)	12	12 (12)	0.146 ± 0.065	0.288	0.325	9	31
FAB	Fabrianese	130	122 (30)	0.102 ± 0.092	0.364	0.343	32	155
FRB	Frabosana	24	24 (24)	0.044 ± 0.031	0.355	0.356	29	273
GDP	Gentile di Puglia	167	158 (30)	0.034 ± 0.043	0.376	0.370	38	371
IST	Istriana (Carsolina)	81	78 (30)	0.121 ± 0.083	0.341	0.332	28	88
LMN	Lamon	23	22 (22)	0.056 ± 0.062	0.350	0.357	22	101
LPG	Alpagota	32	32 (30)	0.069 ± 0.068	0.358	0.351	37	579
LTD	Laticauda	11	11 (11)	0.085 ± 0.101	0.350	0.347	11	89
MAS	Massese	163 (1086)[a]	161 (30)	0.057 ± 0.021	0.346	0.351	35	142
MER	Merinizzata Italiana	134	129 (30)	0.037 ± 0.032	0.371	0.370	38	250
NTC	Noticiana	17	14 (14)	0.082 ± 0.041	0.323	0.340	14	72
ODL	Delle Langhe	163	157 (30)	0.094 ± 0.053	0.337	0.338	35	192
PAM	Dell'Amiata	16	16 (16)	0.069 ± 0.071	0.367	0.358	18	165
PCC	Pecora di Corteno	19	17 (17)	0.080 ± 0.061	0.347	0.344	17	63
PMR	Pomarancina	14	14 (14)	0.044 ± 0.028	0.351	0.366	15	122
PNA	Nera di Arbus	65	63 (30)	0.078 ± 0.072	0.362	0.344	38	459
SAR	Sarda	515 (3047)[a]	495 (30)	0.066 ± 0.044	0.348	0.346	38	351
SMN	Sambucana	30	30 (30)	0.039 ± 0.023	0.359	0.366	37	273
SPV	Sopravissana	34	33 (30)	0.052 ± 0.061	0.370	0.366	34	153
SVR	Savoiarda	15	13 (13)	0.105 ± 0.062	0.329	0.340	13	101
TCL	Tacola	51	50 (30)	0.023 ± 0.026	0.366	0.366	40	1699
VCN	Vicentina (Foza)	17	16 (16)	0.077 ± 0.058	0.333	0.345	16	71
VLC	Valle del Belice	28	26 (26)	0.057 ± 0.048	0.360	0.356	31	375

[a] The number in parenthesis refers to the initial sample size, before filtering procedures reported in Materials and methods section.

Nine breeds were excluded from analysis because including fewer than ten individuals after quality control and exclusion of relatives: Cornella bianca, Garfagnina bianca, Lacaune, Moscia Leccese, Schwarznasenschaf, Suffolk, Sciara-Moscia calabrese, Villnoesser schaf-Fiemmese, and Zerasca.

Abbreviations: F_{ROH}: inbreeding based on runs of homozygosity; He: expected heterozygosity; Ho: observed heterozygosity; Ne: effective population size.

Using Plink 1.9 software [390], we performed a quality control on this dataset, excluding individuals with a call rate below 95% and those directly related (as identified by an in-house script based on Mendelian errors), as well as SNPs located on sex chromosomes and with a call rate below 95%. For population structure analysis, we applied a minor allele frequency (MAF) filter of 0.1%, and pruned for linkage disequilibrium (LD) using Plink *--indep-pairwise* (50, 10, 0.5) function. To balance breed sample sizes, we excluded populations with fewer than 10 individuals and reduced the number of subjects to a maximum of 30 (30 being the median size per breed) using the *bite.kmeans.sampling* function of the BITEV2 v. 2.1.2 R package's [391]. The resulting dataset included 816 individuals from 34 populations (Table 4.3.1 and Supplementary Table S4.3.1).

4.3.3.3 Population structure and phylogenomic relationships

To investigate population structure, we performed a multidimensional scaling analysis (MDS) with Plink v1.9 using *--mds-plot eigvals –cluster*, setting a number of dimensions equal to the number of individuals. To better visualize both local and global structures and relationships, we further reduced dimensionality using a Potential of Heat-diffusion for Affinity-based Transition Embedding (PHATE) algorithm, as implemented in phateR v1.0.7 library [492], using the first 20 principal components (PCs) from the MDS and applying the following parameters: knn = 34 (equal to the number of analyzed breeds), decay = 100, and gamma = 0.

Phylogenetic trees were constructed using population-level bootstrapped Reynolds distances, calculated using an in-house script, and individual-level bootstrapped identity-by-state (IBS) distances, calculated with PHYLIP v3.697 [493]. The trees were visualized using ggtree v3.10.1 R package [494]. Moreover, we used Treemix v1.13 [158] to investigate historical gene flow, testing models with 0 to 20 migration events. Migrations were also assessed using *f3* statistics.

After phasing data with Beagle v4.1 [495], we analyzed haplotype sharing based on identity-by-descent (IBD) with RefinedIBD v3.1 [415], applying a 40 Mb long sliding-window with 0.15 Mb trimming. We retained segments that were at least 1.5 Mb long and with a minimum LOD score of 3.0. Segments shared between individuals of different breeds were analyzed, and pairwise medians calculated. A value of 0 was assigned to pairs of populations sharing no segments. The top 5% haplotype sharing among breeds was visualized using circlize v0.4.16 R package [416].

The individual genetic background was analyzed with ADMIXTURE v1.3 [161], testing a number of clusters (K) from 2 to 35. The best-fitting model was selected based on the lowest five-fold cross-validation error (c-v). For each individual, ancestry fractions (Q-values) for each cluster were calculated.

4.3.3.4 Genetic diversity and inbreeding

Genomic diversity was assessed for each population by calculating observed and expected heterozygosity (Ho and He, respectively) using Plink v1.9. Plink was also used to detect runs of homozygosity (ROHs) with a sliding window approach. As suggested by Meyermans *et al.* (2020) [136], no MAF or LD pruning was applied for this analysis; however, we excluded direct relatives and duplicated animals but did not balanced breed sizes, resulting in a dataset of 2,066 individuals and 45,740 SNPs. The following parameters were used: homozyg-density 73, homozyg-gap 500, homozyg-kb 1000, homozyg-snp 49, homozyg-window-het 0, homozyg-window-missing 2, homozyg-window-snp 49. Specifically, the minimum number of SNPs defining both a ROH and the window size was calculated using L parameter, whereas the density parameter was identified as the minimal value that maximized genome coverage, which, with this setting, reached 98.4% [136]. A ROH-based inbreeding coefficient (F_{ROH}) was calculated for each individual according to McQuillan's formula [133], both for all detected ROH segments and by ROH length class (1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, and >16 Mb), allowing for an estimation of the timing of inbreeding events [135].

The effective population size (N_e) of all populations was calculated using two LD-based software tools: SNeP v1.1 [140], which analyzed SNPs spaced between 280 Kb and 20 Mb and applied the Sved & Feldman recombination rate correction [412]; and GONE v1.0 [496], using default parameters. In both cases, only generations 1 to 100 were plotted.

4.3.3.5 Exploring recent evolution in Italian sheep breed genome

To explore the genomic changes that occurred in Italian sheep populations over recent decades, we compared our data with those from the Biovita project [73], which includes 492 sheep from 20 Italian breeds sampled from 2002 to 2009. For consistency, we merged it with our balanced dataset, applying the same quality filtering, including the exclusion of relatives and the sample size balancing of the Altamurana (ALT) breed, which was sampled in two different locations (see [73]), thus resulting in more than 30 individuals (that was set as the maximum breed size). The final dataset consisted of 1,281 individuals and 39,418 SNPs (Supplementary Table S4.3.1). Among the included breeds, 18 were present in both the datasets, two (Pinzirita – PNZbv and Leccese – LCCbv) only in the Biovita dataset, and 16 only in ours.

On this dataset, we performed MDS, Admixture, F_{ROH}, and heterozygosity analyses as previously described. Additionally, we used PLINK 1.9 to conduct Fst analysis on the breeds common to both datasets, in order to identify genomic regions that have changed the most over time. For the SNPs that, for each comparison, were associated with the top 1% absolute Fst values, we annotated and analyzed genes located within a ±20 kb window around the associated loci, corresponding, approximately, to the distance at which LD halves in sheep species [497]. A gene ontology (GO) enrichment analysis was performed for the set of genes associated with each breed. Using GeneCodis v.4 platform [498], these genes were compared against a background set of genes that were intercepted by the windows around all the possible SNPs of the chip after quality control. GO terms were identified based on the annotation of both *Homo sapiens* and *Bos taurus*, it being the closest available species to sheep, and a significant threshold of p-values adjusted with Benjamini-Hochberg correction of 0.05 was applied. Significant GO terms were grouped according to semantic similarity with GO-Figure! v1.0.2 [499]. Additionally, a list of sheep quantitative trait loci (QTLs) was retrieved from the Sheep QTLdb (Release 56, including 5,417 QTL data) [424]. Those QTLs that fell within the window around the identified SNPs were analyzed. To account for the different number of annotated QTLs per type, we assessed whether the identified SNPs for each breed were significantly enriched in known ovine QTLs. To do this, we determined the total number of SNPs on the genotyping array (N) or among the identified SNPs for each breed (n) that overlapped any QTL annotated in sheep as well as the total number of SNPs on the array (K) or among the identified SNPs for each breed (k) overlapping each specific QTL type. Enrichment p-values were calculated using the hypergeometric test through *phyper(k-1, K, N-K, n, lower.tail = FALSE)* function in R, which tests the probability of observing at least k overlapping SNPs in the breed-specific set. Multiple testing correction was applied using the Benjamini–Hochberg false discovery rate (FDR) method with *p.adjust* R function.

To investigate the possible influence of climate change on genomic variation, we also examined the correlation between Fst values of all SNPs and changes in 20 climatic variables (Supplementary Table S4.3.2) across the breeding ranges of the analyzed populations. Specifically, we retrieved the annual means of 19 bioclimatic variables corresponding to those of the WorldClim dataset and an aridity index from the "Global bioclimatic indicators from 1979 to 2018 derived from reanalysis" via the Copernicus platform [500,501]. These data covered the period 1979–2018 at a resolution of 0.5° x 0.5°.

For each variable, we calculated the median value in the first half of the time span (1979–1998), which we associated with the historical Biovita samples, and in the second half (1999–2018), associated with the more recent samples from our study. We then computed the difference (Δ) between these two periods for each pixel across the study area. Using farm geolocation data described in Bionda *et al.* (2024) [321], we extracted the average values for each period and their differences at each farm location, considering a 10km-diameter buffer around them, and then calculated the breed-level means. This approach yielded, for each breed, a Δ (change) value for each of the 20 climatic variables, representing the environmental shift between the sampling periods. We then tested the correlation between Fst values at each SNP and the corresponding Δ values of the climatic variables using Pearson correlation. Resulting p-values were adjusted for multiple testing using Benjamini-Hochberg procedure as implemented in *p.adjust* function from stats R package. We further investigated SNPs that showed a statistically significant association with at least one climatic variable and that also fell within the top 1% of Fst values for at least one breed. Genes and QTLs within a ±20 kb window around the SNPs associated with each climatic variable, were analyzed as described above.

4.3.3.6 Comparison of Italian local breeds and foreign breeds with Italian herd books

To assess the possible influence on the genomic background of Italian local breeds, a comparison was also done with publicly available data of breeds of foreign origin recognized in Italian herd books [70,502,503], namely: Berrichon du Cher (BRC_FR), Charollais (CHA_FR), Île-de-France (IDF_FR), Lacaune (LAC_FR and LAM_FR for dairy and meat varieties, respectively), Mouton d'Ouessant (OUE_FR), and Romanov (ROM_FR) from France; Suffolk, originated in Great Britain (however, only data sampled in France were available, and therefore called it SUF_FR); Texel, originated in the Netherlands (GTX_NL and STX_NL for German and Scottish Texel, and TEX_FR for Texel sampled in France); and East-Friesian from Germany (EFW_DE and EFB_DE for white and brown variety, respectively). A maximum of 30 unrelated animals per population was included.

We merged these data both with the present Italian sheep dataset (obtaining a final dataset of 1,114 sheep and 37,597 SNPs after quality control described above), and with the Biovita dataset (final dataset of 753 individuals and 32,997 SNPs) to investigate possible differences between the relationship of foreign breeds with present and past Italian population

(Supplementary Table S4.3.1). Specifically, we performed MDS, IBD-based haplotype sharing, and admixture analyses.

4.3.3.7 Local ancestry inference analysis

To investigate introgression in Nera di Arbus (PNA) and Gentile di Puglia (GDP) at the chromosome-level, local ancestry inference (LAI) was analysed using ELAI v1.01, which uses a two-layer hidden Markov model [504]. Haplotypes in the reference population are used to define features of each small genomic region in the target populations. In the LAI analysis, GDP and PNA breeds were considered as targets as they appeared significantly more introgressed by the Merinizzata Ttaliana (MER) and Sarda (SAR) than in the past GDPbv and PNAbv samples, which therefore were considered as reference populations.In particular, to verify that the introgressed population in GDP was the MER, and not the IDF_FR, we also performed MDS, MDS, IBD-based haplotype sharing, admixture, and supervised admixture analyses on a subset of breeds, that were selected among those appearing closer to the GDP in the initial admixture.

LAI analyses were performed using the following parameters: 20 expectation maximization steps (-s), 2 upper clusters (-C), 10 lower clusters (-c), and 50 previous generations before the admixture event (-mg). Those SNPs that either were not identified in a given population (--exclude-miss1) or for which the position was not recorded in the SNP position file (--exclude-nopos) were excluded from the analysis. The 99th percentile of local introgression proportion for each reference, such as MER and GDPbv in the LAI analysis of GDP and SAR and PNAbv in the LAI analysis of PNA, were considered highly introgressed. An ideogram illustrating the distribution of highly introgressed regions (99th percentile) in both target populations (GDP and PNA) was created using the Rideogram v0.2.2 R package [505]. We further investigated the regions spanning 20 kb upstream and downstream of each highly introgressed SNPs to identify the associated genes and QTLs, as previously described.

4.3.4 Results

4.3.4.1 Trend in population and farm size

Overall, the registered sheep population in Italy has shown a consistently negative trend, both in terms of farms and, even more markedly, in animal numbers. This decline is mainly attributable to the reduction in the number of animals belonging to breeds under selection programs (Figure 4.3.2a). In contrast, the overall trend for breeds under conservation programs showed a substantial increase in population size from 2012 to 2019, followed by a slow decline. As for farms, the trend has been variable, with some periods showing growth in the number of registered farms (2012–2015 and 2023), while others displayed negative (2014–2017) or stable patterns (Figure 4.3.2b). Consequently, the percentage of the national sheep population represented by breeds under conservation increased from 7% to 30%, while the proportion of farms rose from 30% to 55%. Trends in animal and farm registrations for each breed are shown in Supplementary Figure S4.3.1.

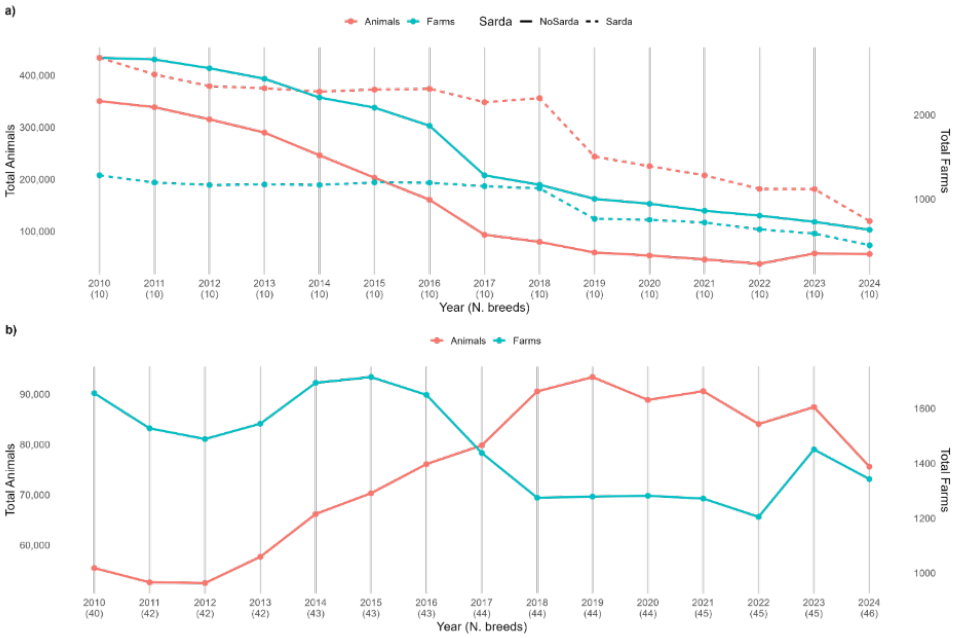


Figure 4.3.2 Trend in the number of registered animals and farms for breeds under selection programs, differentiating between Sarda and other breeds (a), and under conservation programs (b).

More than half of the breeds (29) experienced a contraction of over 10% in the number of farms, with seven losing at least half of their holdings and three becoming formally extinct. Conversely, about one-third of the breeds (20) showed an increase of at least 10% in farm numbers. Regarding animal numbers, population size increased in approximately half of the breeds (26), whereas more than one-third (22) declined by at least 10%. Among these, 18 breeds experienced a reduction of more than half of their initial population size. The average growth rate was negative for 11 breeds, positive for 24, and ranged between 0.95 and 1.05 for the remainder.

Table 4.3.2 Demographic data of Italian sheep breeds.

Breed	N. farms 2024	ΔFarms	N. animals 2024	ΔAnimals	Growth rate	Ne	ΔF	Risk
North-western Italy								
BERGAMASCA	70	-15 (-17.6%)	17063	1083 (6.8%)	1.09	579	0.09	NR
BIELLESE	16	-14 (-46.7%)	306	-1402 (-82.1%)	0.91	68	0.74	C
BRIANZOLA	25	-12 (-32.4%)	1518	471 (45%)	1.04	353	0.14	E
BRIGASCA	8	-7 (-46.7%)	1179	37 (3.2%)	1.1	113	0.44	E
DELLE LANGHE[b]	27	-58 (-68.2%)	1840	-1190 (-39.3%)	0.97	225	0.22	E
FRABOSANA	43	-17 (-28.3%)	4267	1846 (76.2%)	1.05	560	0.09	V
GARESSINA	0	-3 (-100%)	0	-92 (-100%)	0.92	0		Ex
PECORA CIUTA	19	18 (1800%)[a]	545	544 (54400%)[a]	2.42	264	0.19	E
PECORA DI CORTENO	12	-2 (-14.3%)	326	101 (44.9%)	1.04	106	0.47	C
ROSSET	40	-7 (-14.9%)	214	124 (137.8%)	1.61	210	0.24	C
SALTASASSI	0	-2 (-100%)	0	-24 (-100%)	1.05	0		Ex
SAMBUCANA	56	3 (5.7%)	3445	1871 (118.9%)	1.06	523	0.1	V
SAVOIARDA	8	2 (33.3%)	263	182 (224.7%)	1.1	67	0.75	C
TACOLA	107	58 (118.4%)	13720	10765 (364.3%)	1.13	2182	0.02	NR
North-eastern Italy								
ALPAGOTA	42	-9 (-17.6%)	1459	209 (16.7%)	1.02	345	0.15	E
BROGNE	32	7 (28%)	1220	204 (20.1%)	1.03	426	0.12	E
CORNELLA BIANCA	8	3 (60%)	299	192 (179.4%)	1.1	95	0.53	C
CORNIGLIO	13	-3 (-18.8%)	1297	590 (83.5%)	1.05	413	0.12	E
ISTRIANA-CARSOLINA	6	0 (0%)	423	-8 (-1.9%)	1.01	69	0.73	E
JURASCHAF-GIURASSICA	26	-16 (-38.1%)	5	-267 (-98.2%)	0.85	0		Ex
LAMON	19	10 (111.1%)	257	143 (125.4%)	1.11	127	0.39	C
PLEZZANA	3	-1 (-25%)	336	242 (257.4%)	1.11	135	0.37	C
SCHNALSERSCHAF	27	-9 (-25%)	35	-315 (-90%)	0.89	0		Ex
SCHWARZ BRAUNES BERGSCHAF	51	-129 (-71.7%)	36	-1725 (-98%)	1.13	11	4.55	C
SCHWARZNASENSCHAF	31	27 (675%)	282	269 (2069.2%)	1.4	206	0.24	C
TIROLER BERGSCHAF	83	-324 (-79.6%)	33	-4813 (-99.3%)	0.71	8	6.65	C
TIROLER STEINSCHAF	3	2 (200%)[a]	0	-6 (-100%)[a]	1.34	0		Ex

VICENTINA-FOZA	14	11 (366.7%)	186	88 (89.8%)	1.05	128	0.39	C
VILLNOESSER SCHAF-FIEMMESE	67	-45 (-40.2%)	579	-639 (-52.5%)	0.97	142	0.35	E
Central Italy								
APPENNINICA[b]	113	-27 (-19.3%)	5147	-4390 (-46%)	0.96	969	0.05	V
DELL'AMIATA	46	42 (1050%)	3061	2983 (3824.4%)	1.72	476	0.11	E
FABRIANESE[b]	29	-25 (-46.3%)	1525	-1754 (-53.5%)	0.95	317	0.16	E
GARFAGNINA BIANCA	29	16 (123.1%)	1391	1168 (523.8%)	1.18	182	0.28	E
MASSESE[b]	65	-9 (-12.2%)	6121	-3108 (-33.7%)	0.98	778	0.06	V
MERINIZZATA ITALIANA[b]	113	-79 (-41.1%)	7821	-19187 (-71%)	0.92	1424	0.04	NR
NOSTRANA	0	-1 (-100%)[a]	0	-1 (-100%)[a]	1	0		Ex
POMARANCINA	31	4 (14.8%)	1334	654 (96.2%)	1.06	236	0.21	E
QUADRICORNA	2	0 (0%)[a]	52	0 (0%)[a]		50	1	C
SOPRAVISSANA	60	10 (20%)	5449	-245 (-4.3%)	1	797	0.06	V
Southern Italy								
ALTAMURANA	6	1 (20%)	317	-74 (-18.9%)	1.01	149	0.34	C
BAGNOLESE	144	30 (26.3%)	12983	1404 (12.1%)	1.02	2059	0.02	NR
DI BENEVENTO-QUADRELLA	1	0 (0%)[a]	10	0 (0%)[a]	1.03	4	13.89	C
GENTILE DI PUGLIA	40	11 (37.9%)	3999	244 (6.5%)	1.02	983	0.05	V
LATICAUDA	68	9 (15.3%)	2955	-58 (-1.9%)	1	562	0.09	E
MOSCIA LECCESE	21	5 (31.2%)	690	-751 (-52.1%)	0.96	115	0.44	E
TRIMETICCIA DI SEGEZIA	1	0 (0%)[a]	18	18 (Inf%)[a]	0.94	10	5	C
SCIARA-MOSCIA CALABRESE	1	0 (0%)	10	9 (900%)	1.3	6	7.81	C
TURCHESSA	10	6 (150%)	2222	2156 (3266.7%)	1.34	275	0.18	E
ZERASCA	17	-11 (-39.3%)	851	181 (27%)	1.21	156	0.32	E
Isles								
BARBARESCA[b]	18	-12 (-40%)	594	-1270 (-68.1%)	0.94	280	0.18	E
COMISANA[b]	31	-550 (-94.7%)	1755	-50174 (-96.6%)	0.79	345	0.14	E
NERA DI ARBUS	88	29 (49.2%)	7141	4764 (200.4%)	1.09	1013	0.05	NR
NOTICIANA	1	0 (0%)	312	310 (15500%)	1.99	35	1.43	E
PINZIRITA[b]	8	-253 (-96.9%)	0	-24280 (-100%)	0.6	0		Ex
SARDA[b]	452	-831 (-64.8%)	119614	-314165 (-72.4%)	0.92	25739	0	NR
VALLE DEL BELICE[b]	180	-1027 (-85.1%)	15036	-191182 (-92.7%)	1.09	20	2.5	C

[a] Breeds that were recognized after 2010. For these breeds, ΔFarms and ΔAnimals refers to the difference between the recognition year and 2024.

[b] Breeds under selection programs; all other breeds are under conservation programs.

FAO extinction risk categories: NR = Not at risk; V = Vulnerable; E = Endangered; C = Critical; Ex = Extinct.

According to Ne estimates, one-quarter of populations (14) are at short-term risk of extinction (Ne < 50), while more than half (29) are at long-term risk (Ne between 50 and 500). Only five populations (Sarda, Tacola, Bagnolese, Merinizzata Italiana, and Nera di Arbus) showed Ne values exceeding 1000. For the seven populations with Ne = 0, ΔF could not be estimated. Among the remaining populations, the average ΔF was 1.07 ± 2.5%, with 37 populations showing values below 0.5% and five exceeding 3% (Table 4.3.2).

According to the FAO classification, only 11% of breeds (6) are not at risk of extinction, whereas six are considered vulnerable, 20 endangered, and 17—about 30%—critical. Furthermore, seven populations should be regarded as extinct based on 2024 registrations (Table 4.3.2).

4.3.4.2 Population structure and phylogenomic relationships

The population structure emerging from the first three PCs of the MDS analysis and PHATE (Figure 4.3.3a and Figure 4.3.3b, respectively) strongly reflects the geographical origin and/or distribution of sheep breeds across the Italian peninsula. Notably, the PHATE plot closely resembles PC1 and PC3 of the MDS, whereas PC2 mainly separates the Delle Langhe (ODL) sheep, and to a lesser extent two other Piedmontese breeds—Frabosana (FRB) and Savoiarda (SVR)—from the rest of the individuals.

Reynolds distances among populations, represented in Figure 4.3.3c, similarly divide the populations into two main branches: one grouping the insular and southern Italian breeds (top), and the other grouping the northern ones (bottom). However, ODL, FRB, and SVR, as well as Istriana-Carsolina (IST), are located near the central-southern populations, reflecting the pattern seen in the MDS.

When analysing individual identity-by-state (IBS) distances (Figure 4.3.3d), these same populations appear at the base of the tree, while all the others follow a south-to-north gradient. Individuals from the same breed are generally placed contiguously and without substantial intermixing with other populations, with a few exceptions: Biellese (BLS), Tacola (TCL), and Bergamasca (BGS) subjects are intermixed to each other, with part of the latter population also located between the Pecora di Corteno (PCC) and Sambucana (SMN) breeds.

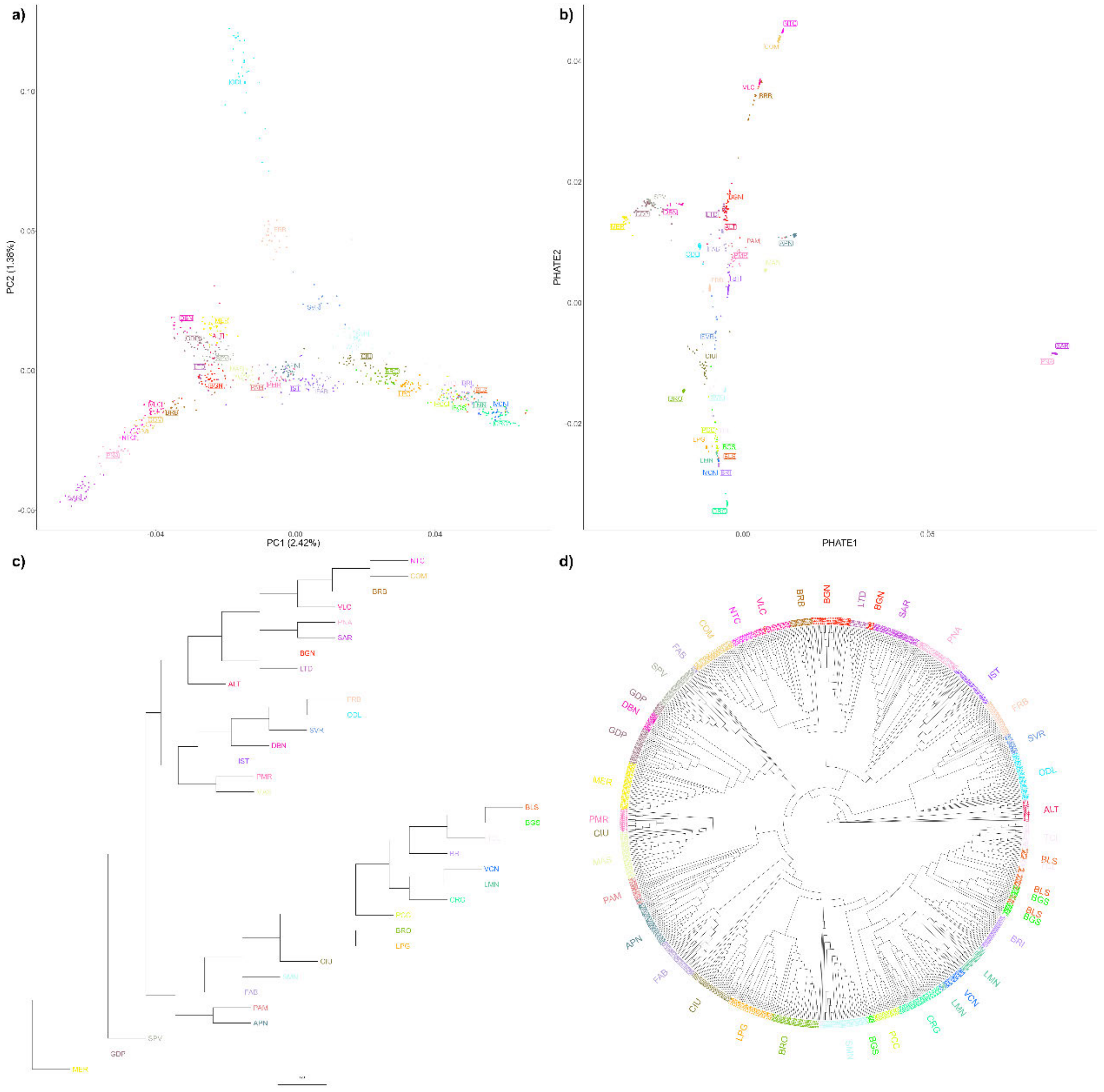


Figure 4.3.3 Italian sheep demographic structure. First two principal components (PCs) of multidimensional scaling analysis (a) and PHATE (b). Each point represents a subject, each color a breed. Dendrograms based on bootstrapped population-level Reynolds distances (c), bootstrapped individual-level identity-by-state distances (d).

Gene flow among populations was investigated using TreeMix. A number of four migration events was chosen as the optimal configuration, and the results are shown in Figure 4.3.4a. The strongest migration signals were observed from SMN to the node grouping the Piedmontese breeds (ODL, FRB, SVR) and from the node ancestral to Pecora Ciuta (CIU) and other northern breeds to SVR. Additional gene flows were detected from Merinizzata Italiana (MER) to CIU and from the base of the CIU branch to Appenninica (APN). However, the *f*3 test did not identify any statistically significant admixture events.

As shown in Figure 4.3.4b, haplotype sharing is most common between geographically close breeds, especially among northern populations. In particular, TCL, BLS, BGS, Corniglio (CRG), and Vicentina-Foza (VCN) present significant sharing with several other northern breeds. Long shared segments were also found between the two Sicilian breeds Noticiana (NTC) and Comisana (COM), and between Sarda (SAR) and Nera di Arbus (PNA) from Sardinia. Interestingly, MER-CIU and ODL-Altamurana (ALT) pairs also show extensive haplotype sharing, despite having different geographic origins, as already highlighted in the TreeMix results.

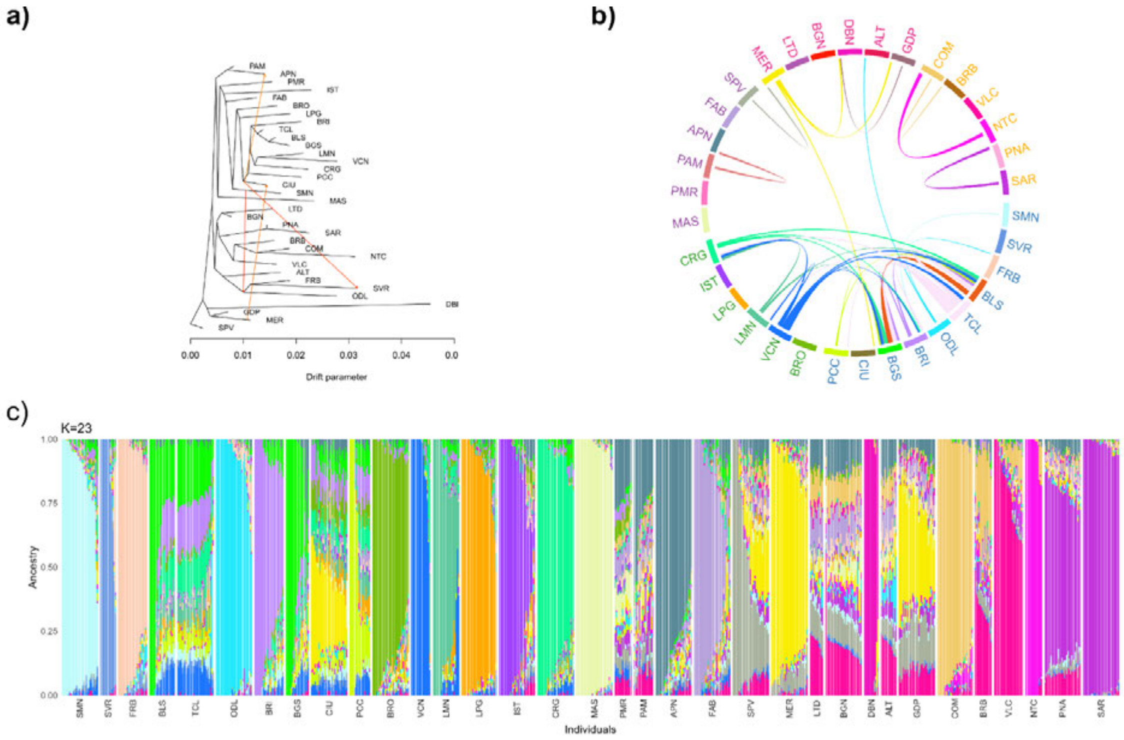


Figure 4.3.4 Gene flow and genomic background of Italian sheep breeds. a) TreeMix analysis with four migration events, represented by arrows colored according to the ancestry percentage received from the donor. b) Top 5% longest identity-by-descent-based haplotype sharing among breeds. Breed names were ordered and colored by geographic location. c) Admixture best-fitting model, with 23 clusters (K). Breeds have been ordered by geographic location; each bar represents an individual, and each color represents a cluster.

At low K values (Supplementary Figure S4.3.2), Admixture analysis clustered populations according to their geographical distribution, with the ODL cluster emerging early (K = 3). The lowest c-v was obtained at K = 23 (Figure 4.3.4c), where most of the populations are characterized by distinct clusters, although many exhibit varying degrees of admixture.

Consistent with previous analyses, BLS and BGS share similar ancestry, as do SAR and PNA sheep, both originating from Sardinia. Some populations appear highly admixed, with proportions similar to those of other breeds within the same geographic range. This is the case, for example, of Pomarancina (PMR) and Dell'Amiata (PAM) or Bagnolese (BGN), Laticauda (LTC) and ALT. Notably, ALT differs from the latter group because it also shows a background component similar to ODL. When a specific ALT cluster emerges (K = 25), it becomes predominant not only in ALT but also in BGN and LTD (together with COM), as well as in Barbaresca (BRB) (with COM and Valle del Belice-VLC) and Gentile di Puglia (GDP) (with MER and, to a lesser extent, Sopravissana-SPV).

4.3.4.3 Genetic diversity and inbreeding

The average F_{ROH} in Italian sheep populations is 6.56±6.1%, ranging from a value of 2.31% in TCL to 14.55% in Di Benevento (DBN) breed (Table 4.3.1). Several populations display high levels of recent inbreeding (related to ROH longer than 16 Mb), sometimes despite relatively low levels of total inbreeding, as observed in BGS and SPV (Supplementary Figure S4.3.3a).

Estimates of Ne varied widely between methods: GONE returned values between 31 and 1699, while SNeP gave estimates from 9 to 40. These extreme estimates corresponded to the most (DBN) and least (TCL) inbred populations, respectively (Table 4.3.1 and Supplementary Figure S4.3.3b).

4.3.4.4 Exploring recent evolution in Italian sheep breed genome

The comparison between the current dataset and the one generated within the Biovita project, which includes animals sampled about 20 years ago, through MDS analysis shows that most of the populations shared between the two datasets cluster closely, with no observable separation (Figure 4.3.5a). The only exception concerns the ALTbv individuals, which partially overlap with the current ALT and partially diverge along PC1. However, it should be noted that the Biovita dataset included two ALT subpopulations, one from Foggia and one from Bari, whereas the vast majority of recent samples came from Bari province and none from Foggia.

In the Admixture analysis, which identified the lowest c-v at K = 28 (Figure 4.3.5c, Supplementary Figure S4.3.4), several breeds present very similar backgrounds across datasets, such as APN, BGN, COM, Alpagota (LPG), LTD, Massese (MAS), ODL, SAR, SPV, and VLC. In the case of BGS and BLS, the overall ancestral composition appears similar, with a shared prevailing cluster; however, intra-dataset individuals show stronger similarity to each other than to their counterparts in the other dataset. Fabrianese (FAB) sheep appear more admixed than FABbv. The same applies to GDP, although in this case the difference is mainly due to a marked introgression from MER in our samples, which is absent in GDPbv. Lastly, some populations show internal substructures. Consistent with the MDS results, ALTbv is divided into two subpopulations, both of which are significantly present in ALT. IST background is composed of two main ancestry clusters, one unique to IST and the other predominant in ISTbv and present in a minority of current IST individuals. PNAbv features a distinctive cluster that is still evident, though not dominant, in PNA, which appears genetically closer to SAR/SARbv population.

These observations are further supported by the comparison of F_{ROH} between the same breeds in the two datasets (Figure 4.3.5b). In general, F_{ROH} values are higher in the current dataset than in the Biovita older data for the same breed. The greatest difference is observed in IST, now among the most inbred breeds, whereas ISTbv had one of the lowest inbreeding levels. Conversely, VLC and GDP in the current dataset are less inbred and exhibit higher heterozygosity than their Biovita counterparts, greatly depending on the increase in recent inbreeding.

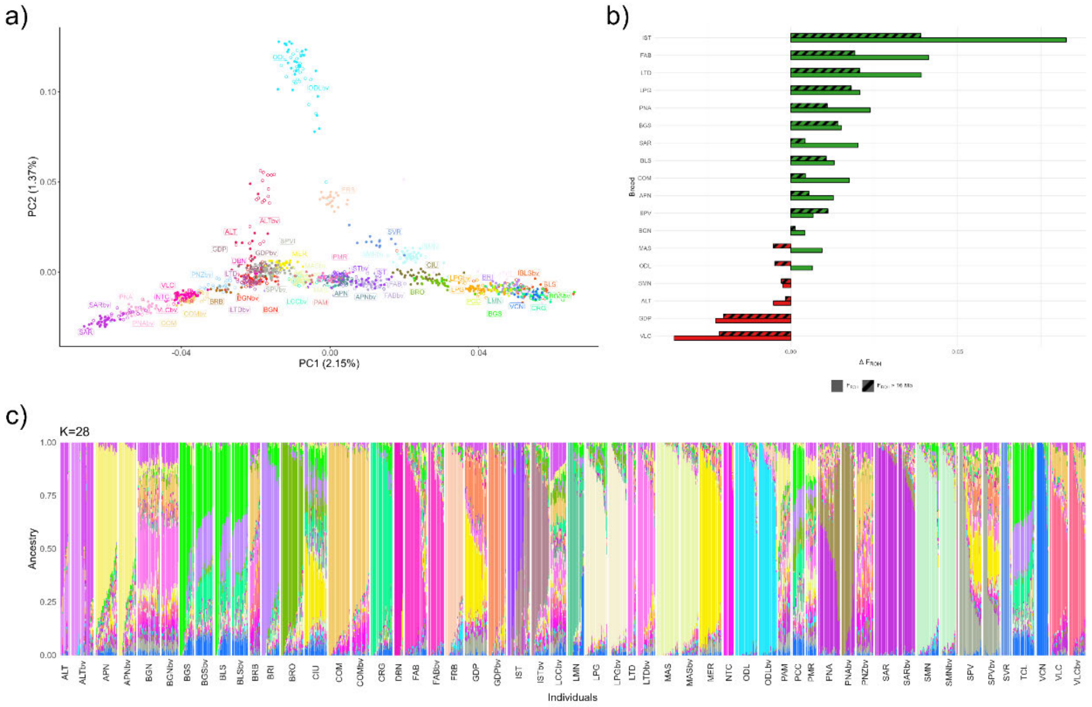


Figure 4.3.5 Comparison of Italian sheep breeds sampled 20 years apart. a) Multidimensional-scaling analysis; empty and full points represent samples from Biovita and current dataset, respectively. b) Difference in F_{ROH} between subjects of the same breed from the Biovita and current dataset. Both overall F_{ROH} and that associated with recent inbreeding events (ROH longer than 16 Mb) were included. c) Best-fitting model of Admixture analysis. In all plots, codes including 'bv' refer to samples from the Biovita project, collected approximately 20 years ago. Abbreviations of breeds only present in the Biovita dataset: LCCbv = Moscia Leccese; PNZbv = Pinzirita.

4.3.4.5 Comparison of Italian local breeds and foreign breeds with Italian herd books

The MDS plot including Italian sheep from the present-day dataset and the breeds of foreign origin recognized in Italian herd books (hereafter referred to as "foreign breeds") (Figure 4.3.6a) shows a clear separation of the two groups along PC1, while PC2 distributes the breeds along a geographic gradient. Texel and Friesian breeds appear clearly isolated from the Italian sheep, whereas the French breeds are positioned more closely. In particular, the two Lacaune populations (LAC_FR and LAM_FR) cluster with central Italian breeds, and the CIU and the Romanov (ROM_FR) show partial overlap. Additionally, the MER is clearly shifted toward the group of the French breeds: Île-de-France, Berrichon du Cher, and Mouton d'Ouessant (IDF_FR, BRC_FR, OUE_FR, respectively). However, PC3 clearly isolates OUE_FR from all other populations, indicating a markedly distinct genomic composition.

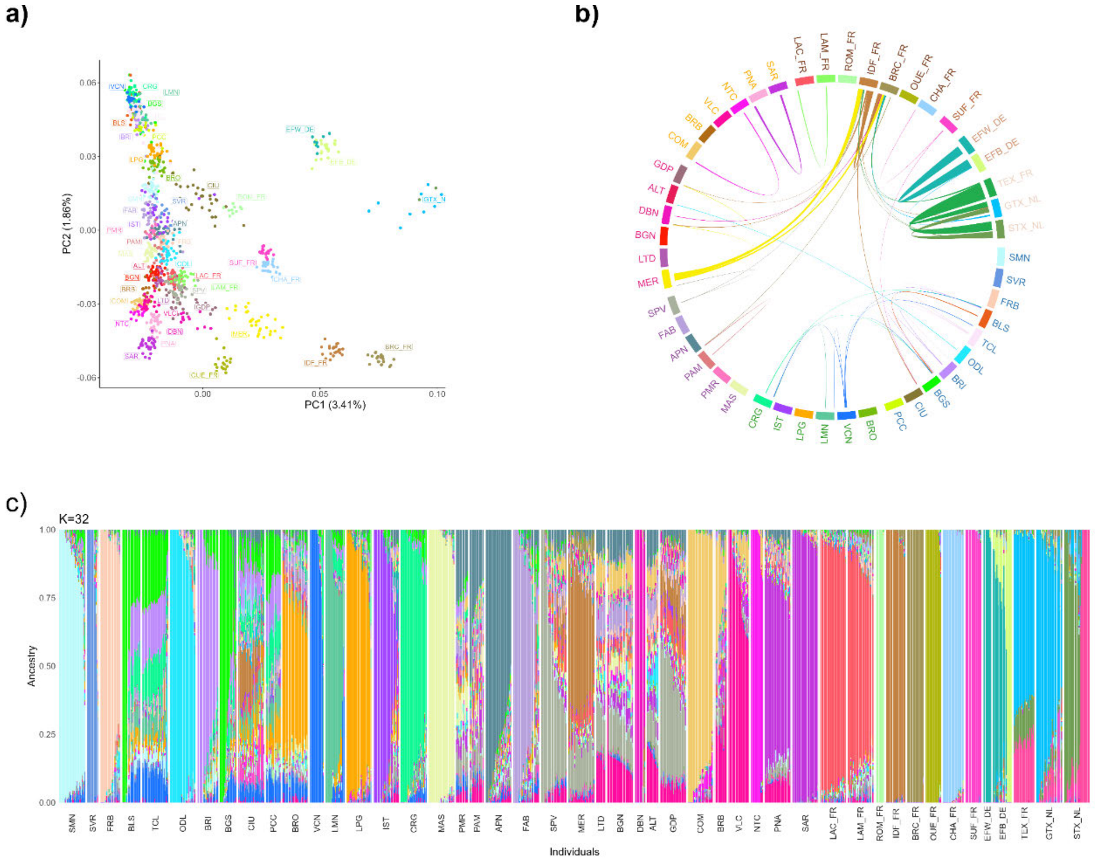


Figure 4.3.6 Populations structure and genomic background: a comparison between Italian and foreign breeds. a) Multidimensional-scaling analysis. b) 5% longest identity-by-descent-based haplotype sharing. Breeds are ordered by geographic location. c) Best-fitting model of Admixture analysis. Breed names are ordered and colored by geographic location.

The results of haplotype sharing and Admixture analyses (Figure 4.3.6b and Figure 4.3.6c, respectively) partially reflect the MDS findings. In general, we observed minimal introgression from most foreign breeds, with only a few exceptions. The breed showing the highest level of haplotype sharing with non-Italian breeds—particularly with IDF_FR and, to a lesser extent, BRC_FR—is the MER. This breed no longer forms its own distinct cluster but instead exhibits a genomic background composed of approximately half of the cluster maximized in IDF_FR. This component is also present in SPV and GDP, likely as a result of MER introgression. As also seen in the MDS, the CIU breed shows a foreign component, primarily derived from the Suffolk (SUF_FR) and IDF_FR breeds; however, the latter could result from MER introgression, as observed in the case of GDP.

In contrast, the same analyses conducted on the Biovita dataset reveal markedly less introgression and haplotype sharing between Italian and foreign breeds. Significant sharing is limited to the presence of Friesian populations (EFB_DE and EFW_DE) in ISTbv, and of IDF_FR in SPVbv, while it is almost entirely absent in GDPbv. However, it is important to note that several breeds showing high levels of introgression from foreign breeds in the present-day dataset, such as MER and CIU, are not included in the Biovita dataset.

4.3.4.6 Identifying highly differentiated genomic regions via Fst

An Fst comparison between current and older Biovita populations was performed for the 18 breeds common to both datasets. For each breed pair, the SNPs within the top 1% of absolute Fst values were identified, and the corresponding genes and QTLs were investigated. The results are reported in Table 4.3.3 and Supplementary Table S4.3.3.

Among the identified regions, several encompassed QTLs. Across almost all breeds, we detected QTLs associated with weight, milk yield, milk protein and fat yield, horn type, *M. paratuberculosis* susceptibility, teat number, and faecal egg count. To account for potential biases due to the unequal number of annotated QTLs across categories, we performed a QTL enrichment analysis (Figure 4.3.7a) for each breed. The results revealed significant associations between SNPs and fertility-related QTLs in ALT, APN, BGN, COM, and MAS. QTLs linked to udder health were detected in GDP and PNA; to parasite infestation in SAR, FAB, LTD SPV, LPG, BLS, and COM; and to respiratory diseases in LTD, COM, and BGS. Wool-related QTLs were found in COM, IST, LTD, ODL, and SPV, while FAB and IST showed enrichment for QTLs associated with milk and cheese production. Several breeds also displayed QTLs related to morphological traits, including body size in BLS, COM, IST, SAR, and VLC.

Table 4.3.3 Results of Fst comparison among individuals of the same breeds sampled 20 years apart and correlation with difference in climatic variables.

Breed	Top 1% Fst	N. significant SNPs	N. genes	N. QTL	N. SNPs associated with environmental variables	N. Genes associated with environmental variables
ALT	0.16 - 0.37	388	152	24		
APN	0.10 - 0.23	393	179	20	BIO09: 2 BIO16: 3	BIO09: 1 BIO16: 1
BGN	0.14 - 0.40	393	178	30		
BGS	0.21 - 0.47	391	172	23	BIO09: 25	BIO09: 22
BLS	0.18 - 0.40	390	157	28	BIO09: 5 BIO16: 4	BIO09: 8 BIO16: 2
COM	0.13 - 0.31	390	179	14		
FAB	0.16 - 0.38	391	174	27		
GDP	0.28 - 0.58	392	162	20		
IST	0.26 - 0.53	391	173	32	Aridity annual mean: 2 BIO16: 117	Aridity annual mean: 3 BIO16: 112
LPG	0.12 - 0.33	389	169	17	BIO11: 12 BIO16: 1	BIO11: 10
LTD	0.22 - 0.51	391	170	22		
MAS	0.17 - 0.41	384	166	8	Aridity annual mean: 7	Aridity annual mean: 8
ODL	0.15 - 0.38	386	153	23	BIO16: 1	BIO16: 1
PNA	0.23 - 0.54	389	169	34		
SAR	0.12 - 0.51	387	165	24		
SMN	0.12 - 0.30	391	190	20	BIO16: 7	BIO16: 6
SPV	0.14 - 0.36	393	165	29	BIO16: 6	BIO16: 4
VLC	0.17 - 0.35	390	185	16		

However, after applying Benjamini–Hochberg (BH) correction for multiple testing, the only significant signals were: *M. paratuberculosis* susceptibility in BGS; body circumference in BLS; pleurisy and staple length in COM; gastrointestinal nematode resistance in SAR; tail length in PNA; horn type and chest width in IST; and entropion in VLC.

A total of 5,638 genes were found in the region surrounding the identified SNPs (Supplementary Table S4.3.4). However, using these genes, only a few significant GO terms were found only for some breeds (Supplementary Table S4.3.5), mainly related to neurological development and functionality (APN, BGS, COM, FAB, LTD, LPG, and ODL); metabolic processes such as nitric oxide biosynthesis (SAR), arginine catabolism (SAR), UPD-glucose transport (ODL), peptide biosynthesis (ODL); immunity (ODL and LTD); cell adhesion (LTD and LPG); regulation of dopamine receptor signalling (ODL); and tissue and organ development (LPG and ODL).

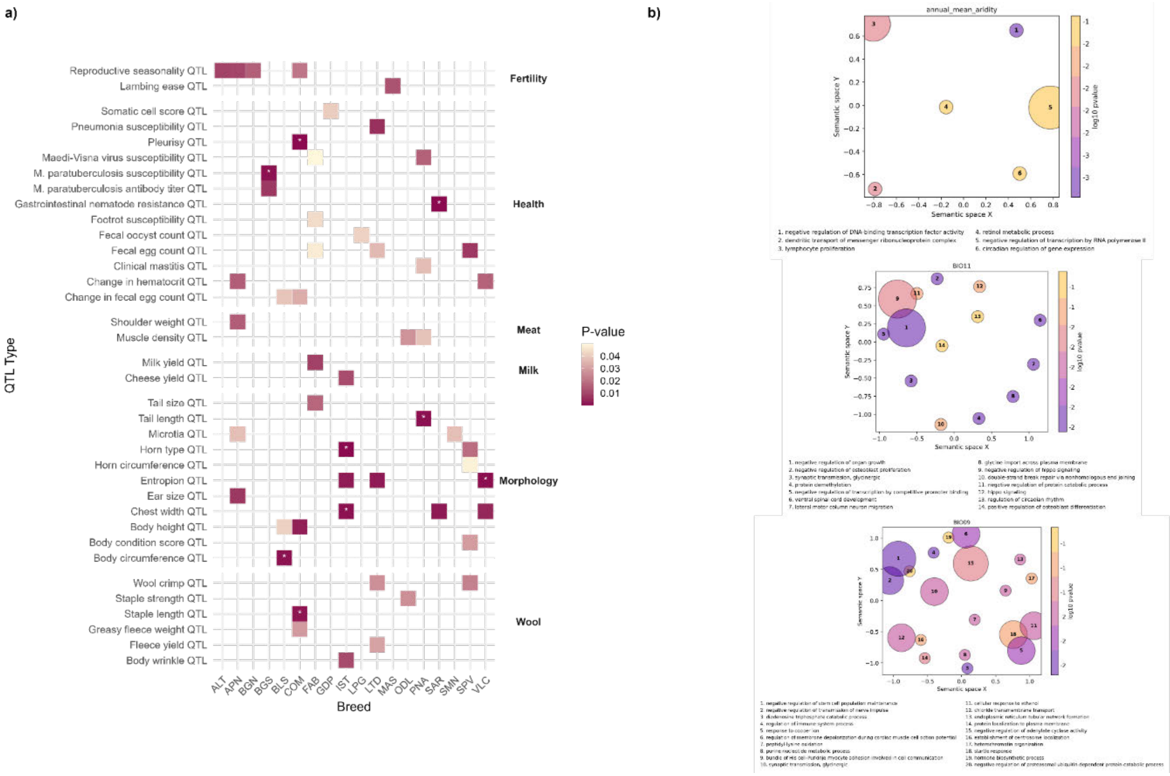


Figure 4.3.7 Results from the temporal comparison of subjects from the same breed sampled 20 years apart. a) Significantly enriched QTLs by breed; QTLs with significant adjusted p-values are indicated with *. b) Semantic clustering of significant GO-terms by environmental variable; results are referred only to GO-terms identified using *Bos taurus* annotation.

A total of 182 SNPs resulted significantly correlated with differences in at least one environmental variable and included in the top 1% Fst distribution of at least one breed comparison (Supplementary Table S4.3.6). The SNPs thus identified were correlated with aridity annual mean (IST and MAS breeds), BIO09-mean temperature during driest quarter (APN, BGS, and BLS), BIO11-mean temperature during the driest quarter (LPG), and, with the highest number, BIO16-Precipitation during wettest quarter (APN, BLS, IST, LPG, ODL, SMN and SPV). Among the 178 genes located near these SNPs (Supplementary Table S4.3.7), the majority (112) were related to BIO16 and differentiating IST from ISTbv. Among these latter genes, some were previously reported as involved in environmental adaptation.

For example, *CD109* was identified as differentiating Iranian sheep living in cold versus hot desert areas [506], and *ST3GAL* was associated with adaptation to heat stress in Egyptian sheep [507]. In addition, we identified three aquaporin genes—*AQP2*, *AQP5*, and *AQP6*—which play key roles in renal transport and urine concentration [47,508,509], as well as *EPAS1*, a hypoxia-related gene identified as a selection signature in high-altitude-adapted animals of several species [257,264].

RACGAP1 was found to be associated with isothermality in Italian goats and to differentiate breeds living in hot versus cold climates [74]; *ADAMTS20* was reported to distinguish goats with different coat colors [244]; and *NPAS3* was associated with local adaptation in goats [510]. In the same study, *FTO*—which we also identified among the top 1% Fst values for the SMN breed—was found to be significant. Several other studies have investigated *FTO* methylation under heat stress [511,512], and linked the gene to growth, meat production and quality, and feed efficiency [513–516], as well as fat metabolism, including adipose accumulation in the sheep tail [517,518].

Additionally, among the genes correlated with BIO09 and showing high Fst values for the BGS breed, we found *RTN1*, which encodes reticulum-associated organelles known as reticulons. This gene was previously linked to sheep adaptation to heat stress [507].

GO enrichment analysis identified 10 significant GO terms associated with genes correlated with the aridity annual mean variable when using *Bos taurus* annotation, and 20 when using human annotation (Supplementary Table S4.3.8). Semantic clustering (Figure 4.3.7b) revealed pathways related, among others, to immune system function (e.g., lymphocyte proliferation), retinol metabolism, circadian regulation of gene expression, regulation of triglyceride metabolism and androgen receptor signaling, and blood vessel development.

For genes correlated with BIO16, only one GO term was significant when using *Homo sapiens* annotation. However, this term is of particular interest, as it relates to renal water transport and involves two aquaporin genes, *AQP2* and *AQP6*.

Among the GO term clusters (Figure 4.3.7b) associated with genes correlated with BIO11 (16 for *Bos taurus*, 56 for *Homo sapiens* annotation), several are related to organ growth and osteoblast proliferation (including negative regulation of organ growth, positive and negative regulation of osteoblast proliferation and differentiation, and Hippo signaling), protein demethylation and catabolism, regulation of circadian rhythm, DNA repair, vocalization and walking behavior, glycine transport and glycinergic synaptic transmission, as well as numerous pathways associated with neurological development and function.

Finally, 31 significant GO terms were identified for BIO09, exclusively using *Bos taurus* annotation (Supplementary Table S4.3.8 and Figure 4.3.7b). These include terms related to immune system regulation, response to copper and zinc ions, purine metabolism, peptidyl-lysine oxidation, regulation of stem cell population maintenance, cardiac function (e.g., membrane depolarization and His-Purkinje fiber adhesion), regulation of adenylate cyclase activity, glycinergic synaptic transmission, negative regulation of nerve impulse transmission, startle response, and hormone biosynthesis.

4.3.4.7 Exploring introgression in Gentile di Puglia and Nera di Arbus breeds

As described above, the current PNA population appears to be more strongly introgressed by SAR compared to the same population in the Biovita dataset. Similarly, the present-day GDP shows signs of introgression from MER, with only a small portion of its genomic background shared with GDPbv. However, interpreting the situation in GDP is complicated by the evident introgression of IDF_FR into the MER breed.

Therefore, to determine whether the GDP was introgressed by MER or directly by IDF_FR, we created a subset of populations showing signs of introgression in GDP and MER, including IDF_FR and BRC_FR, along with Rambouillet (RMB_FR)—selected based on anecdotal evidence of possible crossbreeding and the occasional use of rams from French breeds by some GDP breeders.

In the MDS, GDP clusters with other Italian populations, while MER lies between the Italian sheep and IDF_FR. RMB_FR, instead, appears completely isolated along PC1. The unsupervised admixture analysis at K = 11 (the value with the lowest c-v) mirrors the results obtained from the full dataset: MER and GDP do not form specific clusters but are clearly admixed populations. The MER background is predominantly composed of the IDF_FR component, followed by SPV, whereas the GDP background resembles MER's but with stronger contributions from GDPbv and SPV. Haplotype sharing analysis shows that GDP shares relatively short segments with both IDF_FR and MER, with the latter being more prominent. Finally, in the supervised admixture analysis, where specific components were assigned to all breeds except GDP, results confirmed that the main sources of introgression into GDP were MER and SPV, rather than direct input from IDF_FR. Based on this, LAI was performed using MER as the reference population (Supplementary Figure S4.3.5).

The overall mean of local ancestry proportions of GDP are 0.64 ± 0.08 for MER (compared to 0.31 ± 0.06 IDF_FR, data not shown) and 0.36 ± 0.08 for GDPbv. Among the SNPs classified as highly introgressed (99^{th} percentile), 399 and 395 were found using MER and GDPbv as reference (Figure 4.3.8a and Supplementary Table S4.3.9), intercepting 177 and 199 genes, respectively (Supplementary Table S4.3.10).

Several genes involved in hair follicle development and wool type differentiation were found in GDP non-introgressed regions, including genes (e.g., *APCDD1*) previously reported in relation to high wool quality [519,520]. In contrast, MER-introgressed regions contain the *VDR* gene, also involved in hair follicle regulation [521].

Most of the genes found in non-introgressed regions are related to fertility. These include *BMPR1B* and *UNC5C*, known to regulate ovulation rate and litter size in sheep [522–524], *TCP1*, associated with male fertility in cattle [525], and *TDRD5*, involved in germ cell development [526]. Additionally, *DLG1*, which influences litter size [527], and *VDR* [528] were found in MER-introgressed regions.

GDP non-introgressed regions were enriched in genes related to fat metabolism, including *LPIN1*, involved in triglyceride and fatty acid synthesis in the mammary gland and in fat tail deposition in sheep [529,530], and *SLC4A7*, associated with fat deposition in cattle [531]. Genes associated with meat production traits were identified in both introgressed and non-introgressed regions. In MER-introgressed regions, genes included *ALDH1A1* (meat tenderness and juiciness [532]), *DLG1* (feed intake [533]), and *SLC16A7* (body growth [534]). In GDP non-introgressed regions, *APCDD1*, associated with beef meat quality [535], was found.

Finally, some genes are involved in environmental adaptation. *SLC16A7*, related to drought adaptation in cattle [536], and *KHDRBS2* [510] were located in introgressed regions. In contrast, *SOD2*, fundamental for oxidative stress protection and lipopolysaccharide response [537–540], was found in GDP non-introgressed regions.

Additionally, GDP regions introgressed from MER are enriched in QTLs related to production traits (e.g., fleece yield, milk protein yield) and tail size, whereas regions where GDPbv predominates are associated with udder health (e.g., clinical mastitis) and teat number (Figure 4.3.8c).

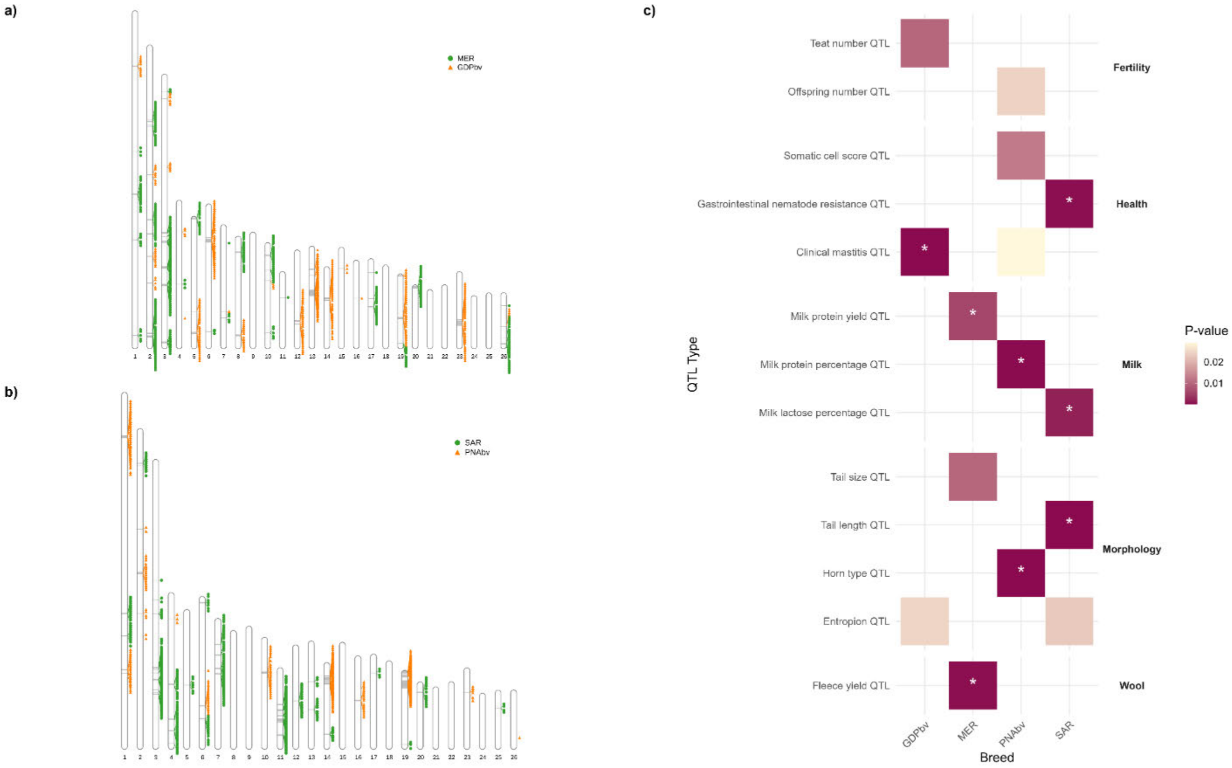


Figure 4.3.8 Results of local ancestry inference (LAI) on two introgressed breeds. a) Ideogram of highly introgressed regions (99th percentile) of Gentile di Puglia (GDP), comparing older GDP samples (GDPbv, orange triangles) and Merinizzata Italiana (MER, green circles). b) Ideogram of highly introgressed regions (99th percentile) of Nera di Arbus (PNA), comparing older PNA samples (PNAbv, orange triangles) and Sarda (SAR, green circles). c) Enriched QTLs included in GDP genomic regions associated with GDPbv or introgressed by MER and enriched QTLs included in PNA genomic regions associated with PNAbv or introgressed by SAR. QTLs with significant adjusted p-values are indicated with *.

Regarding the proportion of local ancestry of PNA, the overall means are 0.65 ± 0.09 and 0.35 ± 0.09 for SAR and PNAbv, respectively. Among the highly introgressed SNPs, 395 and 396 have been identified using SAR and PNAbv as references, respectively (Figure 4.3.8b, Supplementary Figure S4.3.6, and Supplementary Table S4.3.9), intercepting 374 and 194 genes (Supplementary Table S4.3.10).

Several genes in regions not introgressed from SAR and still associated with the older genomic background of PNA are related to fiber development and follicle biology. Among them, *EGFR* is essential for hair development and follicle integrity [541–545] and has been associated with fleece type in sheep [546]. *EGFR* acts in part via the β-catenin pathway and, accordingly, *CTNNB1*, another gene retained in PNA, is linked to fiber diameter in Merino sheep [547]. On the other hand, several fiber-related genes were found in regions that are highly introgressed from SAR. These include a large number of keratin genes (*KRT20*, *KRT24*, *KRT32*, *KRT34*, *KRT35*, *KRT36*) [548–552], as well as *RERE* [553] and *STAT3* [521], all strongly associated with wool quantity and quality.

Regarding milk production, among the genes located in introgressed regions is *STAT5A*, widely known for its role in mammary gland development and its influence on milk performance and composition, also through binding with caseins [554–558]. Conversely, *ACSF3*, found in a PNAbv-associated region, has been linked to goaty milk flavor [559], and *TGFBR2* is involved in mammary morphogenesis and development [560].

Additionally, some PNAbv regions harbor genes related to mastitis, such as *C9*, *FYB* [561], and *MYD88* [562]. The latter is also implicated in both inflammatory responses and resistance to small ruminant lentiviruses [563,564]. Other immune-related genes found in PNAbv-associated regions include *FOCF1*, a candidate for pneumonia resistance [565]. In contrast, introgressed regions contain *STAT5A* and *CSF3*, both associated with mastitis resistance in cattle [566,567], the latter being also linked to gastrointestinal nematode resistance [568].

Finally, both PNAbv and introgressed regions contain genes related to fitness traits, such as fertility and environmental adaptation. Indeed, in PNAbv regions, *CTNNB1* is associated with litter size in goats and sheep [483,569], whereas *CBFA2T3* and *DNAJA2* have been associated with heat tolerance in cattle [570–572], and *EGFR* is linked to environmental and altitude adaptation in sheep and Tibetan goats [573,574]. From the introgressed background, genes such as *IGFBP4* and *CHST11*, associated with fertility and prolificacy [575–577], were identified, as well as *TOP2A*, involved in feed efficiency in dairy sheep [578], and genes like *NR1D1*, *KRT24*, *BRCA1*, *ADAM22*, and *ACLY*, associated with environmental adaptation in both sheep and cattle [579–583].

The QTL enrichment analysis (Figure 4.3.8c) of introgressed genomic regions revealed that regions introduced into PNA from SAR are primarily associated with traits related to production (e.g., milk lactose percentage), morphology (e.g., tail length), and gastrointestinal nematode resistance. In contrast, regions derived from PNAbv include loci related to milk quality (e.g., milk protein percentage), horn morphology, and—although not significant after multiple testing correction—udder health (e.g., somatic cell score, clinical mastitis) and fertility (e.g., number of offspring).

Significant Gene Ontology (GO) terms were found only for PNA's SAR-introgressed regions, associated with "intermediate filament organization", and PNAbv-specific regions, associated with cardiac function ("bundle of His cell action potential" and "AV node cell action potential", both linked to the *SCN5A* and *SCN10A* genes).

4.3.5 Discussion

4.3.5.1 Demographic trends reveal the extinction risk status of Italian sheep breeds

The Italian ovine sector is characterized by remarkable diversity, shaped over centuries by regional traditions, varied farming systems, and environmental heterogeneity. This long history has produced a complex mosaic of local breeds, each adapted to specific landscapes and serving not only productive purposes but also cultural and ecosystem functions. Understanding this complexity requires integrating demographic, genealogical, environmental, and genomic information, which allows us to capture both the current structure of the sector and the evolutionary forces—natural and human-driven—that have shaped it over recent times.

However, this rich diversity appears to be under pressure: according to official census data from Asso.Na.Pa., both the number of registered sheep and sheep-holding farms in Italy have declined over the past 15 years, reflecting trends reported by ISMEA and ISTAT [53,54], which are based on the *Anagrafe Nazionale Zootecnica* (the official national database, for which registration is mandatory). By contrast, registration in the herd book is not mandatory (currently, sheep recorded in the herd book represent only about 5% of the national sheep population, including 4% Sarda sheep and 9% of other breeds under selection programs, but 45% of those under conservation programs), although such registration is legally required for animals to be considered purebred. For these reasons—and because the national database often contains inconsistent or missing breed information, occasionally fails to distinguish individual farms from aggregated sheep and goat holdings, and even includes farms with no animals recorded—the herd book database is the most reliable source of data for evaluating demographic trends and assessing breed risk status.

When considering only breeds officially classified as under selection, a persistent decline is evident. In contrast, data across all registered breeds under conservation show a period of growth in sheep numbers from 2012 to 2019, followed by a mild decline, while the number of farms remained quite variable. Therefore, overall, sheep belonging to local populations increased by approximately 36% between 2010 and 2024, despite the number of their farms decreasing roughly 19%. Despite still representing only a small portion of the total Italian sheep stock (30%), half of the total registered sheep farms hold individuals from local populations, confirming their fundamental role in the Italian ovine sector. Demographic trends, however, vary considerably by breed, with no clear association with either breeding purpose or geographic area. This suggests that the main factor shaping these dynamics is the variability in subsidy schemes and financial incentives granted to farmers for the conservation of local breeds.

Census-based effective population size (Ne) estimates indicate that a quarter of Italian breeds are at immediate extinction risk and over half at long-term risk according to the 50/500 rule [141]. FAO's more comprehensive risk assessment—considering Ne, number of breeding males and females, population growth rate, and ΔF— reinforces these concerns [36]. Only six breeds (Bagnolese, Bergamasca, Merinizzata Italiana, Nera di Arbus, Sarda, and Tacola) currently fall into a “not at risk” category. 30% of breeds are assessed as critical, and seven appear extinct, having no registered animals in 2024. However, as reported above, there are discrepancies between registered and actual animals; therefore, unregistered individuals that share morphology and genotype, despite not being recognized as purebred according to law (Reg, EU 2016/1012), may still represent important reservoirs of genetic diversity [30]. Proper registration, supported by both morphological and genomic verification, should therefore be encouraged. In fact, the 2025 herd book regulation applies a derogation to these breeds and to those classified as critically endangered, allowing the inclusion in the herd book of animals assessed as conforming to the breed standard, even if their parents are not themselves registered.

4.3.5.1 Genomic investigation of Italian sheep biodiversity

Additional factors that can exacerbate extinction risk include narrow breeding ranges (common among local Italian breeds), environmental threats, and political, social, or market pressures [36,142,373]. Given this complex interplay of demographic, environmental, and management challenges, genomic tools have become pivotal for monitoring and managing breed diversity. Indeed, analyses of heterozygosity, ROH, and genomic Ne allow a more precise quantification of genetic health, providing insights that complement pedigree and census data [120]. Our data show that F_{ROH} levels in Italian sheep vary dramatically by breed, both in terms of global value and the distribution of ROH length classes, which reflects the timing of inbreeding events [135]. For instance, Pecora Ciuta and Bergamasca show moderate inbreeding, but with significant long ROH segments indicating recent inbreeding events. More alarming are breeds like Fabrianese, Istriana, and Di Benevento, which exhibit high recent and overall inbreeding, raising imminent risk of genetic erosion if breeding is not better managed. These inbreeding trends correspond well with trajectories in genomic Ne. Most breeds display a decline in Ne approximately 6 to 40 generations ago, as seen in other domestic animals and sheep breeds [584–586]. Exceptions include Di Benevento, which had persistently low Ne but has risen recently, and Tacola, which remained stable before its recent increase.

These results are supported by the demographic data of these breeds: the Di Benevento was recently recognized as a breed (2021), but with only a few animals and a single farm currently registered to Asso.Na.Pa.; conversely, the Tacola showed a constantly positive trend in the registrations of both heads and farms.

Genomic analyses also allow for the exploration of population structure and relationships among breeds, enhancing our understanding of their origin, evolution, and management. The results of MDS, PHATE, and Reynolds genetic distances reveal a clear geographic differentiation among Italian sheep breeds, consistent with previous studies [70,73,503,587]. However, breed-specific patterns already emerge at this level. For instance, the Delle Langhe breed exhibits an early separation from the other populations and shows genetic affinities with central-southern Italian breeds, particularly the Altamurana. This pattern, previously reported by Ciani *et al.* (2014) [73], may reflect historical crossbreeding practices aimed at improving the dairy aptitude of local breeds through the introduction of Delle Langhe genetics.

Data obtained from these analyses, as well as individual IBS trees, admixture, and haplotype sharing, reveal clear population differentiation among many Italian sheep breeds. Notably, most central-southern breeds appear to be genetically well-distinct from one another. In contrast, several northern breeds—excluding most of the populations from Piedmont and Veneto—share a more similar genetic background and show high levels of introgression from neighboring populations. Overall, the strongest genetic sharing is observed among breeds from the same geographic region.

Among the northern populations, the Biellese and Bergamasca breeds exhibit highly overlapping genetic backgrounds, and strong signals of introgression from these breeds are found in several others, such as the Tacola. These breeds are part of the group of Alpine meat breeds, which share several morphological traits, including the convex "*montonino*" fronto-nasal profile, long pendulous ears (except for Tacola), and a short, thin tail. The Bergamasca breed is thought to have originated in the Bergamo area of northern Italy as early as the 5th century. The modern breed descends from this ancestral population and underwent significant development during the long-range transhumance in the 12th century, when previously isolated sheep flocks—often raised in monastic farms and valley communities—came into contact and interbred. By the 13th–14th century, a population with the key traits of the modern Bergamasca was already well established. In the following centuries, this breed expanded to nearby provinces and eventually into central Italy, where it was raised both in pure form and as a cross with local populations. Indeed, Bergamasca is the largest-sized breed in Italy (rams weigh about 110 kg, and sheep about 80 kg) and consequently rams have been commonly used to improve meat production in other breeds, contributing to the formation of several meat-type breeds such as the Biellese, Varesina, Brianzola, Pecora di Corteno, Lamon, Alpagota, Bellunese, Tirolese, and Appenninica [588–590]. This historical diffusion is consistent with the significant genetic sharing observed in our analyses between Bergamasca and many other Italian meat breeds. It should be noted, however, that in this breed a marked discrepancy has emerged in recent years between the number of animals registered in Lombardy and those actually present. This decline in breeders' interest in registration appears to be linked to the difficulties they face in providing the data required by the association, given that flocks are generally managed under nomadic systems. In 2022, however, registrations increased again following the introduction of financial incentives for the breed in the region, supporting the hypothesis that such incentives are the main driver of fluctuations in local registration trends. The Biellese takes its name from the province where it originated, an important center for the textile industry. Indeed, this breed was initially reared for both meat and wool production; however, its wool, being coarse, was primarily used for carpets and padding. Considered a subtype of the Bergamasca until the early 20th century, it likely underwent intense crossbreeding with it, particularly following a sharp population decline in the 1960s that threatened its survival [48,73]. At present, this population shows a low number of registered animals, which has decreased over the last 15 years. Although historical sources suggest that the Biellese was also crossed with other minor Piedmontese breeds such as the Savoiarda, Frabosana, and Sambucana, our results identify these as genetically well-defined and distinct populations, showing only limited signs of recent introgression. The Tacola sheep, by contrast, is thought to derive directly from the Biellese, a relationship supported by their nearly identical genomic background. However, the Tacola displays a distinctive morphological trait—markedly reduced auricular pinnae—that sets it apart from its presumed ancestor [48]. This characteristic favoured the recognition of the Tacola as a distinct breed on a morphological basis, as happened for other populations, allowing the farmers to access funding dedicated to livestock biodiversity support.

Among central Italian breeds, the Pomarancina and Dell'Amiata display admixed genomic backgrounds dominated by the Appenninica cluster, which is consistent with their derivation from the ancient Apennine population and following crossbreeding aimed at improving meat and wool production [48,591]. The two breeds from the Campania region—Laticauda and Bagnolese—share a complex admixed background, similar to the Altamurana and presenting prominent contributions from breeds from central-southern Italy and Sicily. In particular, as soon as the Altamurana cluster appears, their admixture is dominated by it, with relevant portions also similar to Comisana [48,589].

4.3.5.2 Recent genomic evolution of Italian sheep and their relationship with foreign breeds

To better understand how Italian sheep populations have evolved over recent decades in response to market preferences, political and societal changes, and breeders' decisions, we compared our data with the Biovita dataset, which includes individuals sampled about two decades ago. A concerning result is the general increase in genomic inbreeding observed across most populations. Notably, the Istriana breed, which previously showed among the lowest inbreeding levels, now exhibits high F_{ROH} values. Conversely, a decrease in F_{ROH} was observed in Gentile di Puglia and Valle del Belice, likely reflecting an increasingly admixed genomic background in these populations and/or different sampling strategies.

Although most breeds appear to have retained their genomic identity over time, a higher degree of admixture was observed in several, often not traceable to a single donor population.

In some cases, we detected marked genetic differences or substructures that warrant further investigation. For example, a clear differentiation was observed in the Altamurana breed, consistent with findings reported by Ciani *et al.* (2014) [73], where two distinct Altamurana subpopulations, raised in different locations, were sampled. These two subpopulations are partially distinguishable in the Admixture analysis and both contribute—at varying proportions—to the background of most recent Altamurana individuals analyzed in this study. One of these clusters is also significantly represented in other breeds, such as Bagnolese, Istriana (from the Biovita dataset), Dell'Amiata, Pomarancina, Pinzirita, and Sopravissana. However, it is important to note that at present no Altamurana sheep farms are present in Foggia, the city of origin of one of the Biovita subpopulations. Notable differences were also observed in the Bergamasca and Biellese breeds. In the Biovita dataset, individuals from both breeds shared an admixed background with similar proportions. In contrast, in the recent dataset some individuals showed a uniform genomic background consistent with the main Bergamasca–Biellese cluster, while others retained a more admixed ancestry, like the Biovita samples. Notably, all individuals with a uniform background were sampled in Ravenna, including several from the same farm, which rears both breeds, whereas the more admixed individuals originated both from Ravenna and other provinces, such as Ragusa, Forlì-Cesena, Torino, and Cuneo.

This analysis also allowed to better understand the background of the Sicilian Barbaresca: now raised in Sicily for triple-purpose production (milk, meat, and wool), it originates from the cross between the North African Barbaresca and the local Pinzirita, a process that likely began during the Saracen rule in the 9th century [48]. This is supported by the resemblance of Barbaresca and Pinzirita (which was only present in the Biovita dataset) admixture, despite the first presenting a higher presence of Valle del Belice and Laticauda. Notably, this population shows marked decrease in animal and farm registration in recent years, and thus should be monitored to avoid genetic erosion and excessive introgression.

The Istriana (also known as Carsolina) breed also displayed a complex structure, with one cluster found only in some of the recent individuals, while another cluster was shared by both Biovita and recent samples. This breed originated from crossbreeding between local populations inhabiting the karst regions of the northern Adriatic and sheep introduced by migrants from the Balkans, as documented since the 17th century. The history of this breed has been closely linked to the events of the two World Wars, with the population experiencing a dramatic decline in the post-war period due to the devastation of the territory and the advent of industrial agriculture [59]. Today, a few hundreds of heads are raised in the karst areas of Italy (particularly in Friuli-Venezia Giulia), as well as in Slovenia and Croatia [48].

In the Nera di Arbus sheep, all Biovita individuals shared the same predominant genomic cluster, with limited signs of Sarda introgression. In contrast, most recent samples showed a background largely dominated by Sarda ancestry. Both breeds originated in Sardinia and are used for milk and cheese production, but they differ markedly in morphology: while the Sarda is polled and white, the Nera di Arbus has black skin and fleece and retains some ancestral traits such as horns in both sexes, small ears, and smaller body size. The Sarda is the most widespread Italian sheep breed, whereas the Nera di Arbus, which is less productive, is mainly confined to the southwestern part of Sardinia, but also shows an overall positive trend in the number of registered animals in the last decade, likely thanks to the financial support to its breeding, often one of the major sources of income for these farmers. The Nera di Arbus is considered an ancestral lineage of Sardinian sheep that escaped the intense dairy selection applied to the Sarda. It survived due to its ability to exploit the marginal pastures of the hilly region and because of its value in the local handicraft traditions of Arbus, which utilize both horn and fleece [592]. However, it came close to extinction, and only relatively recent conservation initiatives led to its recovery and official recognition in 2008.

Another case of worrying introgression is that of the Gentile di Puglia. While Biovita samples showed a homogeneous genomic background dominated by a single cluster, recent samples displayed a markedly admixed profile. The dominant ancestry in these modern individuals is derived from the Merinizzata Italiana, followed by residual contributions from the historical Gentile di Puglia and Sopravissana clusters. While it cannot be ruled out that these results may partly be due to sampling of individuals not fully adhering to the breed standard, the fact that all sampled animals were registered to the herd book and that the pattern is consistent across individuals suggests that recent unsupervised crossbreeding with Merinizzata Italiana is a plausible explanation. In contrast, the Sopravissana shows a more stable picture across time: some individuals exhibit a homogeneous background, while others show admixture, mainly with Merinizzata Italiana. The history of these two breeds helps explain these patterns. The Gentile di Puglia originated from an Apulian population renowned for its fine wool since Roman times. During the Spanish domination (15th century), this local population was crossed with Merino rams imported from Spain by the Aragonese and Bourbon monarchies. The resulting breed was involved in horizontal transhumance along the "tratturi" routes between Puglia and the Apennines of Abruzzo and later spread into other central and southern regions, contributing to the development of several local breeds. The Sopravissana, on the other hand, descends from the local Vissana population, which underwent "merinization" between 1750 and 1943. This process involved crossbreeding with Merino rams, especially from the Bergerie Nationale in Rambouillet, during the first half of the 20th century to improve wool quality, after which the exportation of Rambouillet rams largely ceased. Like the Gentile di Puglia, the Sopravissana followed seasonal transhumance routes from Lazio to Umbria and Marche along ancient Roman "consolari" roads. Although both breeds had a triple-purpose aptitude, they were mainly raised for wool. The wool market crisis in the late 19th century severely impacted these breeds. This crisis was caused by urbanization (leading to rural abandonment), the rise of synthetic fibers (reducing wool's commercial value), and the increasing selection for specialized meat or dairy breeds. At the same time, restrictions on pastoral movement hindered transhumance [593]. As a result, breeders were forced to repurpose these populations. Despite the good quality of their milk, low yield and poor milking ease, combined with the spread of more productive dairy breeds (e.g., Sarda and Massese), led to a shift toward meat production. This goal was pursued through intense and often indiscriminate crossbreeding, involving both Italian and foreign meat breeds—such as Württemberger (Germany), Rambouillet, Île-de-France, and Berrichon du Cher (France). Alongside population declines (further aggravated by regulations requiring breeders to cover wool disposal costs (Reg. EC 1069/2009), this led the Gentile di Puglia and Sopravissana to the brink of extinction. Indeed, by the 1980s, surveys revealed that over 80% of Gentile di Puglia and over half of Sopravissana flocks were crossbred to improve production. Consequently, a recovery project was initiated to (i) re-establish and conserve the original purebred populations, through stricter breed standards, classification into conservation programs, and molecular testing for breed verification; and (ii) improve selected crossbred lines to develop new meat breeds, namely, the Merinizzata Italiana and the Trimeticcia di Segezia [593,594]. According to demographic data, both breeds experienced an increase in the number of registered farms and animals during the second decade of this century, but in recent years, while the number of farms has remained stable, the number of animals has declined, highlighting the need for ongoing monitoring of these populations.

Another important insight was gained by introducing the breeds of foreign origin recognized in Italian herd books into the dataset, which allowed us to better characterize their influence on Italian populations. Notably, on average, the introgression of foreign breeds into Italian sheep appeared less pronounced in the Biovita dataset compared to the modern dataset—with the exception of the Istriana breed. However, it is important to note that some of the breeds currently showing the highest levels of introgression were not included in the historical Biovita dataset. For instance, the Pecora Ciuta exhibits strong introgression from both the Île-de-France and Suffolk breeds. However, given that other analyses also indicate extensive introgression from the Merinizzata Italiana, this could contribute to the ancestral proportion derived from the Île-de-France, as better explained in the following section. The Pecora Ciuta, whose name refers to its small size, was traditionally reared in the harsh mountainous areas of Valtellina and Alto Lario. Due to pasture reduction, abandonment of mountain areas, and the introduction of larger breeds—such as Bergamasca and Merino—which were used to crossbreed the Ciuta, the population nearly disappeared. In fact, it was considered extinct as of 2001. However, the breed was later recovered starting from a small group of individuals whose morphology matched the historical descriptions of the Ciuta, and in the last 12 years the number of its registrations has progressively increased [48].

The case of the Merinizzata Italiana also highlights the importance of the composition of the dataset used for analyses such as Admixture. Indeed, in the dataset including only Italian breeds, the Merinizzata Italiana formed a distinct cluster. However, when foreign breeds were included, strong introgression from the French Île-de-France and, to a lesser extent, Berrichon du Cher became evident, along with a significant contribution from the Sopravissana. This result is consistent with earlier studies on Merino and Merino-derived sheep worldwide. In Ciani *et al.* (2015) [503], for example, where only the Rambouillet was included among the French breeds, the Merinizzata Italiana appeared distinct from both French and Italian Merinos. By contrast, in Ceccobelli *et al.* (2023) [587], where Île-de-France and Berrichon du Cher were included, the Merinizzata Italiana clustered more closely with the French Merino-derived breeds, indicating the predominance of their genetic influence. As previously mentioned, the Merinizzata Italiana was developed in the late 1980s from crossbred individuals of Gentile di Puglia and Sopravissana origin. Particular care was taken to select animals crossed with German Merinos, in order to avoid excessive similarity to the Île-de-France, which, despite being widely used in southern Italy at the time, was poorly adapted to local production systems. These selected individuals were subjected to breeding programs aimed at standardizing morphology and improving meat production traits. As a result of these efforts, the Merinizzata Italiana was officially recognized in 1997 [594] and has since become one of the most widespread meat sheep breeds in Italy, despite the negative trend in registrations observed over the last 15 years. Despite clear indications to halt crossbreeding with foreign breeds and to prioritize founders with limited Île-de-France ancestry [594], our findings indicate that Île-de-France remains the predominant genomic ancestry in the modern Merinizzata Italiana. To preserve the breed's original genetic identity—and possibly its environmental adaptation—morphological monitoring by breed experts should be coupled with molecular analyses.

The absence of a Merinizzata Italiana–specific cluster in the admixture analysis complicated the interpretation of the introgression observed in modern Gentile di Puglia individuals, described above. To determine whether this introgression stemmed from the Merinizzata Italiana or directly from foreign breeds such as Île-de-France, we conducted a focused analysis using a subset of breeds that appeared significantly in the background of Gentile di Puglia. This subset included Île-de-France, Berrichon du Cher, and Rambouillet, as these breeds have been reported on farms currently raising Gentile di Puglia. The results clearly indicated that introgression originated primarily from the Merinizzata Italiana, rather than directly from French breeds. To explore this further, we applied local ancestry inference to identify which genomic regions of the current Gentile di Puglia carry signatures of Merinizzata Italiana introgression, and which retain ancestry from the original Gentile di Puglia. We also performed a similar analysis for the Nera di Arbus, comparing modern individuals to those in the Biovita dataset and to the Sarda, in order to assess the extent and distribution of introgression in this breed.

4.3.5.3 Identification of highly differentiated and introgressed genomic regions

We compared individuals of the same breed sampled 20 years apart to identify regions that may have evolved over time under anthropogenic and/or natural selection. Our first focus was on regions related to production traits. Interestingly, we did not observe a clear separation of genes or QTLs linked to different production traits that could reflect the specific breeding purposes of the various sheep breeds. This may be due to the fact that most breeds were originally selected for triple purposes (milk, meat, and wool) and only more recently, after the wool market crisis, directed toward more specialized production. However, for most breeds, there is still no strong selection for a single production trait, as many that were originally maintained for small-scale, family-based farming have largely retained that traditional purpose.

Several genes with high Fst values—or located in regions showing peak Fst values—were associated with meat production in multiple breeds. These included genes related to body weight, growth, and conformation (*WWC1*, *ZNF385B*, *OSBPL3*, *CAMK2B*, *DDP6*) [430,595–601], meat quality (*HSPG2*, *RUNX1*, *GADD45A*) [602–605], or both (*KLF15*, *CTNNA3*, *CACNA2D1*) [598,606–614]. In addition, we identified several enriched QTLs associated with body measurements, weight, and muscle density—traits important not only for meat production but also for resilience during long transhumance—as well as many linked to morphological features such as ear, tail, and horn shape, which are likely under selection because they serve as distinctive identifiers of each breed. Moreover, LAI showed that regions specific to the Gentile di Puglia are also enriched for genes involved in fat metabolism, including *LPIN1* and *SLC4A7* [529–531]. Meanwhile, introgressed regions include QTLs and genes related to meat quality, growth, and feed efficiency—such as *ALDH1A1*, *DLG1*, and *SLC16A7*—which is consistent with the current meat-oriented breeding objectives of both breeds [532–534].

Unexpectedly, genes and enriched QTLs under selection over time that were also associated with milk production and quality were relatively few in all breeds, and included *ART3*, *CACNA2D1*, *CTNNA3*, *DPP6*, *PTK2*, *ZMIZ1*, *ZNF385D*, *SLC15A5*, and *ROBO2* [615,616,625,617–624]. Some considerations can be made for both the Nera di Arbus and the Gentile di Puglia, as introgression may play a role in shaping their milk-related traits. Indeed, the Sarda is known for its higher milk yield compared to Nera di Arbus, whose milk instead typically displays higher fat and protein content. Consistently, introgressed regions in the Nera di Arbus often contain QTLs associated with milk lactose percentage, while regions still reflecting the ancestral background include QTLs related to milk protein content. Although not primarily selected for dairy, the Gentile di Puglia also contributes to traditional cheese production—most notably the PDO *Canestrato Pugliese*, made from its milk—and some Merinizzata Italiana-introgressed regions carry QTLs associated with milk protein and fat content. Taken together, these patterns suggest that introgression may affect milk characteristics in both breeds: in the Nera di Arbus, Sarda introgression could contribute to increased yield but possibly at the expense of traditional quality traits, while in the Gentile di Puglia, Merinizzata Italiana introgression may alter the typical milk composition and, consequently, the characteristics of its dairy products.

In Fst temporal comparison, a number of genes were also related to fiber production, such as *PADI2*, *ROBO2*, *RPS6KC1*, *LAMC2*, *KIF16B*, and *ZNF385D* [626–631]. QTLs linked to wool quality and quantity were enriched in some breeds, particularly in Laticauda, despite this breed not being selected for wool production. However, more considerations can be made for the LAI analyses. Indeed, as previously mentioned, the Nera di Arbus sheep differs from the Sarda not only in clearly observable morphological traits—such as its black fleece and the presence of horns—but also in its ancestral lineage and, more importantly, in its traditional aptitude for wool production, which has historically supported the creation of local handcrafted textiles. Several genes central to this function, such as *EGFR* and *CTNNB1* [546,547], were detected in regions still associated with the older, more ancestral Nera di Arbus background, likely reflecting the retention of ancestral genomic components. Nonetheless, a significant portion of genes involved in fiber development—including several keratin genes, along with *RERE* and *STAT3*—were found in highly introgressed regions [521,553]. This suggests that gene flow from Sarda, a non-wool breed, may have affected the original fiber-related genomic architecture of the Nera di Arbus. In the context of breed conservation, where the maintenance of unique ancestral traits is a central goal, such introgression could compromise local wool quality and thus hinder the valorization of traditional products and reduce the distinctiveness of the breed. Similarly, with regard to the Gentile di Puglia, we identified genomic regions associated with hair follicle development and wool type differentiation within ancestral regions, reflecting its original wool-oriented breeding purpose. While wool is no longer the main productive focus, introgressed regions from the Merinizzata Italiana that include QTLs linked to fleece yield and *VDR*, a gene involved in follicle development [521], could potentially alter the typical fleece traits that characterize the Gentile di Puglia.

Another pivotal aspect of livestock breeding, beyond production itself, is reproduction—both to obtain lambs for meat production, to ensure lactating ewes, and to provide replacements for future generations. It is therefore not surprising that many regions under selection over time harbor genes involved in fertility and litter size (*CHRNA2*, *ENPP3*, *RABL3*, *CDH18*, *HSPG2*, *RUNX1*, *ZMIZ1*, *NCOA1*, *PTK2*) [632,633,642,634–641] and, in some dairy breeds, QTLs for reproductive seasonality or lambing ease. In both Nera di Arbus and Gentile di Puglia, fertility-related genes and QTLs were found both in introgressed and ancestral regions. This overlap reflects the complexity of the breed's genomic landscape and may also point to heterosis-like effects, with introgression increasing genetic diversity at fertility loci and potentially influencing fertility-related traits.

Health-related factors represent a major determinant of livestock productivity, reproductive efficiency, and welfare. Consistently, immunity-related genes and QTLs were among the most frequently associated with SNPs showing high Fst values, and immunity-related GO terms were significantly enriched in the Delle Langhe and Laticauda breeds. These included genes and QTLs related to somatic cell count and mastitis (*CACNA2D1*, *SEMA5A*, *NEGR1*, *PTK2B*, *CTNNA3*) [623,643–646], gastrointestinal parasites (*PTK2B*) [647], and various viral or bacterial diseases. Among these, specific examples include: genes associated with respiratory diseases (*PADI2*) [648] and QTLs for paratuberculosis (in the Bergamasca) and pleurisy (in Comisana); *CTNNA3* gene, found in Altamurana and associated with brucellosis [649]; a QTL in Fabrianese and *HSPG2* gene in Appenninica for footrot [650], which represents a significant cause of lameness in sheep flocks, leading to economic losses due to reduced wool production, poor fertility, and decreased growth rates; *GADD45A*, found in Laticauda, for *Peste des petits ruminants* virus [651]; and both QTLs and *CXCL9* gene for Visna-Maedi [652]. Notably, among the highly differentiated genes in Valle del Belice, we also identified *CXCL10*, which encodes chemokines involved in various infections and infestations [568,652–656]. Moreover, when we analyzed introgression in the Nera di Arbus, we found that some of the PNAbv regions harbor QTLs and genes (e.g., *C9*, *FYB*, and *MYD88*) related to somatic cell count and mastitis resistance, traits essential for udder health [561,562]. Other genes involved in immune response are *MYD88*, a candidate gene for resistance to small ruminant lentiviruses, and *FOCF1*, linked to pneumonia resistance [565]. In contrast, introgressed regions contained *STAT5A* and *CSF3*, both associated with mastitis resistance in cattle [566,567], and genes (*CX3CR1* and *CSF3*) and QTLs involved in immune response and parasite resistance [568].

Alongside selection for productive and fitness-related traits, livestock breeds, especially local populations raised in extensive systems, are also shaped by selective pressures that drive adaptation to their specific environments. This can explain why we found several non-introgressed regions associated to environmental adaptation, both in the Nera di Arbus—such as *CBFA2T3* and *DNAJA2*, associated with heat tolerance in cattle [570–572], and *EGFR*, linked to environmental and altitude adaptation in sheep and Tibetan goats [46,573]—and Gentile di Puglia—in particular *SOD2*, involved in oxidative stress protection [537–540]. However, the presence of genes such as *SLC16A7* and *KHDRBS2*, linked to responses to environmental stressors [510,573], within Merinizzata Italiana-introgressed segments raise concerns about the potential erosion of locally adapted genetic variants that may be crucial for the breed's resilience in the challenging environments of southern Italy. A substantial number of these adaptive genes were also found by Fst within-breed temporal comparison—many detected in Valle del Belice. These included associations with solar radiation (*KLF15*) [657], drought (*CACNA2D1*) [658], and heat tolerance (*C4H4orf22*) [659]. However, most were linked to altitude adaptation, including *AGBL4*, *PDK4* (also relevant to energy metabolism), *PTPRD*, *SLC22A17*, *LONP1*, *ASCC3*, and *CAMK2B* [660–666].

Given these signals, we further explored how climate change and corresponding natural selection have shaped these breeds in recent decades. Specifically, we identified candidate SNPs for environmental adaptation by analyzing their correlation with shifting environmental variables and marked breed genetic differentiation over time. The strongest signals were tied to BIO16 (precipitation during the wettest quarter), followed by aridity annual mean, BIO09 (mean temperature of the driest quarter), and BIO11 (mean temperature of the coldest quarter). Precipitation-related candidates (BIO16) were particularly abundant when comparing the current and historic Istriana populations. Noteworthy among the associated genes many have been previously associated to livestock environmental adaptation, including *CD109* and *ST3GAL*, both implicated in sheep adaptation to arid and hot climates [506,507], and *EPAS1*, a key regulator of oxygen homeostasis under hypoxia and a recognized marker of altitude adaptation in several species [257,264]. We also detected aquaporin genes and enriched GO terms related to renal water transport. Aquaporins are crucial for renal water reabsorption and urine concentration, highlighting the importance of osmoregulation in environments with scarce or unpredictable rainfall and, consequently, the adaptive significance of maintaining water balance in livestock exposed to arid or variable climates [47,508,509].

The results of BIO11 correlation revealed genes linked to organ growth, osteoblast activity, and Hippo signaling. These processes are integral to body-size modulation, a classic adaptive trait shaped by climate. Indeed, according to Bergmann's rule, animals living in hotter climates tend to be smaller, as a reduced body size favors heat dissipation [258]. Alternatively, populations evolved in hot but arid environments can also present large bodies, which reduces metabolic rate and endogenous heat production, but long and thin appendages, which increase surface area and facilitate heat loss [89]. This morphological adaptation aligns with our observation of enriched pathways related to chondrocyte proliferation for BIO09, suggesting an underlying genetic basis for limb elongation in these populations [667]. Moreover, the Hippo pathway, in particular, orchestrates the so-called "pace of life", influencing not only growth, but also sexual maturation timing, lifespan, and metabolism, thus representing a molecular bridge between environmental stimuli and developmental responses [668].

Several ontologies significant for BIO11 are involved neurological development—including glycinergic synapse regulation—and behavioral modulation. Behavioral plasticity enables rapid responses to environmental challenges, allowing animals to modify feeding, foraging, or social behaviors quickly [82]. Glycinergic synapses, serving as key inhibitory transmitters in spinal and brainstem circuits, play a critical role in shaping motor and sensory pathways (e.g. respiration, vision, audition, pain processing), thereby also influencing behavioral output [669].

For BIO09, we also observed enrichment in the regulation of diadenosine triphosphate (Ap_3A) catabolism. Ap_3A belongs to the "alarmones", stress-responsive signaling molecules whose levels rise swiftly with external stress to regulate gene expression, modulate ATP-binding enzyme activity, and stimulate repair and antioxidant pathways [670].

Aridity-related ontologies also revealed negative regulation of DNA transcription, suggesting a strategy of resource conservation under environmental strain: stress-driven epigenetic remodeling in immune cells is one documented mechanism resulting in broad transcriptional suppression and a shift to post-transcriptional control [671].

Other genes and pathways shared across environmental variables involved immune response, metabolic and energy regulation, angiogenesis and cardiac function, circadian rhythm, and oxidative protection. Circadian regulation aligns metabolic, reproductive, and behavioral processes with daily and seasonal environmental cycles, a critical adaptation mechanism [672–674]. Oxidative stress management is equally vital under thermal extremes, with copper and zinc—linked here to BIO09—being essential trace elements for antioxidant enzymes and immune function [675,676]. Purine metabolism, responding dynamically to oxidative and thermal stress, plays a central role in energy management [677].

Selection under aridity also affected retinol metabolism pathways. Vitamin A supports vision, immune function, epithelial integrity, and resistance to oxidative stress—particularly important where UV exposure, heat, and pathogen pressure are high [678]. Immune regulation itself is a core adaptive mechanism: environmental stressors like heat and drought are known to compromise immune responsiveness in livestock [508]. Across climatic gradients, resilient immune capability is vital for survival and sustained productivity, and with climate change expanding parasitic and pathogen threats, selecting for immune-adapted livestock is increasingly important [679].

Metabolic regulation allows dynamic energy adjustment to environmental demands [44]. Triglyceride synthesis and lipid mobilization are central in thermoregulation and energy storage under varying conditions [680]. For example, *FTO*, strongly associated with BIO16 in the Sambucana sheep, is crucial for fat metabolism, feed efficiency, meat quality, and tail fat deposition—a reserve for coping with harsh conditions [515–518]. Variants and epigenetic modification in *FTO* have also been linked to heat and environmental variability in sheep and goats [510–512].

Thermoregulatory demands during heat stress prompt physiological adaptations like increased respiration, heart rate, and microvascular perfusion [22,681]. Indeed, we found GO terms related to cardiac function (BIO09) and microvascular development (aridity annual mean), which contribute to dissipate heat, support tissue perfusion, and preserve homeostasis under thermal and hydric stress.

4.3.6 Conclusions

In conclusion, this study provides a comprehensive analysis of the recent history and genomic evolution of Italian sheep biodiversity, revealing a multifaceted picture of both challenges and dynamics. Our results demonstrate the resilience and adaptability of these breeds, showing that despite demographic pressures, many populations maintain substantial genetic diversity and retain traits shaped by centuries of environmental adaptation. However, they also serve as a clear wake-up call regarding the survival prospects of many Italian breeds, highlighting reductions in population size, declines in genetic variability, and widespread cases of admixture. For example, the evidence of clear introgression in some local breeds underscore the dual nature of gene flow: while it can introduce advantageous alleles that support adaptation or production, it may also alter traits defining breed identity. Balancing the conservation of ancestral genomic signatures with the benefits of adaptive introgression is therefore a central challenge. In this context, breeders' associations are pivotal: by collecting and analyzing farm-level data nationally, they support the management and conservation of genetic resources and foster farmers' engagement in breed improvement. However, the coordinating role of breeders' associations in safeguarding and improving populations does not appear to be fully leveraged and understood, highlighting the need for greater structured efforts and collaboration among all stakeholders in the Italian breeding system: breeders, breeders' associations, and government institutions. Complementing these initiatives with an integrative approach that combines demographic, farm-level, and genomic data is essential to preserve genetic diversity and support the long-term sustainability of Italian sheep biodiversity.

4.3.7 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S4.3.1 Dataset composition for the main analyses performed in the present study.

Supplementary Table S4.3.2 Description of the climatic variables used for correlation analysis of Fst values with climatic change in each population's breeding range.

Supplementary Table S4.3.3 SNPs associated with the highest 1% |Fst| values in each breed comparison (older vs. recent samples of the same breed).

Supplementary Table S4.3.4 Genes associated with the highly differentiated SNPs identified in each breed comparison (older vs. recent samples of the same breed) through Fst (see Table S4.3.3).

Supplementary Table S4.3.5 GO terms associated with the highly differentiated genes identified in each breed comparison (older vs. recent samples of the same breed) through Fst (see Table S4.3.4).

Supplementary Table S4.3.6 SNPs associated with the highest 1% |Fst| values in at least one breed comparison and significantly correlated to at least one environmental variable change.

Supplementary Table S4.3.7 Genes associated with the SNPs identified in correlation analysis of Fst values with climate change (see Table S4.3.6).

Supplementary Table S4.3.8 GO terms associated with the highly identified in correlation analysis of Fst values with climate change (see Table S4.3.7).

Supplementary Table S4.3.9 SNPs in the 99th percentile of most introgressed regions of older Gentile di Puglia population (GDPbv) or Merinizzata Italiana (MER) in Gentile di Puglia (GDP) or of older Nera di Arbus (PNAbv) or Sarda (SAR) in Nera di Arbus (PNA) according to local ancestry inference (LAI) analysis.

Supplementary Table S4.3.10 Genes associated with the most introgressed regions identified in LAI analysis (see Table S4.3.9).

Supplementary Figure S4.3.1 Trends in yearly animal and farm registrations per breed from 2010 to 2024.

Supplementary Figure S4.3.2 Admixture analysis for a number of clusters (K) ranging from 2 to 35.

Supplementary Figure S4.3.3 Genetic diversity of Italian sheep breeds.

a) Boxplot of individual inbreeding coefficients based on runs of homozygosity (F_{ROH}). Each point corresponds to one individual. Boxplots show the median (line), interquartile range

(IQR, box), whiskers extending up to 1.5 × IQR, and points beyond whiskers representing outliers.

b) Barplot of mean F_{ROH} per breed. Colors indicate the contribution of ROH of different length classes. Breeds are ordered by total mean F_{ROH}.

Supplementary Figure S4.3.4 Admixture analysis for a number of clusters (K) ranging from 20 to 30, including recent and older (codes including ‘bv’) samples.

Supplementary Figure S4.3.5 Local ancestry inference with Gentile di Puglia (GDP) as the target population, older GDP samples (GDPbv) as the background, and Merinizzata Italiana (MER) as the candidate source of introgression.

Supplementary Figure S4.3.6 Local ancestry inference with Nera di Arbus (PNA) as the target population, older PNA samples (PNAbv) as the background, and Sarda (SAR) as the candidate source of introgression.

4.4 SPATIO-TEMPORAL GENOMICS OF ITALIAN GOATS: RECENT EVOLUTION, ADAPTATION, AND FUTURE VULNERABILITY

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Submitted to: animal

4.4.1 Abstract

Italy hosts a rich biodiversity of local goat breeds, shaped by its wide variety of climates, landscapes, and traditional farming systems, making the preservation of these locally adapted populations critical for maintaining genetic resources. This study aimed to explore the genomic biodiversity of Italian goats, track recent temporal changes through comparison with samples collected about two decades ago, and investigate the genomic mechanisms underlying environmental adaptation, as well as identify hotspots of possible climatic vulnerability.

Demographic data over the last 15 years show that only five breeds are currently considered not at risk of extinction according to FAO criteria, while 22 breeds are classified as critical or formally extinct (no registered animals in 2024). Medium-density SNP data from 685 goats representing 31 populations were analyzed for population structure, genomic background, and genetic diversity. Comparison with historical samples revealed changes over time, exemplified by Bianca Monticellana and Capestrina, which now display a highly similar and uniform genetic background and higher inbreeding. Northern and central-southern breeds are well separated, with northern populations exhibiting more distinct genomic backgrounds while central-southern populations are generally more admixed. Landscape genomic analyses were conducted on a subset of 693 goats from 32 populations, using LFMM and pRDA approaches together with present and projected (SSPs 2–4.5 and 5–8.5, 2080-2100) climatic variables from WorldClim 2.1. A total of 468 SNPs were identified as putatively adaptive, including five detected by both methods, encompassing genes such as *KPNA1*, *PARP9*, and *LRP8*. Genomic offset analyses highlighted vulnerable areas in the northern fringes of the Alpine region, the eastern Po Valley (unsampled due to limited presence of local goat populations), and the Murgia-Gargano region of Apulia, home to the Garganica breed.

Overall, these results reveal the impact of breeding practices and environmental pressures on Italian goat genomes, provide insights into adaptive genetic variation, and identify populations and regions at greatest risk, emphasizing the need for targeted conservation and management strategies to preserve this unique component of livestock biodiversity.

4.4.2 Introduction

Meeting the growing global demand for food and animal products while preserving biodiversity and ecosystem resilience is a major challenge for modern agriculture [27]. In this context, small ruminants—and goats in particular—play a critical role in sustainable livestock systems. As one of the earliest domesticated species [682], goats are now reared across a wide range of environmental conditions and production systems, from high-input dairy farms to low-input, extensive settings. Their exceptional adaptability, hardiness, and ability to thrive on scarce resources make them particularly valuable in marginal areas and resource-limited regions, where they often represent a primary source of food, income, and livelihood security for rural communities [42,226,320,683,684].

Italy offers a unique case study for investigating goat biodiversity and environmental adaptation. The country hosts 47 officially recognised local goat breeds, managed by the Italian Sheep and Goat Breeders Association (Asso.Na.Pa., www.assonapa.it), and many ecotypes still unrecognised, shaped by centuries of agro-pastoral practices, diverse ecological zones, and regional selection pressures. This genetic richness is matched by a mosaic of landscapes—from alpine valleys to Mediterranean coasts—and a variety of traditional management systems that reflect both environmental constraints and cultural heritage [48,74,256]. These breeds, often raised in extensive or low-input systems, have developed distinct adaptive traits that allow them to survive, reproduce, and remain productive under sub-optimal or highly variable conditions [12]. Furthermore, the strong link between local breeds and their territories contributes to landscape management, prevents land abandonment, and supports traditional economies based on high-quality niche products [35,63,685].

Understanding the genomic basis of adaptation in these breeds is crucial for designing future-oriented breeding and conservation strategies [21,87]. The development of affordable genotyping technologies and the availability of dense SNP and whole genome sequencing data have opened new opportunities to explore the genetic structure of livestock populations and to identify loci under selection [110,686]. International initiatives, such as the Italian Goat Consortium [74,687], AdaptMap [71], and VarGoats [72], have significantly contributed to the generation of large-scale genomic datasets that support such analyses. In addition, CHEESR (2017–2021) and SHEEP&GOAT (2021–2025) are two national projects funded under the National Rural Development Plan (PSRN, submeasure 10.2), coordinated by Asso.Na.Pa. in collaboration with several academic and research institutions. These initiatives were developed to enhance Italian sheep and goat biodiversity, production, and health also through the introduction of genomic tools in the sector.

Among the tools enabled by large-scale genomic datasets, landscape genomics provides a robust framework to investigate how environmental factors influence genetic variation across space. This approach combines genome-wide data with high-resolution environmental variables to detect signatures of local adaptation, typically through genotype–environment association (GEA) methods or spatially explicit models [179,180]. The identification of candidate adaptive loci or genomic regions under selection enables researchers not only to understand evolutionary processes but also to support the development of informed breeding, conservation, and management strategies. In this context, the concept of genomic offset has emerged as a key tool for assessing vulnerability to climate change. Genomic offset estimates the predicted mismatch between the current genomic composition of populations and the allele frequencies that would be theoretically optimal under future climatic conditions. It provides a quantitative measure of adaptive distance, helping identify populations that may face greater challenges in maintaining fitness as their environments shift. This information is especially useful for prioritizing conservation actions, such as in situ preservation, targeted gene flow, or climate-informed breed management [198–200].

While identifying the genomic signatures of adaptation is essential, it is equally important to monitor how livestock populations change over time in response to both environmental and human-driven forces. Local animal populations are not fixed entities—they are constantly shaped by climatic variability, political shifts, economic pressures, and breeder decisions

[371,373,487,488]. As such, preserving their genetic resources requires not only a snapshot of current diversity, but also a temporal perspective that captures how genetic structure and relationships have evolved. Monitoring these changes through genomic analyses provides critical insight into the balance between maintaining breed identity and adapting to new challenges. This is particularly relevant for understanding the relationships among populations, identifying cases of introgression or fragmentation, and evaluating how well current management practices support the long-term viability and distinctiveness of each breed [113,688]. Taken together, these approaches offer the opportunity to move beyond static assessments of genetic resources and toward a more dynamic and predictive understanding of livestock diversity.

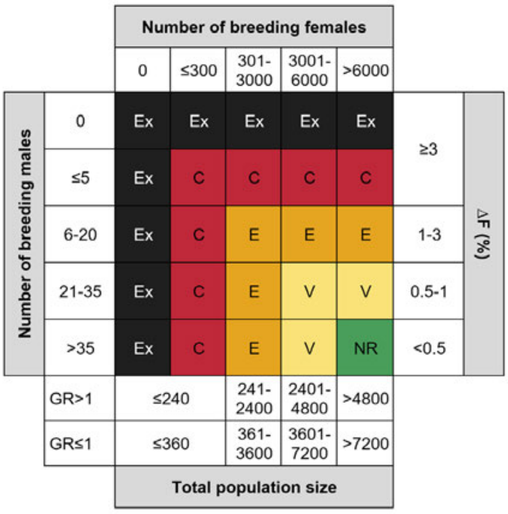
Within this framework, the present study provides an updated and comprehensive characterisation of the genetic diversity and structure of Italian goat populations, using newly generated SNP data for 31 breeds. It incorporates a temporal comparison between individuals sampled approximately 20 years ago and those sampled more recently, offering insight into how the genomic makeup of these populations has changed over time. In parallel, it applies landscape genomics approaches to explore genotype–environment relationships and estimate genomic offset under projected climate scenarios. Owing to the remarkable diversity of local breeds reared in extensive systems and the wide range of climatic conditions, Italy represents an exceptional natural laboratory for investigating livestock genomic adaptation, giving this study a broader relevance beyond the national context. By integrating genomic, environmental, and temporal dimensions, this work contributes to a deeper understanding of how Italian goat populations are responding to ongoing challenges and provides a knowledge base for their effective conservation and climate-resilient management.

4.4.3 Materials and methods

4.4.3.1 Demographic data

Census data were obtained from Asso.Na.Pa. and comprised the number of officially registered farms and live animals recorded on December 31st of each year from 2010 to 2024.

For each breed, the proportional change in farm and animal numbers was calculated as the difference between the values recorded in 2024 and those recorded in 2010; for breeds officially recognized after 2010, the first year of recognition was used as the baseline. The annual growth rate was estimated from the logarithmic difference in animal numbers between two consecutive censuses, standardized by the time interval in years, excluding years with zero registrations [36]. For 2024, the expected rate of inbreeding increase (ΔF) was computed as 1/(2*Ne), where Ne represents the effective population size, estimated using Wright's formula [36,144]. Risk status for each breed was then determined according to the FAO criteria [36], which take into account the number of breeding females and males, total population size, growth rate, and ΔF (Figure 4.4.1).



		Number of breeding females						
		0	≤300	301-3000	3001-6000	>6000		
Number of breeding males	0	Ex	Ex	Ex	Ex	Ex	≥3	ΔF (%)
	≤5	Ex	C	C	C	C		
	6-20	Ex	C	E	E	E	1-3	
	21-35	Ex	C	E	V	V	0.5-1	
	>35	Ex	C	E	V	NR	<0.5	
	GR>1	≤240		241-2400	2401-4800	>4800		
	GR≤1	≤360		361-3600	3601-7200	>7200		
		Total population size						

Figure 4.4.1 Criteria for the definition of breed risk categories according to FAO. For each breed, the risk category was assigned based on the least favorable parameter. Abbreviations: GR = Growth rate; ΔF = Inbreeding rate; NR = Not at risk; V = Vulnerable; E = Endangered; C = Critical; Ex = Extinct.

4.4.3.2 Sampling

A total of 5,888 goats from 34 Italian breeds were sampled using nasal swabs and genotyped with the GoatSNP60 or GoatSNP65 v2/v3 BeadChips (Illumina Inc., San Diego, CA). Specifically, 5,584 samples from 30 populations were collected within the CHEESR and SHEEP&GOAT projects, 232 samples from 16 breeds were obtained within the Agritech project, and data for the 72 Comune di Sicilia/Mascaruna (CCS) goats were retrieved from Bionda *et al.* (2023) [407] and Floridia *et al.* (2025) [689] (Supplementary Table S4.4.1).

For most breeds, the sampling strategy involved the collection of 11 to 60 animals registered in the herd book, with one male and two unrelated females selected per farm. In some breeds, such as Camosciata delle Alpi (CTP) and Saanen (SNN)—where the sampling was also intended to support the development of genomic breeding values—additional related and unrelated individuals were collected for further analyses.

4.4.3.3 Dataset and quality control

As mentioned above, the sampling of SNN and CTP goats followed different criteria compared to other breeds, including the inclusion of relatives and French and Dutch bucks used for artificial insemination in Italy. For this reason, we pre-selected these breeds, only retaining Italian animals that were enrolled in the main section of the herd book and, for males, with positive parentage verification. The resulting dataset comprised 2,793 goats from 34 different populations (Table 4.4.1 and Supplementary Table S4.4.1).

Table 4.4.1 Dataset composition and main results of genomic biodiversity analysis on Italian goat breeds.

Breed Code	Breed Name	N. raw	N. QC	N. balanced	He	Ho	FROH	Ne (SNeP)	Ne (GONE)
AET	Argentata dell'Etna	29	28	27	0.407	0.406	0.023 ± 0.045	35	411
BDL	Bionda dell'Adamello	29	29	27	0.396	0.389	0.051 ± 0.052	32	233
BNM	Bianca Monticellana	29	27	27	0.349	0.355	0.113 ± 0.053	30	340
CAP	Capestrina	18	17	17	0.369	0.373	0.083 ± 0.079	19	173
CCR	Ciociara Grigia	12	12	12	0.396	0.403	0.039 ± 0.042	13	120
CCS	Comune di Sicilia/ Mascaruna	72	66	27	0.406	0.403	0.043 ± 0.072	29	133
CPS	Capestrina	60	57	27	0.410	0.403	0.028 ± 0.04	34	470
CTN	Cilentana Nera	28	27	27	0.409	0.415	0.011 ± 0.012	35	918
CTP	Camosciata delle Alpi	1097 (3202)*	916	27	0.414	0.408	0.047 ± 0.033	33	323
DRP	Di Potenza	33	31	27	0.410	0.404	0.035 ± 0.052	31	160
DRT	Di Teramo	11	9	9	0.383	0.407	0.033 ± 0.032	8	32
FDM	Fulva dei Monti Picentini	17	14	14	0.365	0.387	0.071 ± 0.046	14	61
FNS	Frisa Valtellinese	25	23	23	0.378	0.381	0.054 ± 0.037	26	117
GRG	Girgentana	25	25	25	0.351	0.339	0.133 ± 0.089	27	117
GRM	Campobasso Grigia Molisana	9	9	9	0.387	0.409	0.028 ± 0.041	9	50
GRN	Garganica	127	122	27	0.408	0.391	0.057 ± 0.06	31	199
LRN	Lariana/ Di Livo	21	21	21	0.398	0.405	0.013 ± 0.017	26	506
MCH	Pezzata Mochena	20	18	18	0.393	0.410	0.022 ± 0.022	21	173
MLT	Maltese	32	30	27	0.384	0.374	0.088 ± 0.077	30	147
MSN	Messinese	65	61	27	0.414	0.409	0.019 ± 0.029	35	1526
NCT	Nicastrese	155	149	27	0.413	0.408	0.025 ± 0.034	34	465
NPT	Napoletana	9	8	8	0.345	0.370	0.106 ± 0.098	7	32
ORB	Orobica	29	27	27	0.343	0.344	0.094 ± 0.031	33	220
RCN	Roccaverano	21	19	19	0.408	0.414	0.027 ± 0.04	22	233
RCR	Rustica di Calabria	63	61	27	0.412	0.404	0.028 ± 0.039	34	529
RSM	Rossa Mediterranea/ Derivata di Siria	36	33	27	0.409	0.403	0.038 ± 0.039	32	173
SNN	Saanen	536 (1540)*	451	27	0.415	0.410	0.049 ± 0.037	28	123
SRR	Sarda	118	117	27	0.414	0.404	0.03 ± 0.039	34	490
VLD	Valdostana	20	19	19	0.366	0.378	0.056 ± 0.015	23	213
VLS	Vallesana	18	17	17	0.359	0.356	0.112 ± 0.084	18	76
VRZ	Verzaschese	15	15	15	0.377	0.378	0.057 ± 0.054	17	138

Cilentana Fulva, Cilentana Grigia, and Jonica were excluded because, after quality control and exclusion of relatives (QC), less than 7 individuals were retained.

*For Camosciata delle Alpi and Saanen, the number of individuals before pre-screening (see main text) is reported in parentheses.

Abbreviations: He = Expected heterozygosis; Ho = Observed heterozygosis; FROH = Inbreeding based on runs of homozygosity; Ne = Genomic effective population size, calculated with SNeP or GONE software.

Quality control was performed using PLINK v1.9 [390], filtering out SNPs located on sex chromosomes and those with a call rate below 95%. Individuals with more than 5% missing genotypes or identified as closely related (based on Mendelian errors, detected using an in-house script) were also excluded. Furthermore, SNPs with a minor allele frequency (MAF) below 0.1% and those in linkage disequilibrium (LD) were pruned using the --indep-pairwise 50 10 0.5 option in PLINK. To prevent over- or underrepresentation of certain breeds, we excluded populations with fewer than 8 animals (Jonica, Cilentana Fulva, and Cilentana Nera), and subsampled the remaining breeds to a maximum of 27 individuals (corresponding to the dataset's breed size median), using the *bite.representative.sampling* function from the BITEV2 R package [391]. The final dataset included 685 goats from 31 populations and 46,878 SNPs.

4.4.3.4 Population structure and phylogenomic relationships

A multidimensional scaling (MDS) analysis was conducted with PLINK using the number of dimensions equal to the number of individuals. A further dimensionality reduction to better visualize both local and global relationships was performed by applying PHATE [492] using the first 20 principal components (PCs) from the MDS, with the following parameters: knn = 31, decay = 100, and gamma = 0.

Reynolds distances among populations were calculated using an in-house script and used to generate a NeighbourNet with SplitsTree [413], which was plotted with phanorn and tangle R packages [690,691]. Bootstrapped identity-by-state (IBS) distances among individuals were computed with PHYLIP [493] and the related tree was visualised using the ggtree R package [494]. TreeMix [158] was run allowing 0 to 10 migration events. Additionally, we computed *f3* statistics.

Ancestral genomic components were inferred with ADMIXTURE v1.3 [161], testing ten models for a number of clusters (K) from 2 to 25. The best-fitting model was selected as the one with the median five-fold cross-validation (cv) error across all runs. For each K, the run with the lowest cv was plotted.

After phasing the dataset (pruned for MAF but not for LD) with Beagle v4.1 [495], identity-by-descent (IBD) based haplotype sharing was analyzed using RefinedIBD v3.1 [415] with default parameters. We computed the pairwise median of shared segments between individuals from different populations, assigning a value of 0 in the absence of shared haplotypes. The top 5% longest haplotype sharing values were visualised using the circlize R package [416].

Genomic diversity analyses were carried out on the unphased dataset, without breed size balancing or LD pruning [136], including 2,458 individuals and 48,573 SNPs. Observed and expected heterozygosity (Ho and He) and McQuillan's inbreeding coefficient based on runs of homozygosity (F_{ROH}) were estimated [133]. ROHs were detected with PLINK v1.9 using the following parameters: --homozyg-gap 500, --homozyg-kb 1000, --homozyg-window-het 0, --homozyg-window-missing 2. The minimum number of SNPs required to define both a ROH and the window size (--homozyg-snp and --homozyg-window-snp, respectively) was estimated to be 42 according to L parameter [136]. Density parameter (--homozyg-density) was set as 55, corresponding to the lowest value that maximized genome coverage, which under these conditions reached 98.2% [136].

Effective population size (Ne) based on LD was estimated using SNeP v1.1 [140], analyzing SNPs at 280 Kb to 20 Mb distance and applying and Sved & Feldman recombination rate modifier [412]. Ne was also estimated using GONE v1.0 [496] with default settings.

4.4.3.5 Comparison with older data

The balanced dataset described above was compared with data of goats sampled in 1995-2010, including the publicly available Italian Goat Consortium (IGC2) dataset [74], which includes 1,071 samples genotyped with the GoatSNP50 BeadChip (Illumina Inc., San Diego, CA), and newly generated data from the Agritech project for Orobica (ORB, n = 42), Bionda dell'Adamello (BDL, n = 37), Verzaschese (NVE, n = 25), Lariana (LRN, n = 38), SAA (n = 17), and CTP (n = 23) goats born between 1995 and 2010, genotyped with the GoatSNP65 BeadChip (Illumina Inc., San Diego, CA). Samples from the Bezoar, Montecristo, and Maltese x Sarda populations from IGC2 were excluded, whereas Maltese sampled in Sardinia or elsewhere, as well as Rossa Mediterranea and Derivata di Siria were merged, as they are different denominations for the same breed. After merging the datasets, we applied the same quality control, filtering, and breed size balancing procedures described above, resulting in a final dataset of 46,807 SNPs and 1,358 goats from 37 breeds: 22 breeds were included in both the "present" and "older", nine only in the present one, and six only in the older one (Supplementary Table S4.4.1).

The following analyses were then repeated, focusing on comparisons between contemporary and historical samples of the same breeds: MDS, ADMIXTURE (testing up to 27 K), and F_{ROH} estimation.

4.4.3.6 Landscape genomics

4.4.3.6.1 Dataset and quality control

For the landscape genomics analysis, only recent animals with geographic coordinates were considered. Saanen and Camosciata delle Alpi goats were excluded due to their rearing in intensive or semi-intensive systems, making them less exposed to environmental pressures. The resulting dataset included 1,159 animals from 32 breeds.

Quality control followed the same approach as above: excluding SNPs and individuals with low call rates, closely related individuals, and sex chromosome SNPs, along with pruning for MAF and LD. Additionally, to avoid population structure bias, the seven overrepresented breeds were reduced to a maximum of 35 individuals, selected based on geographic distribution, ensuring at least one individual per farm. The final dataset comprised 693 animals and 46,255 SNPs (Supplementary Table S4.4.1).

4.4.3.6.2 Climatic variables

Environmental data were obtained from the WorldClim v2.1 database at a 30-second resolution (~1 km^2), covering the period 1970–2000. We extracted elevation and the average values of 19 bioclimatic variables [692] described in Supplementary Table S4.4.2. Additionally, monthly data on precipitation, minimum temperature and maximum temperatures were used to calculate median seasonal values, considering December, January, and February for winter season; March, April and May for spring season; June, July, and August for summer season, and September, October and November for autumn season. Environmental data were assigned to each sample using the *extract* function from the raster R package [355].

To avoid collinearity, a subset of environmental variables was selected [179]: *exclude* function from usdm R package [693] was applied to recursively exclude variables with VIF > 5; subsequently, *findCorrelation* function from caret R package [694] was applied to exclude the least number of variables so that the maximum Pearson correlation coefficient was 0.75.

Projections of the environmental variables were retrieved at a resolution of 30 seconds from WorldClim v2.1 for the period 2081-2100 and referred to the global climate model ACESS-CM2 [695,696], and the shared socio-economic pathways (SSP) 2-4.5 (moderate climate change) and 5-8.5 (severe climate change) [697].

4.4.3.6.3 Population structure

An MDS analysis was performed using PLINK v1.9 as previously described. Additionally, ancestral population structure was inferred using the *snmf* function of the LEA R package [194], running 30 replicates for K values from 1 to 25. Cross-entropy (ce) was calculated for each replicate and K value. The best-fitting K was defined as the one with the lowest median ce. To identify the most parsimonious K, we used the *calculate.fda* function of the doremi R package [698], fitting ce as a function of K and calculating its first derivative. We then computed the absolute difference between the derivative at each K and the derivative at the best K (i.e., |Δf'(K)|). We selected the lowest K value among those with |Δf'(K)| less than or equal to the third quartile as the most parsimonious K. Missing genotypes were imputed using the mode method from the impute function in LEA, based on the replicate with the lowest ce at the most parsimonious K.

4.4.3.6.4 Gene-environment association

To identify SNPs associated with selected focal environmental variables, we combined two complementary GEA approaches: univariate latent factor mixed model (LFMM) and multivariate partial redundancy analysis (pRDA).

Introduced by Frichot *et al.* (2013) [190]. LFMM is a Bayesian univariate mixed models that account for neutral population structure by incorporating a user-defined number of latent factors as random effects, inferred simultaneously with genomic–environmental associations using a Markov Chain Monte Carlo algorithm, while environmental predictors are modeled as fixed effects. In the present study, LFMM was conducted using the lfmm R package v2.0 [699]. The *lfmm_ridge* function estimated regularised effect sizes using a number of latent factors equal to the most parsimonious K. The *lfmm_test* function produced p-values for each GEA, calibrated using the genomic control method. These p-values were then transformed into q-values using the qvalue R package [700]; SNPs with an FDR < 0.05 were considered significant.

pRDA is a constrained ordination method that models multivariate linear relationships between genotype and environment by constructing canonical axes through PCA on fitted genetic values constrained by environmental predictors. Unlike standard RDA, pRDA incorporates neutral genetic structure as a conditioning variable, improving robustness under isolation by distance or low dispersal and reducing spurious associations caused by spatial autocorrelation or population structure [192]. Here, pRDA was performed with the *rda* function from the vegan R package [349], using the latent score matrix from LFMM as a covariate (conditioning matrix). Due to computational constraints, we could not apply *anova.cca* function and thus we deemed significant the least number of axes so that the sum of explained variance was at least 70% and/or the explained variance was at least 10%.-SNPs were deemed significant if their score exceeded the mean by at least 3 standard deviations on at least one of the significant canonical axes.

Genes located within ± 26.6 kb of these SNPs were identified, 26.6 kb being half of the average gap between consecutive analysed SNPs. Gene ontology (GO) enrichment analysis was carried out using GeneCodis v4 [498], comparing these genes to a background set including all genes associated with all SNPs available in the GEA dataset. GO terms were assigned according to the *Bos taurus* annotation, as it is the closest reference species. A significant threshold was set to 0.05 after Benjamini-Hochberg correction.

4.4.3.7 Local genomic offset

To estimate individual local genomic offset (g.o.) metrics, we first calculated a current Adaptive Index (AI), which reflects the relationship between putatively adaptive genetic variation and present-day environmental conditions. We performed a redundancy analysis (RDA) using only the SNPs identified as candidates in previous GEAs as the response matrix, and the standardised environmental variables used for GEAs as the explanatory matrix. Significant RDA axes were determined using the *anova.cca* function with 999 permutations and a significance threshold of $p < 0.01$.

For each of the spatial pixels in the study area (i.e., Italy) and significant RDA axis, the current AI was computed as: $ev * \sum_{i=1}^{n} b_i a_i$, where a_i is the loading of environmental variable i on the selected RDA axis, and b_i is the standardised value of that variable at the pixel, and ev is the variance explained by the RDA axis. All pixel-level environmental maps were standardized with the same center and scale factor as the present individual-level matrix.

To evaluate whether individuals could be grouped based on similar AI values, we applied K-means clustering to individual AI scores. On ten random subsamples of 10,000 pixels, we applied *KMeans_rcpp*, testing 2 to 10 models, and *silhouette_of_cluster* functions from ClusterR package [701], and selected the number of clusters consistently associated with the highest silhouette score.

Next, we predicted future AI values using climate projections under two alternative SSPs. Each environmental variable was weighted according to its loading from the initial RDA, thus reflecting its contribution to SNP-environment associations under current conditions, and the future AI was calculated following the same procedure as for the current AI, but using the future values of standardized environmental variables.

Local g.o. was then computed as the Euclidean distance between the current and future AI values for each pixel across the study area. To test whether the two climate change scenarios or individual groups of individuals were associated with different average local genomic offsets, we performed Kruskal-Wallis and Dunn test through *kruskal_test* and *dunn_test* functions from rstatix [702], after assessing normality and variance homogeneity with Shapiro and Levene tests, respectively.

To identify potential spatial outliers (i.e., individuals with significantly different genomic offset values compared to their neighbours) and/or local clusters (i.e., groups of geographically close individuals with consistently higher or lower offset), we evaluated the local spatial autocorrelation of genomic offset values. Specifically, the rgeoda's [703] *local_moran* function was used to calculate local Moran's I statistics based on individual genomic offsets (averaged across the two climate scenarios), using spatial weights derived from the *knn_weights* function across a range of K values from 2 to 100. Significance was determined using 999 conditional permutations, with p-values adjusted using the Benjamini–Hochberg method. A threshold of 0.01 was used to identify statistically significant results.

4.4.4 Results

4.4.4.1 Census data

Census data reveal that over the past 15 years (2010–2024), the number of registered goat farms has steadily declined, with an overall reduction of 32%. In contrast, the number of registered animals exhibited a more variable trend: an initial decline of 16% between 2012 and 2014 was followed by a 40% increase up to 2018, and then a subsequent decline of 23% in the last four years.

A similar pattern was observed when focusing solely on breeds under conservation programs (Figure 4.4.2A). Although the number of farms decreased by 28%, the total number of registered animals increased by 5% compared to 2010, despite a downward trend in the past five years. However, notable differences emerged among individual breeds (Supplementary Figure S4.4.1). For instance, Alpina, Jonica, Maltese, Rossa Mediterranea, and Valdostana showed a consistent decline over the entire period. In contrast, breeds such as Cilentana Grigia, Di Potenza, Di Teramo, Grigia Valle Lanzo Fiurina, Nicastrese, and Pomellata showed an overall increasing trend. An interesting case is the Girgentana breed, which experienced a sharp decline in both animals and farms in 2017–2018; while the number of farms then stabilised, the number of animals peaked abruptly in 2019 before slowly returning to 2018 levels. Similarly, the Garfagnana breed saw a marked increase between 2012 and 2013, after which farm numbers remained stable, but animal numbers declined. It is worth noting that nine local breeds were newly recognized during the study period. However, no registrations were reported in 2024 for ten breeds, suggesting a possible extinction risk.

A different scenario emerged for the three breeds under selection programs (Figure 4.4.2B). The Sarda goat maintained a relatively stable number of farms and showed an overall positive trend in animal numbers—despite a recent decline. However, given the limited number of registered bucks, the Sarda was reclassified as "breed under conservation programs" in 2025. In contrast, both Saanen and Camosciata delle Alpi experienced a negative trend in farm numbers and, especially after 2018, in animal numbers as well. Compared to 2010, Camosciata delle Alpi lost 37% of farms and 30% of animals, while Saanen suffered losses of 60% and 64%, respectively.

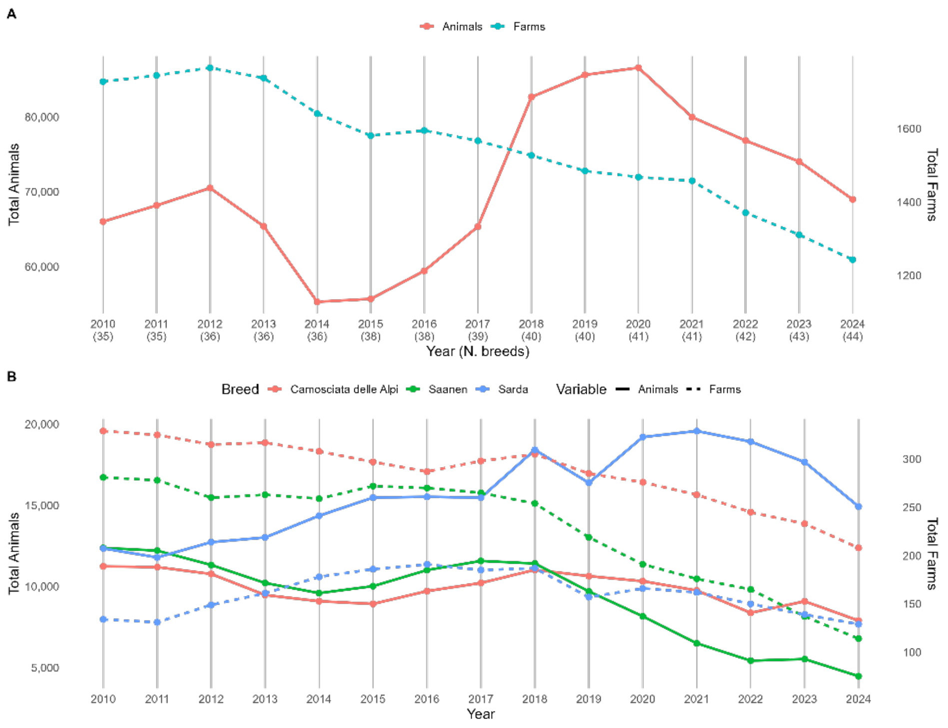


Figure 4.4.2 Trends in animal and farm registration for Italian goat breeds under conservation (A) and selection (B) programs.

Estimates of census-based effective population size (Ne), often used to assess extinction risk, indicated that 21 breeds (45%) had Ne < 50, representing a short-term risk of extinction, including ten with Ne equal to 0. Another 16 breeds (34%) had Ne values between 50 and 500, indicating a long-term risk, while 10 breeds (21%) had Ne > 500, including five with values exceeding 1,000 (Table 4.4.2).

Table 4.4.2 Main results on census data analysis for Italian goat breeds.

Breed	N. farms (2024)	ΔFarms	N. animals (2024)	ΔAnimals	Growth Rate	Ne	ΔF	Risk
Northwestern Italy								
ALPINA	6	-138 (-95.8%)	1	-609 (-99.8%)	0.70	0		Ex
BIONDA DELL'ADAMELLO	63	-48 (-43.2%)	1501	-522 (-25.8%)	0.98	384	0.13	E
CAMOSCIATA DELLE ALPI	208	-121 (-36.8%)	7900	-3341 (-29.7%)	0.98	942	0.05	NR
FRISA VALTELLINESE	43	-29 (-40.3%)	1561	223 (16.7%)	1.02	385	0.13	E
GRIGIA VALLE LANZO FIURINA	42	40 (2000%)	756	752 (18800%)	2.35	214	0.23	E
LARIANA O DI LIVO	15	-52 (-77.6%)	736	-896 (-54.9%)	0.97	122	0.41	E
MANTELLATA POSTERIORE	3	0 (0%)*	0	0 (NaN%)*		0		Ex
OROBICA O DI VAL GEROLA	98	19 (24.1%)	3036	1079 (55.1%)	1.04	677	0.07	E
ROCCAVERANO	32	-8 (-20%)	1570	711 (82.8%)	1.05	437	0.11	E
SAANEN	114	-167 (-59.4%)	4472	-7907 (-63.9%)	0.93	314	0.16	V
SEMPIONE	3	0 (0%)	27	11 (68.8%)	1.08	7	6.75	C
VALDOSTANA	6	-199 (-97.1%)	13	-1674 (-99.2%)	0.74	4	13.54	C
VALLESANA	17	0 (0%)	410	265 (182.8%)	1.09	154	0.32	E
VERZASCHESE	8	-23 (-74.2%)	609	-317 (-34.2%)	0.98	132	0.38	E
Northeastern Italy								
PASSEIRER GEBIRGZIEGE	0	-1 (-100%)*	0	-1 (-100%)*	1.00	0		Ex
PEZZATA MOCHENA	26	6 (30%)	229	117 (104.5%)	1.09	49	1.02	C
Central Italy								
BIANCA MONTICELLANA	12	-21 (-63.6%)	512	-543 (-51.5%)	0.98	92	0.55	E
CAPESTRINA	18	-23 (-56.1%)	291	-125 (-30%)	0.99	39	1.29	C
CIOCIARA GRIGIA	12	-16 (-57.1%)	142	-199 (-58.4%)	0.97	47	1.06	C
DI MONTECRISTO	0	-1 (-100%)*	0	-4 (-100%)*	0.94	0		Ex
GARFAGNANA	18	10 (125%)	165	-78 (-32.1%)	1.28	34	1.47	C
Southern Italy								
CAMPOBASSO GRIGIA MOLISANA	3	-2 (-40%)	119	-126 (-51.4%)	0.99	23	2.19	C
CILENTANA FULVA	8	-3 (-27.3%)	362	160 (79.2%)	1.06	76	0.66	E

CILENTANA GRIGIA	11	4 (57.1%)	55	27 (96.4%)	1.10	38	1.33	C
CILENTANA NERA	40	1 (2.6%)	1649	590 (55.7%)	1.04	344	0.15	E
DELL'ASPROMONTE	113	-39 (-25.7%)	13597	-1682 (-11%)	1.02	2753	0.02	NR
DI BENEVENTO VALFORTORINA	6	5 (500%)*	27	26 (2600%)*	1.68	19	2.68	C
DI POTENZA	59	51 (637.5%)	3189	3065 (2471.8%)	1.30	672	0.07	V
DI TERAMO	4	3 (300%)	128	127 (12700%)	1.53	59	0.85	C
FULVA DEGLI ALBURNI	1	0 (0%)*	0	0 (NaN%)*		0		Ex
FULVA DEI MONTI PICENTINI	0	-1 (-100%)*	0	0 (NaN%)*		0		Ex
GARGANICA	44	13 (41.9%)	3983	2282 (134.2%)	1.10	760	0.07	V
GRIGIA DEGLI ALBURNI	0	-1 (-100%)*	0	0 (NaN%)*		0		Ex
GRIGIA DEI MONTI PICENTINI	0	-1 (-100%)*	0	0 (NaN%)*		0		Ex
JONICA	3	-13 (-81.2%)	18	-817 (-97.8%)	0.80	0		Ex
NAPOLETANA	2	0 (0%)	83	43 (107.5%)	1.67	29	1.73	C
NICASTRESE	72	29 (67.4%)	6316	3589 (131.6%)	1.07	1118	0.04	NR
POMELLATA	4	3 (300%)	59	57 (2850%)	1.36	18	2.73	C
RUSTICA DI CALABRIA	97	1 (1%)	11166	772 (7.4%)	1.01	2388	0.02	NR
SCREZIATA	1	0 (0%)*	0	0 (NaN%)*		0		Ex
Isles								
ARGENTATA DELL'ETNA	110	51 (86.4%)	2910	1035 (55.2%)	1.04	1241	0.04	E
GIRGENTANA	20	-25 (-55.6%)	768	-704 (-47.8%)	1.00	190	0.26	E
MALTESE	34	-56 (-62.2%)	643	-2384 (-78.8%)	0.97	66	0.76	E
MESSINESE	114	-5 (-4.2%)	7097	-675 (-8.7%)	1.00	1869	0.03	NR
ROSSA MEDITERRANEA	18	-43 (-70.5%)	780	-1847 (-70.3%)	0.93	126	0.4	E
SARDA**	129	-5 (-3.7%)	14930	2582 (20.9%)	1.02	128	0.39	V
SARDA PRIMITIVA	57	16 (39%)	4478	1228 (37.8%)	1.03	958	0.05	V

*Breeds that were recognized after 2010. For these breeds, ΔFarms and ΔAnimals refers to the difference between the recognition year and 2024.

**Breed under selection programs; all other breeds are under conservation programs. It should be noted that Sarda breed has been reclassified as "breed under conservation program" in 2025.

Abbreviations: ΔFarms/Animals = Absolute and percentage difference in the number of registered farms/animals from 2010 (or year of recognition) to 2024; Ne = census-based effective population size; ΔF = census-based inbreeding rate; FAO extinction risk categories: NR = Not at risk; V = Vulnerable; E = Endangered; C = Critical; Ex = Extinct.

ΔF values could not be calculated for the 10 breeds with no registrations in 2024. Among the remaining breeds, ΔF was below 0.5% for 22 breeds, between 0.5–1% for 4 breeds, between 1–3% for 3 breeds, and above 3% for Valdostana and Passeirer Gebirgziege (Table 4.4.2).

According to the FAO criteria [36], only five breeds (11%) are currently not at risk of extinction: Camosciata delle Alpi, Aspromontana, Messinese, Nicastrese, and Rustica di Calabria. Five breeds, including the Sarda goat—which, despite being the largest Italian breed, had only 32 registered bucks in 2024—are classified as vulnerable. Alarmingly, 15 breeds (32%) are considered endangered, 12 (26%) are critical, and the 10 breeds with no registrations in 2024 should be formally regarded as extinct (Table 4.4.2).

4.4.4.2 Italian goat population biodiversity

The population structure inferred from the MDS analysis (Figure 4.4.3A) revealed a clear north-to-south separation of Italian goat populations along PC1, which accounted for 3.52% of total genetic variation. PC2 distinguished Bianca Monticellana (BNM) and Capestrina (CAP) from other populations, with some overlap from Ciociara Grigia (CCR). PC3 further isolated the Orobica (ORB) population and, to a lesser extent, the Girgentana (GRG).

The PHATE analysis provided a smoother representation of geographic gradients. While PC1 still reflected the north-south divide, PC2 distinguished central breeds (BNM, CAP, CCR) and partially separated insular (Sicilian, Sardinian, and Maltese) populations (Figure 4.4.3B).

The two main branches of the NeighbourNetwork based on Reynolds genetic distances separated northern and southern populations, with the Di Teramo (DRT) and the Pezzata Mochena (MCH) being the most central breeds. Further grouping could be observed, mostly connecting breeds with similar geographical origin (Figure 4.4.3C). Individual IBS distances (Figure 4.4.3D) showed that most individuals grouped with their respective populations along a geographic continuum. Notable exceptions included the intermixing of: Sicilian breeds Argentata dell'Etna (AET) and Messinese (MSN); BNM, CAP, and CCR; and Cilentana Nera (CTN), Di Potenza (DRP), and Garganica (GRN). Moreover, some MSN individuals clustered near the Comune di Sicilia/Mascaruna (CCS); three Roccavrano (RCN) were near

Bionda dell'Adamello (BDL); and a few Lariana (LRN) goats grouped with northern breeds such as ORB, Frisa Valtellinese (FNS) and Verzaschese (VRZ). Additionally, several DRP individuals were positioned between Napoletana (NPT) and Rustica di Calabria (RCR).

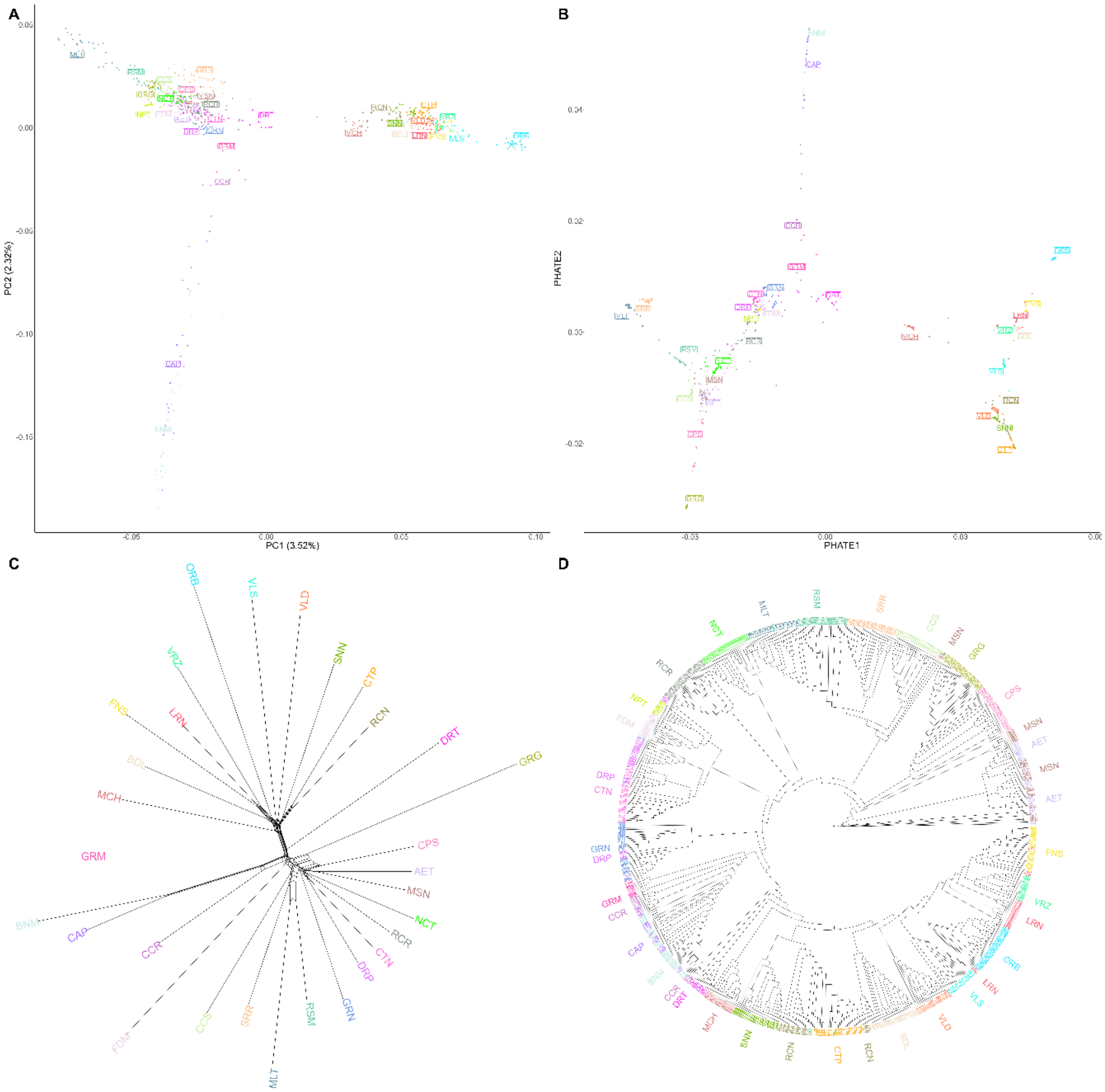


Figure 4.4.3 Genetic population structure of Italian goat breeds.

First two principal components (PCs) of multidimensional scaling analysis (A) and PHATE (B). Each point corresponds to a subject and points are colored by breed.

Reynold distance-based neighbournet (C) and individual identity-by-state dendrogram (D).

Treemix identified one main migration event connecting the RCN to the Sarda (SRR) population (Supplementary Figure S4.4.2). *f3* statistics confirmed significant admixture from

BNM to CAP and from both to CCR; from GRG to Aspromontana (CPS); from ORB and FNS or VRZ to LRN; and from Maltese (MLT) to MSN (Supplementary Table S4.4.3).

Haplotype sharing analysis (Figure 4.4.4A) highlighted substantial sharing between BNM and CAP, confirming their close genetic relationship. Other sharing events, typically involving shorter haplotypes, were mainly restricted to geographically proximate populations. Only one case of sharing with a cosmopolitan breed was detected, between RCN and Camosciata delle Alpi (CTP).

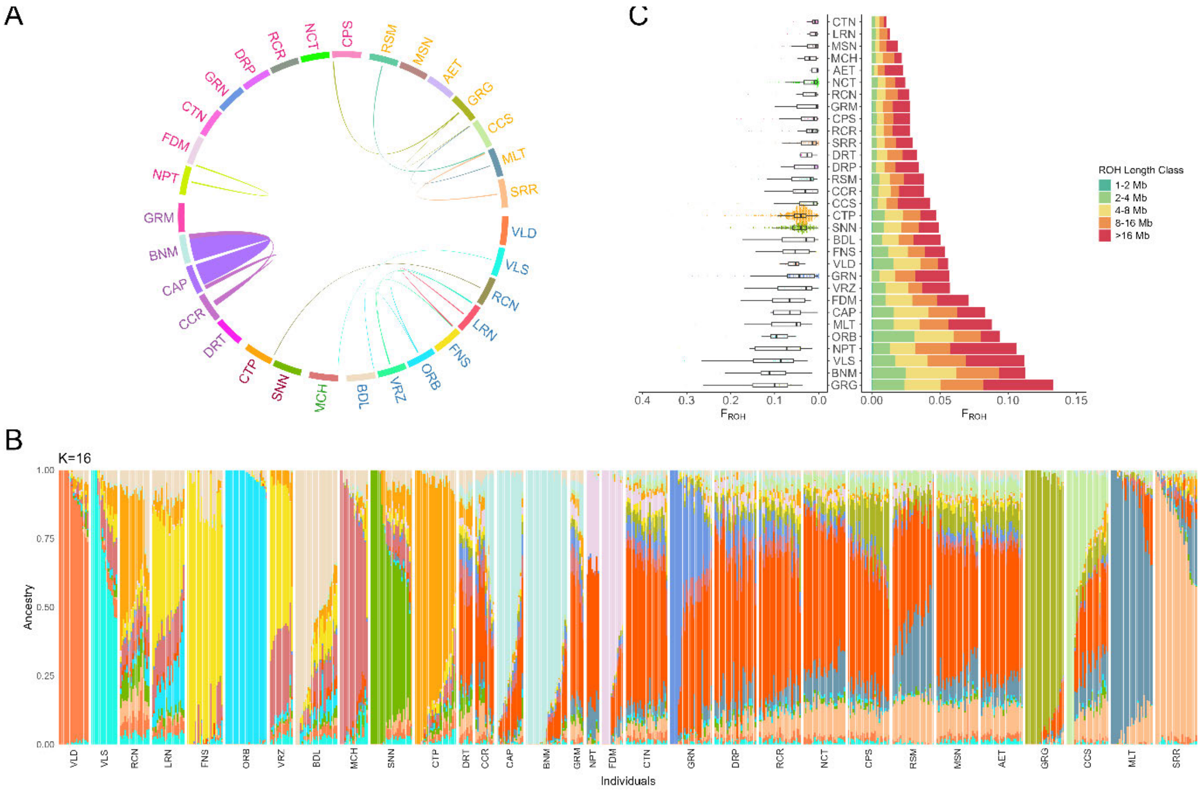


Figure 4.4.4 Genomic background and diversity of Italian goat breeds.

A) Identity-by-descent (IBD) haplotype sharing, showing the top 5% longest segments shared among breeds; breed names are colored by microregion of origin.

B) Admixture analysis with the best-fitting model (K = 16). Each bar represents an individual, and each color indicates the proportion of ancestry assigned to a specific cluster. Breeds are ordered geographically.

C) Inbreeding estimated from runs of homozygosity (F_{ROH}), shown at the individual level (boxplot, left) and as breed averages (barplot, right). In the barplot, colors represent the contribution of different ROH length classes, and breeds are ordered by average total F_{ROH}.

Admixture analysis supported these findings. At K = 2, a clear north-south ancestry gradient was observed. At K = 3 and 4, distinct clusters emerged for BNM-CAP and ORB (Supplementary Figure S4.4.3). The lowest median cross-validation error was observed at K = 16 (Figure 4.4.4B), but it should be noticed that cv for K 15-17 were all very close (Supplementary Figure S4.4.4). At this resolution, most of northern populations displayed defined, breed-specific ancestries, while most southern populations shared a common ancestral component—here referred to as the "Mediterranean component"—with varying degrees of introgression. Three northern populations exhibited a composite background: the VRZ was mainly composed by FNS and MCH; a similar admixture was observed with the LRN, which however also displayed shared ancestry with other northern populations, such as ORB and BDL. When, at K = 17, a cluster was identified for VRZ, it also appeared as the predominant component in LRN and as introgressing component in some FNS; in this model the MCH cluster disappears, and the breed appears mainly related to BDL, with relevant portions also from the Mediterranean component and VRZ. A different pattern was instead found for the RCN, where most individuals showed clear introgression from the specialized dairy breed CTP. In the central-southern region, CAP and BNM had nearly identical profiles, which separated from neighboring breeds. Among southern populations, FDM, GRG, MLT, and SRR formed unique clusters, with signs of reciprocal introgression between the latter two. GRN and CCS also formed specific clusters, though with substantial individual variation. All the other populations displayed similar admixed pattern, with the Mediterranean component largely dominating the background usually followed by the same clusters that were maximized in other southern populations. In particular, MSN, AET, RCR, and NCT shared a very similar pattern, with evident sharing with CCS, GRG, SRR, and MLT in addition to the Mediterranean component. In contrast, other populations presented other secondary ancestries: for example, FDM cluster was present in other breeds from Campania (CTN and NPT, the latter obtaining its cluster at K = 17, despite the limited sample size), whereas GRN and BDL cluster in breeds from central Italy (GRM and CCR). Interestingly, the background of DRT shared ancestry also with MCH and CTP, whereas GRG cluster was particularly evident in CPS, consistent with other results.

Inbreeding, estimated via ROH, varied considerably among populations (Table 4.4.1). F_{ROH} ranged from 1.1 ± 1.2% in CTN to 13.3 ± 8.9% in GRG, with an average value across all the individuals of 4.6 ± 4.5%. Differences in the distribution of ROH length classes reflected contrasting demographic histories. Populations such as GRG, NPT, and Vallesana (VLS) exhibited high F_{ROH} values predominantly due to long ROH (>16 Mb), indicative of recent inbreeding. In contrast, ORB and BNM showed elevated inbreeding, but spread across all ROH classes. Some populations with moderate overall inbreeding, such as AET and CCS, still harboured individuals with signs of recent consanguinity. The specialised dairy breeds Saanen (SNN) and CTP showed moderate F_{ROH} values with uniform ROH distributions.

Effective population size (Ne) estimates obtained from SNeP and GONE varied in absolute values (8–38 for SNeP and 34–1142 for GONE), ranking of the populations was largely superimposable, with a few exceptions, such as the Lariana, which positioned among the breeds with the highest Ne only using GONE software (Table 4.4.1). Considering the most recent generations, NPT and DRP had the lowest Ne values, while MSN had the highest. Temporal Ne trajectories from GONE (Supplementary Figure S4.4.5) revealed breed-specific dynamics that aligned with ROH-based inbreeding trends. For example, DRT showed Ne values much higher than all the other populations up to about 4 generations ago, when a sharp decline occurred, whereas ORB experienced a long-term decline beginning ~65 generations ago, followed by partial recovery and stabilization ~40 generations ago.

4.4.4.3 Comparison with older samples

The MDS plot (Figure 4.4.5A) obtained from the merged dataset closely mirrored the one based on contemporary samples alone, showing a clear south-to-north geographic gradient along PC1 and primarily distinguishing BNM and CAP along PC2. Notably, individuals from the same population consistently clustered together regardless of sampling period, indicating strong temporal continuity and preservation of population identity over time.

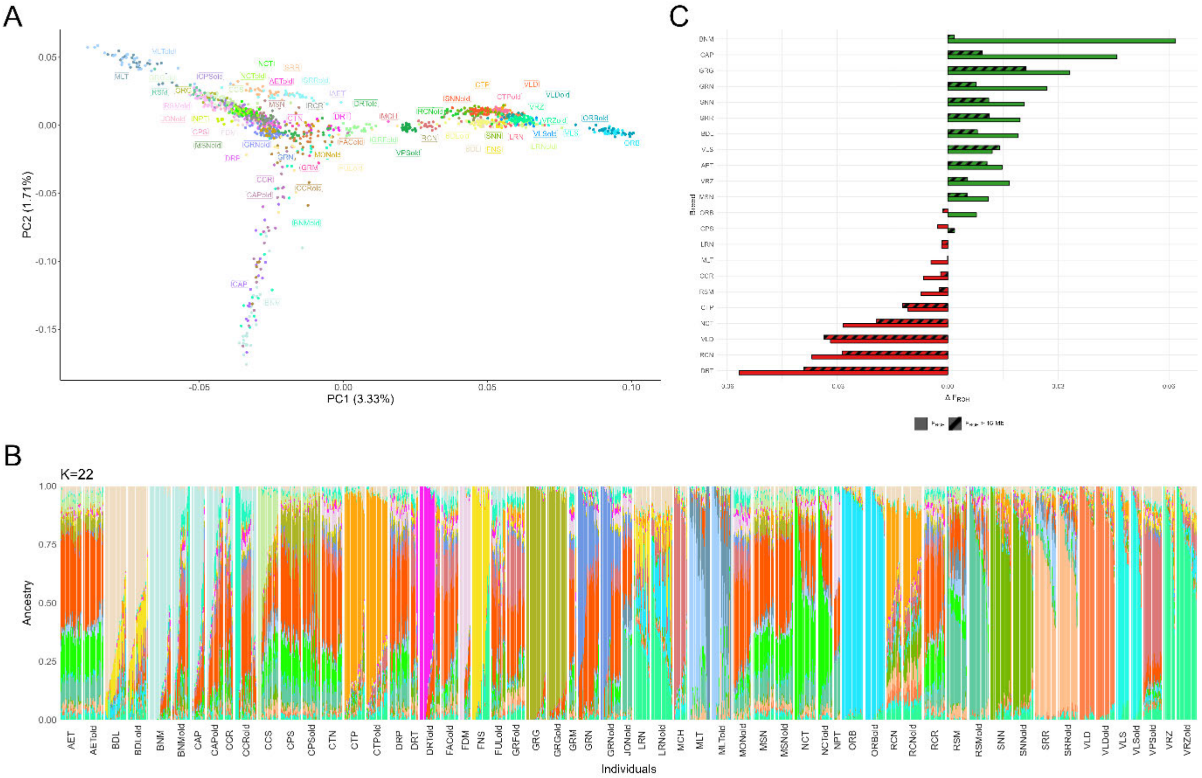


Figure 4.4.5 Comparison of recent samples with goats sampled 20 years ago.

A) First two principal components (PC) of multidimensional scaling analysis; open points represent older samples and filled points represent samples from the current dataset.

B) Admixture analysis with the best-fitting model with 22 clusters (K). Codes including 'old' refer to the older dataset. Codes for breed only present in the older dataset: FACold = Facciuta della Valnerina; FULold = Fulva del Lazio; GRFold = Garfagnana; JONold = Jonica; MONold = Capra di Montefalcone; VPSold = Capra della Val Passiria.

C) Differences in F_{ROH} between individuals of the same breed from the older and current dataset, including both overall F_{ROH} and the component associated with recent inbreeding (ROH > 16 Mb).

Admixture analysis identified K = 22 as the best-fitting model (Figure 4.4.5B and Supplementary Figure S4.4.6). In line with the MDS results, most populations showed minimal differences between historical and contemporary samples. Notably, we observed that, in addition to the Mediterranean component, two other clusters were largely present in Southern and Central breeds, respectively: one maximized in certain NCT individuals, and another shared by both MCH and IGC2 Val Passiria (VPSold). The latter cluster constituted the largest portion of the genetic background in VPSold and, even more prominently, in MCH, but VPSold also exhibited contributions from BRZ, BDL, and the Mediterranean component, whereas MCH showed a minor input from VLS. Some notable differences emerged between older and contemporary populations for certain breeds. Compared to historical samples, current BNM and CAP individuals displayed greater genetic uniformity, with higher Q-values for their characteristic ancestral component, while the opposite was true for Rossa Mediterranea (RSM). To a lesser extent, SRR also exhibited increased introgression from MLT. The LRN population remained highly admixed across timepoints but showed more evident introgression from FNS in recent samples. The most marked changes were observed in CCR and DRT: while a distinct ancestral component was evident in part of the older individuals, contemporary samples exhibited a more admixed genetic background. Interestingly, two separate clusters were primarily associated with the MLT population and were present in both time periods; these clusters appeared to reflect sampling location, distinguishing goats from Calabria or Sicily *vs* those from Sardinia, reflecting differentiation due to geographic isolation and consequent genetic drift.

Temporal differences in inbreeding were also observed across populations (Figure 4.4.5C). A few populations showed a reduction in F_{ROH} over time, i.e., CTP, NCT, VLD, RNC, and DRT, which all previously exhibited elevated levels of recent inbreeding. Conversely, several populations showed an increase in F_{ROH}, especially BNM, CAP, and GRG. Among them, GRG also increased in F_{ROH} associated with long ROH, and so did, for example VLS, whereas this was minimal for BNM and CAP, despite the overall F_{ROH} increase of 4.5 to 6%.

4.4.4.4 Landscape genomics

4.4.4.4.1 Genomic structure

The 693 goats, belonging to 32 breeds, selected for the GEA dataset mainly represented populations from the Alpine region, central–southern Italy, as well as Sicily and Sardinia (Figure 4.4.6A). No samples were available from the central–northern regions, where the number of goat farms is generally low, particularly when excluding specialized dairy breeds. In this area, the main local breed is the Garfagnina, which unfortunately was not included in the present dataset. This breed registered 165 animals in 2024 and, according to our analyses, presents an admixed genomic background, mainly composed of the Mediterranean component and the cluster related to MCH and VPSold, consistent with previous findings [74,254].

The plot obtained from the first two components of the MDS was largely consistent with that from the dataset used for biodiversity analysis. The primary differentiation was observed along PC1, separating northern from southern populations, whereas PC2 distinguished BNM, CAP, and partly CCR breeds (Supplementary Figure S4.4.7A). According to the median ce values across Ks (Supplementary Figure S4.4.7B), the sNMF analyses indicated K = 13 as the best-fitting model (lowest ce), and K = 7 as the most parsimonious solution. At K = 13, most central–southern breeds shared a predominant ancestral component (the "Mediterranean component"), whereas most northern breeds displayed a distinctive background (Supplementary Figure S4.4.7C). At K = 7, only a few breeds remained clearly distinct (FNS, GRG, MLT, ORB, BNM–CAP, and VLD–VLS), while all others showed an admixed background (Supplementary Figure S4.4.7D).

4.4.4.4.2 Environmental data

Pruning of the environmental matrix for collinearity resulted in the identification of a subset of seven variables used for the GEA analysis, as reported in Table 4.4.3 and Supplementary Figure S4.4.8. This subset included variables related both to precipitation (prec_autumn, prec_spring), temperature (tmax_summer, BIO8), and their daily or seasonal variation (BIO3, BIO4, BIO15).

Table 4.4.3 Subset of environmental variables used for GEA analyses.

Variable code	Definition	|r|>0.75
BIO3 (%)	Isothermality Corresponds to 100*BIO2/BIO7, namely the ratio between mean diurnal range (mean of monthly difference between maximum and minimum temperature) and temperature annual range (difference between maximum temperature of warmest month and minimum temperature of coldest month)	BIO2 (0.83)
BIO4 (°C)	Temperature seasonality Corresponds to the standard deviation of the monthly mean temperature multiplied by 100	BIO7 (0.91) BIO6 (-0.83) BIO9 (-0.76) BIO11 (-0.80) tmax_summer (-0.77) tmin_winter (-0.82) tmin_autumn (-0.78)
BIO8 (°C)	Mean temperature of wettest quarter Corresponds to the mean of monthly mean temperature during the three consecutive months with the highest precipitation	
BIO15 (%)	Precipitation seasonality Corresponds to the annual coefficient of variation of the monthly precipitation	BIO14 (-0.82) BIO17 (-0.81)
prec_spring (mm)	Total precipitation during spring Calculated as the median value of the average total precipitation in March, April, and May	BIO12 (0.93) BIO13 (0.82) BIO16 (0.78) BIO17 (0.77)
prec_autumn (mm)	Total precipitation during autumn Calculated as the median value of the average total precipitation in September, October, and November	BIO12 (0.87) BIO13 (0.94) BIO16 (0.98)
tmax_summer (°C)	Maximum temperature during summer Calculated as the median value of the average maximum temperature in June, July, and August	BIO1 (0.94) BIO10 (0.96) BIO11 (0.89) BIO5 (1.00) BIO6 (0.84) BIO8 (0.77) tmax_winter (0.91) tmax_spring (0.93) tmax_autumn (0.95) tmin_winter (0.83) tmin_spring (0.90) timin_summer (0.91) tmin_autum (0.87) elev (-0.87)

4.4.4.4.3 GEAs

LFMM analysis was performed using seven latent factors, corresponding to the number of K inferred as the most parsimonious solution in the sNMF analysis. At a 0.05 FDR threshold, six SNPs were significantly associated with environmental variables: four with BIO8, one with prec_spring, and one with a combination of BIO4, BIO8, and tmax_summer (Table 4.4.4 and Supplementary Figure S4.4.9).

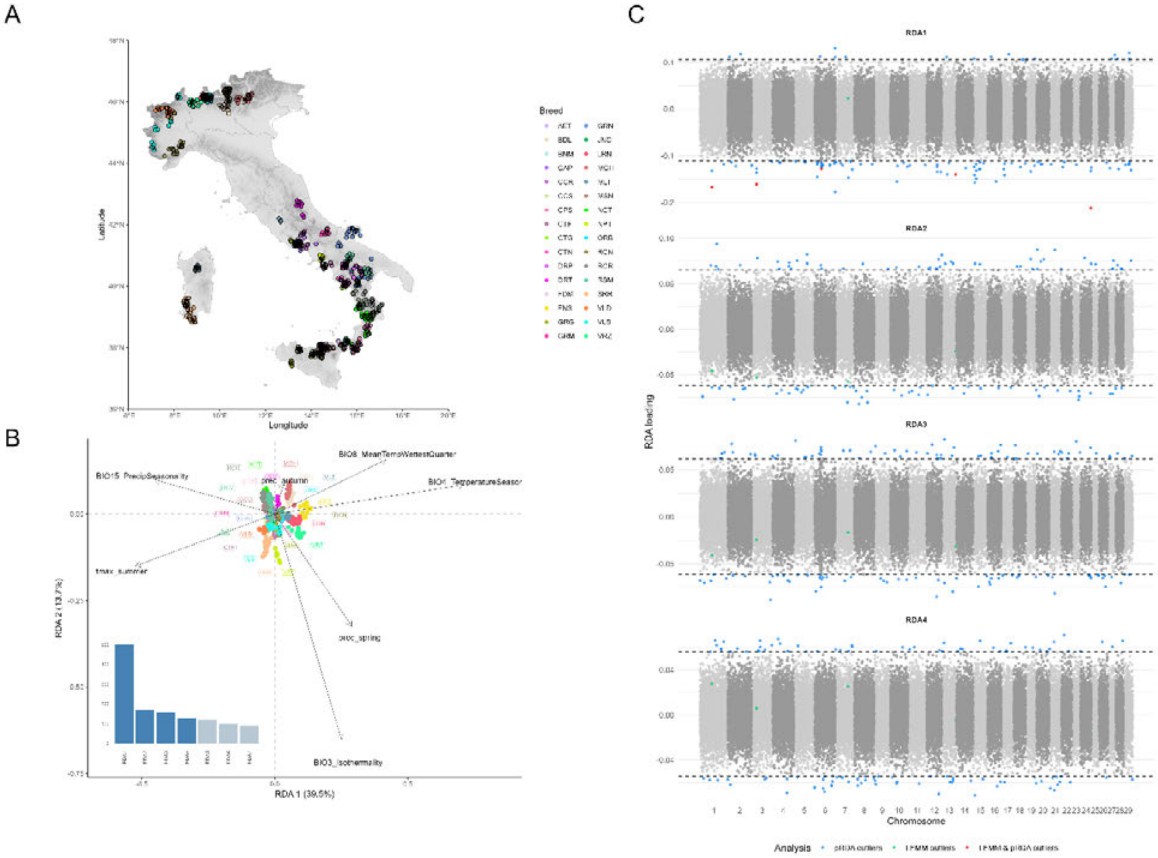


Figure 4.4.6 Genomic–environment association (GEA) analyses in Italian goats.

A) Geographic distribution of samples used for GEAs. A small jitter was applied to the points to display individuals sampled from the same location.

B) Partial redundancy analysis (pRDA): biplot of individuals and environmental variables' loadings on the first two axes, and scree plot of axis inertia, with significant axes shown in dark blue.

C) Manhattan plot of SNP scores on the significant RDA axes. Blue points indicate SNP outliers for the given RDA axis; red points mark putatively adaptive SNPs identified by both LFMM and the corresponding pRDA axis; green points mark those identified only by LFMM.

Table 4.4.4 Significant SNPs from LFMM analysis.

SNP	Position	Associated variable (LFMM)	Canonical axis (pRDA)	Genes
snp2286-scaffold1069-2525871	1:66591843	BIO8	RDA1	*KPNA1*, *PARP9*
snp44030-scaffold595-5911614	3:27914663	BIO8	RDA1	*LRP8*
snp26756-scaffold281-914407	6:37569967	BIO8	RDA1	
snp27546-scaffold2948-75069	7:71850309	prec_spring	-	*MXD3*, *RAB24*, *NSD1*
snp22907-scaffold2282-191752	13:78652699	BIO8	RDA1	
snp58824-scaffold960-1184341	24:59375683	BIO4, BIO8, tmax_summer	RDA1	

The pRDA model had an adjusted R^2 of 1.8%. Based on the screeplot and explained variance, the first four canonical axes were considered significant (Figure 4.4.6B). RDA1 (39.5% of variance explained) was mainly driven by BIO4, tmax_summer, and BIO15 (scores = 0.71, −0.52, −0.45, respectively). RDA2 (13.7%) was driven by BIO3 (−0.65); RDA3 (12.6%) by BIO15, BIO8, and prec_spring (0.60, −0.49, 0.47); and RDA4 (10.2%) by tmax_summer and prec_spring (0.87, 0.55).

In total, we identified 111, 107, 134, and 116 SNPs with outlier loadings across the four axes, corresponding to 467 unique outlier SNPs (Supplementary Table S4.4.4). Notably, five of the six SNPs identified with LFMM were also recovered among the pRDA outliers (Figure 4.4.6C). Three genes were identified within the windows surrounding consensus SNPs: *KPNA1*, *PARP9*, and *LRP8*. In addition, the consensus SNP on chromosome 24 was located in close proximity to, but not within, the *MC4R* gene. Considering all 467 SNPs detected by either LFMM or pRDA, we identified 284 associated genes (Supplementary Table S4.4.5). When analysing all genes together, no significant GO terms were detected. In contrast, focusing only on the subsets of genes associated with individual RDA axes revealed five significant terms for RDA1-related genes and 52 for RDA4-related genes (Supplementary Table S4.4.6).

4.4.4.5 Genomic offset

Given the low number of consensus SNPs, we used the union of SNPs detected by either LFMM or pRDA for genomic offset (g.o.) estimation. An adaptively enriched RDA was performed using these SNPs and the standardized present-day environmental matrix, yielding an adjusted R^2 of 9.8%. Based on permutation tests, all seven canonical axes were retained for g.o. calculation (Table 4.4.5).

Table 4.4.5 Summary of the explained variance and variables associated with adaptively enriched RDA canonical axis.

Canonical axis	Explained variance	Associated variables (score)
1	55.5%	BIO4 (0.71) tmax_summer (-0.52) BIO15 (-0.45)
2	14.4%	BIO3 (-0.65)
3	12.3%	BIO15 (0.60) prec_spring (-0.49) BIO8 (0.47)
4	9.5%	prec_autumn (0.87) prec_spring (0.55)
5	4.3%	BIO3 (0.51)
6	2.3%	BIO (0.63) tmax_summer (0.63)
7	1.8%	BIO4 (0.41)

For each pixel of the Italian map, we calculated a present adaptive index (AI), which allowed us to identify two main adaptive groups: Group 1, covering northern and central-eastern Italy, and Group 2, encompassing southern and central-western Italy as well as the islands (Figure 4.4.7A). Notably, when single-axis adaptive indexes were inspected individually (Supplementary Figure S4.4.10), the distribution appeared to be mainly driven by the south-to-north gradient of RDA1 (since AI was weighted by the explained variance of each axis). However, most of the other axes showed distinct values strongly associated with altitude, with higher or lower values along the Alps and Apennines. From a climatic perspective, Group 2 was characterized by higher and more stable annual temperatures, but lower and more seasonally variable precipitation (Figure 4.4.7B). With the exception of CCR, all breeds were assigned to a single group (Table 4.4.6).

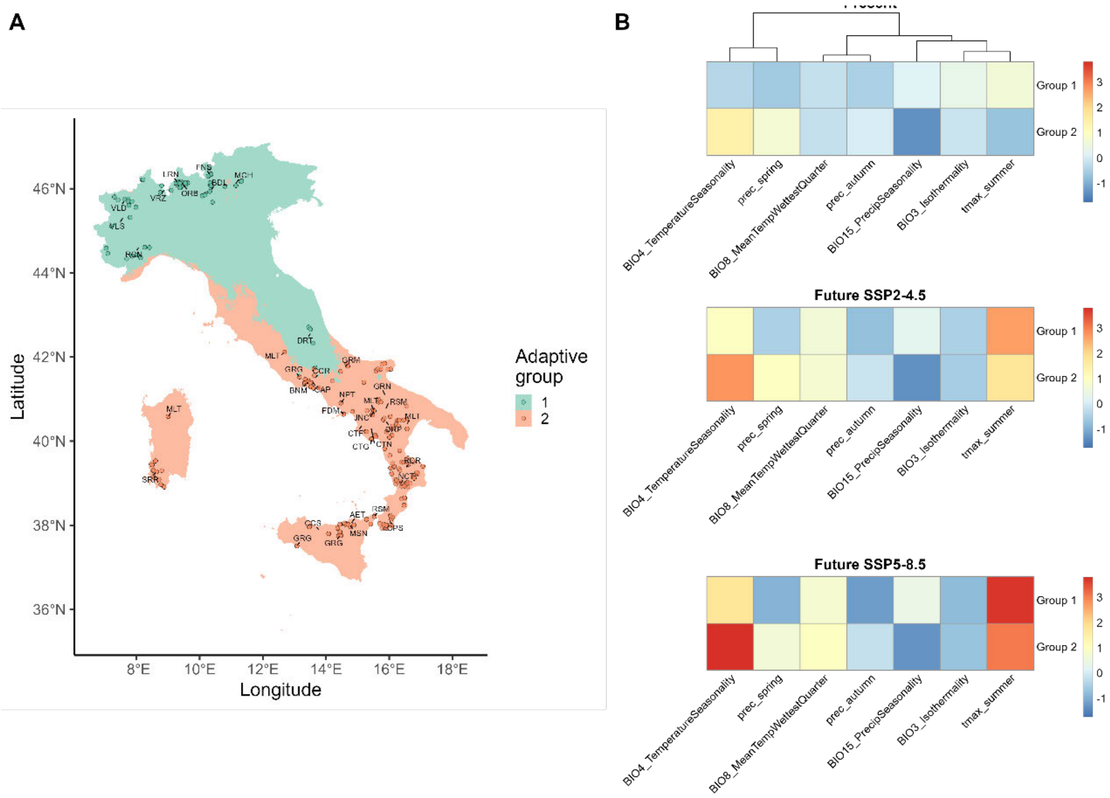


Figure 4.4.7 Main adaptive groups according to the current adaptive index across all axes (A) and heatmaps of the average scaled values of present and future (two scenarios) environmental variables (B).

The Euclidean distance between present and future AIs was used to calculate a local g.o. for each pixel under both the moderate (SSP 2-4.5) and severe (SSP 5-8.5) projected climate scenarios. Under the moderate scenario, g.o. values ranged from 0.06 to 1.22 (mean ± sd: 0.23 ± 0.11), with higher values observed in some Alpine areas along the northern borders of Italy, in eastern Veneto/Friuli-Venezia Giulia, and in a pronounced hotspot in the eastern Po Valley. In contrast, the southernmost part of Italy and Sicily showed lower values (Figure 4.4.8A). In this scenario, g.o. was most strongly correlated with BIO8 ($r = -0.56$) and, to a lesser extent, with tmax_summer ($r = 0.23$) (Supplementary Figure S4.4.11). Since only a few breeds were located in areas with the highest g.o. values, the populations with the highest average g.o. were all from southern or insular Italy: AET, Cilentana Fulva (CTF), and SRR (Table 4.4.6). When adaptive groups were compared, they were significantly different, with Group 2 showing slightly higher values (0.21 ± 0.07) than Group 1 (0.19 ± 0.08) (p-value = 4.56e-08, Figure 4.4.8B).

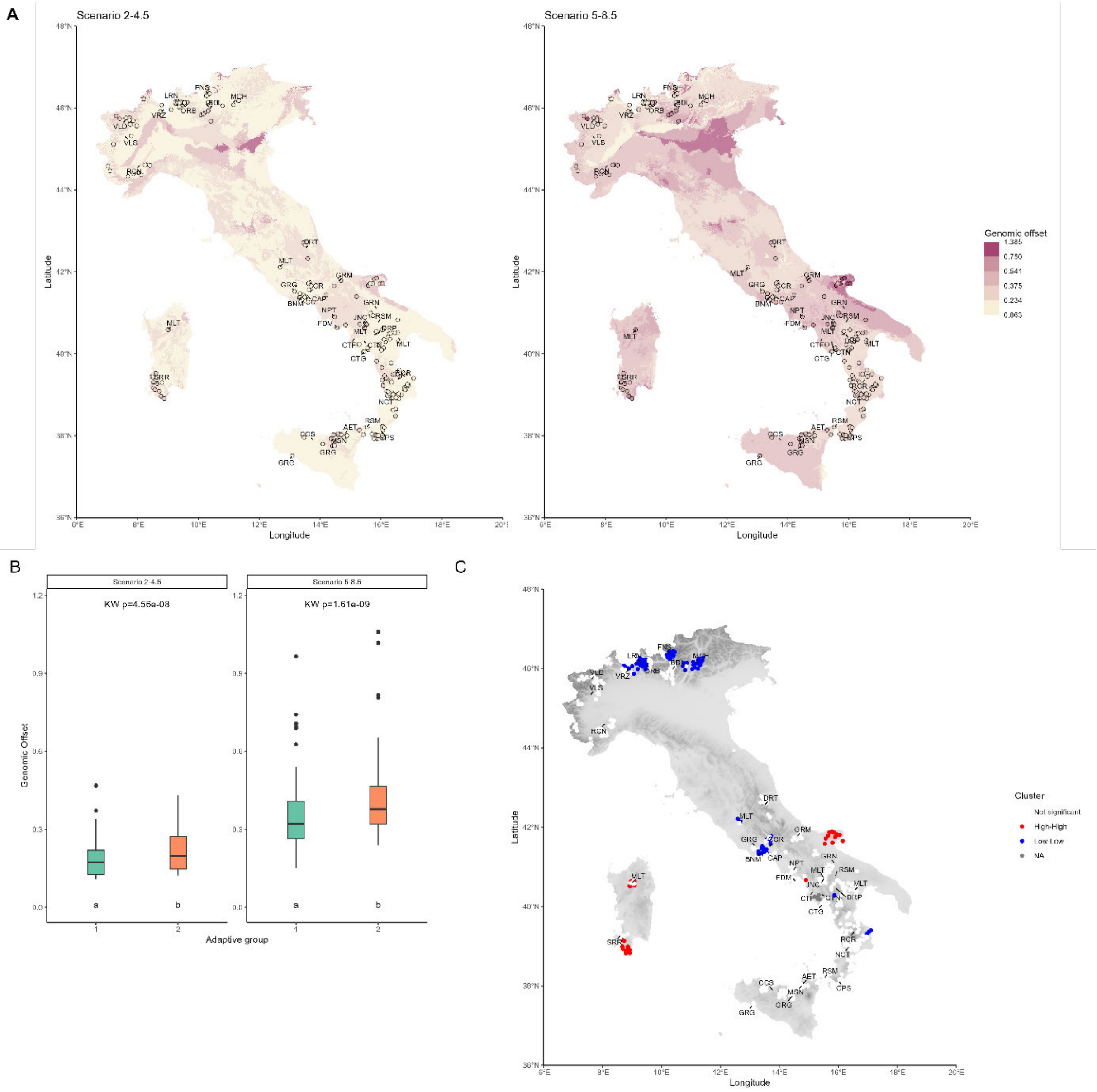


Figure 4.4.8 Genomic offset estimation.

A) Genomic offset estimated under moderate (2–4.5) and severe (5–8.5) scenarios.

B) Differences in genomic offset values among individuals assigned to different adaptive groups, based on the Kruskal–Wallis test.

C) Statistically significant hot- and cold-spots of expected genomic vulnerability identified through local autocorrelation analysis. Only individuals consistently assigned to the same group across all tested K values are colored. Results for each tested K are shown in Supplementary Figure S4.4.13. A small jitter was applied to display overlapping points.

Table 4.4.6 Italian goat breed attribution to adaptive groups, based on the current adaptive index, and mean ± standard deviation genomic offset (g.o.) under moderate (2–4.5) and severe (5–8.5) scenarios.

Breed	Breed name	N	Centroid	Adaptive groups	g.o. 2-4.5	g.o. 5-8.5
AET	Argentata dell'Etna	27	(14.657, 38.038)	2	0.261 ± 0.067	0.394 ± 0.058
BDL	Bionda dell'Adamello	29	(10.386, 45.968)	1	0.222 ± 0.112	0.511 ± 0.192
BNM	Bianca Monticellana	27	(13.389, 41.388)	2	0.131 ± 0.006	0.344 ± 0.095
CAP	Capestrina	16	(13.53, 41.384)	2	0.141 ± 0.036	0.307 ± 0.096
CCR	Ciociara Grigia	12	(13.662, 41.497)	1 (25%), 2 (75%)	0.203 ± 0.076	0.391 ± 0.129
CCS	Comune di Sicilia/Mascaruna	35	(13.786, 37.878)	2	0.135 ± 0.005	0.464 ± 0.001
CPS	Capestrina	35	(16.053, 38.157)	2	0.191 ± 0.036	0.333 ± 0.017
CTF	Cilentana Fulva	5	(15.079, 40.443)	2	0.278 ± 0.073	0.487 ± 0.12
CTG	Cilentana Grigia	3	(15.444, 40.05)	2	0.197 ± 0.001	0.350 ± 0.009
CTN	Cilentana Nera	26	(15.431, 40.29)	2	0.208 ± 0.031	0.404 ± 0.069
DRP	Di Potenza	31	(15.949, 40.469)	2	0.179 ± 0.039	0.347 ± 0.076
DRT	Di Teramo	9	(13.499, 42.608)	1	0.238 ± 0.031	0.361 ± 0.066
FDM	Fulva dei Monti Picentini	14	(14.558, 40.637)	2	0.272 ± 0	0.505 ± 0
FNS	Frisa Valtellinese	26	(10.33, 46.338)	1	0.127 ± 0.006	0.311 ± 0.006
GRG	Girgentana	22	(13.886, 38.199)	2	0.218 ± 0.089	0.417 ± 0.047
GRM	Campobasso Grigia Molisana	8	(14.622, 41.772)	2	0.240 ± 0.015	0.401 ± 0.018
GRN	Garganica	35	(15.866, 40.994)	2	0.258 ± 0.114	0.598 ± 0.333
JNC	Jonica	2	(15.517, 40.718)	2	0.266 ± 0	0.419 ± 0
LRN	Lariana/Di Livo	21	(9.301, 46.147)	1	0.194 ± 0.05	0.161 ± 0.011
MCH	Pezzata Mochena	18	(11.143, 46.127)	1	0.117 ± 0.007	0.322 ± 0.029
MLT	Maltese	23	(11.408, 40.747)	2	0.237 ± 0.067	0.469 ± 0.11
MSN	Messinese	35	(14.659, 37.967)	2	0.267 ± 0.077	0.396 ± 0.061
NCT	Nicastrese	36	(16.324, 38.97)	2	0.226 ± 0.044	0.311 ± 0.036
NPT	Napoletana	8	(14.474, 40.906)	2	0.260 ± 0	0.494 ± 0
ORB	Orobica	24	(9.478, 46.095)	1	0.165 ± 0.034	0.337 ± 0.133
RCN	Roccaverano	19	(8.096, 44.654)	1	0.193 ± 0.016	0.456 ± 0.046
RCR	Rustica di Calabria	35	(16.534, 39.392)	2	0.215 ± 0.041	0.303 ± 0.029
RSM	Rossa Mediterranea/Derivata di Siria	31	(15.737, 39.601)	2	0.181 ± 0.039	0.397 ± 0.083
SRR	Sarda	35	(8.66, 39.229)	2	0.272 ± 0.064	0.533 ± 0.081
VLD	Valdostana	18	(7.627, 45.718)	1	0.253 ± 0.123	0.402 ± 0.164
VLS	Vallesana	17	(7.617, 45.368)	1	0.214 ± 0.061	0.32 ± 0.069
VRZ	Verzaschese	15	(8.838, 45.974)	1	0.186 ± 0.037	0.259 ± 0.078

As expected, under the severe scenario, g.o. values ranged from 0.09 to 1.39 (mean ± sd: 0.43 ± 0.16), significantly higher than in the moderate scenario. Areas with high g.o. under the moderate scenario—such as the northern Italian border and eastern Po Valley—again stood out, but with broader spatial extent. Additional vulnerable areas were also identified along the western ridge of the Ligurian and Tosco-Emilian Apennines, as well as in the Murgia-Gargano area of Apulia. The northern territory appeared highly heterogeneous, with some areas, such as the plains of Piedmont and Lombardy, showing the lowest g.o. values (Figure 4.4.8A). As in SSP 2-4.5, the main driver of g.o. was BIO8 ($r = -0.58$), followed by BIO15 ($r = 0.32$) (Supplementary Figure S4.4.12). The complex geographical pattern of g.o. was reflected in average breed-level g.o., as the most vulnerable populations were spread across very different regions: BDL in the north, GRN in the south, and SRR in Sardinia (Table 4.4.6). As in the moderate scenario, Group 2 showed significantly higher values (0.41 ± 0.14) than Group 1 (0.35 ± 0.14) (p-value = 1.61e-09), but in this case, variability was higher within both groups (Figure 4.4.8B).

Finally, we performed a local spatial autocorrelation analysis on individual g.o. values averaged across the two scenarios, testing different numbers of clusters. Individuals consistently assigned to a group across all tested models are shown in Figure 4.4.8C. Several breeds included a substantial proportion of individuals consistently placed in the low–low group, both from northern Italy (FNS, LRN, MCH, VRZ) and central Italy (BNM, CAP, CCR). In contrast, about half of GRN and one third of SRR and MLT were always classified in the high–high group. For the remaining individuals, group assignment stabilized only at higher K values, revealing vulnerability hotspots mainly in breeds from Sardinia, Campania, the Gargano area (Apulia), some areas of Sicily, and among BDL goats. Conversely, some goats from northern, central, and Calabrian populations appeared as cold spots. Interestingly, some BNM, CCR, NCT, and ORB were included in the low–high group, suggesting lower vulnerability compared to neighbouring goats, whereas some AET, MSN, RSM, and SRR showed higher vulnerability than their neighbours (high–low group) (Supplementary Figure S4.4.13).

4.4.5 Discussion

Local livestock breeds are vital components of agricultural biodiversity, providing resilience to environmental changes, important ecosystem services, and contributing to food security. The creation and evolution of these breeds are influenced by a complex interplay of factors, including environmental conditions, socio-cultural practices, historical breeding preferences, policies, and management strategies [142,276,373]. Monitoring these populations is essential to understand how demographic changes, management practices, and environmental pressures impact their genetic resources over time. Integrating genomic tools with temporal comparisons offers a comprehensive approach to assess changes in diversity, population structure, and inbreeding [110], while landscape genomics enables the identification of genomic regions underlying adaptation and potential vulnerability [94]. Such information is critical for prioritizing conservation actions, supporting sustainable breeding strategies, and safeguarding the long-term resilience of livestock biodiversity, aligning with FAO's strategic objectives to conserve and sustainably use animal genetic resources [26,704].

4.4.5.1 Italian goat demography, biodiversity, and recent evolution

Census data from the last 15 years (2010–2024) reveal a concerning decline in registered goat farms, with an overall reduction of about one third. In contrast, the number of registered goats has shown more variability, likely reflecting the fluctuations in regional and national financial incentives supporting local breeds. Demographic information also allows the estimation of extinction risk, according to FAO criteria [36]. Alarmingly, only five breeds (11%)—including the cosmopolitan Camosciata delle Alpi—are currently not considered at risk of extinction, whereas 15 (32%) are classified as endangered and 12 (26%) as critical. Moreover, 10 breeds had no registered animals or farms in 2024, thereby meeting the criteria for formal extinction. For these populations, particular efforts should be dedicated to identifying genomic material outside the official herdbooks, while actively promoting registration among potential farmers who may still maintain these breeds, following appropriate phenotypic and genomic evaluations [30].

Beyond the identification of these suitable individuals, genomic analyses are also essential for monitoring existing populations, as they improve the accuracy of effective population size

(Ne) and inbreeding estimates, and provide valuable insights into the history and evolutionary trajectories of each population, particularly when comparisons with historical datasets are possible [110,120].

Our results highlight the strong relationship between geography and genomic composition, as previously reported in several studies [72,74,244,687,705], with a clear differentiation between northern breeds and central–southern and insular populations. We also confirm previous findings that genetic sharing occurs primarily among breeds within the same breeding areas [74]. This is particularly evident for central–southern populations, likely due to the long history of horizontal transhumance, which allowed flocks from different regions to meet and intermix [256]. This process likely explains the high levels of admixture observed among these populations—although some breeds remain clearly distinct—all of which share a predominant ancestry component that can be described as the 'Mediterranean component' [689].

In contrast, northern breeds have historically practiced vertical transhumance, moving seasonally from plains to mountains within relatively restricted areas, which limited gene flow and exchanges with flocks from other valleys [256]. However, extending the dataset beyond Italian borders would make it possible to investigate potential gene flow among breeds across the entire Alpine arc, whether raised in Italy or in neighbouring countries. In this study, northern breeds were distinguished into three main groups by several analyses: one including specialized (Camosciata delle Alpi and Saanen) and north-western breeds (Roccaverano and Valdostana); one with breeds from Lombardy (Frisa Valtellinese, Bionda dell'Adamello, Verzaschese, Vallesana, Lariana, and Orobica, the latter being particularly distinct); and the Pezzata Mochena. Camosciata delle Alpi and Saanen are two dairy breeds originally from Switzerland that have become widely distributed across continents due to their high milk production. In Italy, they are commonly raised in intensive or semi-intensive systems, mainly in central and northern regions, and artificial insemination is used in about 10-15% of animals, often with French semen. Our analyses indicate moderate levels of inbreeding, with no evidence of excessive recent inbreeding. Despite their productive success, both breeds have shown a marked decline in registrations over the past 15 years, with losses of about one-third of Camosciata delle Alpi and two-thirds of Saanen.

The replacement of local breeds with specialized ones is a major driver of breed erosion [12]. However, crossbreeding with Camosciata delle Alpi and Saanen is reported to be limited in Italian local goats [706], a finding that is confirmed by our results. Indeed, only one breed showed clear signs of introgression from Camosciata delle Alpi: the Roccaverano goat. This Piedmontese breed is reported to be of mixed origin, as suggested by its variable appearance, with some hypotheses linking it to goats introduced by Saracen soldiers during invasions in the region [48]. Robiola di Roccaverano cheese is the first recognized Italian PDO product made with goat milk (blended with cow milk) and accepts milk only from Roccaverano, Camosciata delle Alpi, and their crosses. Interestingly, TreeMix also identified possible migration between Roccaverano and Sarda breeds, partially supported by the presence, although limited, of the Sarda cluster within the Roccaverano background.

Another distinctive north-western breed is the Valdostana, raised in Valle d'Aosta and made famous by traditional non-cruel fights, called "Batailles de Chèvres", events that since 1981 attract tourists and provide an economic opportunity for farmers. Morphologically similar to other Alpine populations, the Valdostana is distinguished by its large size and stocky conformation, concave profile, and especially its very developed saber-shaped, ibex-like horns, present in both sexes and likely selected specifically for these competitions [48,707]. Genomic analyses confirm a distinct background for this breed, despite only average inbreeding values. However, registrations have declined dramatically: from about 1,500 heads across 200 farms in 2010 to only a single farm with 13 heads today.

Lombardy populations were all clearly distinguishable from one another. In particular, the Orobica exhibits a distinct genomic composition, as previously reported [708,709]. This may be explained either by its origin, which some authors link to goats brought by charcoal workers from southern Italy that crossed with local Alpine populations, or by the strong isolation of the valleys where it is traditionally raised [48,708]. While a southern origin may account for the Orobica's morphological traits—such as long hair and long diverging and slightly twisted horns, especially developed in males—, isolation likely explains the very high inbreeding levels observed, which however show only limited signs of recent events, suggesting effective recent management. It is worth noting that, despite exhibiting typical characteristics such as horns and long hair, this breed faced challenges in obtaining official recognition due to the variability of its coat colour, which was achieved only after extensive studies on its morphology and farming systems [708,710,711].

Another case of polychrome goat is the Lariana (or Di Livo), a population raised in mountainous areas around Como. Its origins are uncertain, but it is believed to have descended from an original primary population ("Di Livo") that was later crossed with goats from other populations in the region, as farmers with limited resources often acquired surplus animals—frequently belonging to different breeds or crosses—from surrounding farms. This, combined with limited selection for morphological traits and uniformity, likely explains both its variable appearance and its complex genomic background, which includes contributions from many northern breeds, particularly Verzaschese (reported to have largely replaced much of the local population), Bionda dell'Adamello, Orobica, and Frisa Valtellinese (whose contribution appears to have increased over the last 20 years, likely to improve Lariana meat production). In addition to the spread of more standardized breeds, particularly Verzaschese, and the difficulties in preserving highly variable breeds [48], the Lariana population has declined, a trend exacerbated by wolf predation and the expansion of luxury economic and touristic development of Lake Como, which are increasingly driving farmers to abandon pastoral activities in the region.

Interesting results were also obtained for the Pezzata Mochena, which takes its name both from the Bavarian population that settled in some valleys of Trentino-Alto Adige around the 14th century and from the typical black-and-white patched coat that characterizes it. At the beginning of the 21st century, this population was on the verge of extinction, but regional projects and dedicated associations succeeded in obtaining official recognition and in promoting its recovery [48], although demographic data still classify it as critically endangered. Interestingly, this breed was consistently placed among northern populations, though slightly shifted towards those raised in central Italy. Moreover, when the Val Passiria (or Passeirer Gebirgsziege), another morphologically variable breed from the same eastern-Alpine region for which no registration data are currently available, was included in the dataset, the two appeared highly similar, confirming the hypothesis of historical crosses between them [48].

Among central Italian breeds, Bianca Monticellana and Capestrina stood out in our analysis for their early separation from other populations, while at the same time showing a clearly shared genomic background with each other and, to a lesser extent, with Ciociara Grigia. Despite being distinguishable by coat color—white in the Bianca Monticellana, black (sometimes with Swiss markings) in the Capestrina, and gray in the Ciociara Grigia—all three breeds originated in Lazio and are commonly raised together in the same flocks or share grazing areas. This could explain their genomic similarities, already reported elsewhere [712]. These findings suggest that Bianca Monticellana and Capestrina may represent two varieties of the same population, potentially warranting consideration as a single breed. Interestingly, our results also indicate that recently sampled Capestrina and Bianca Monticellana show a much more uniform background than individuals from the IGC2 dataset, together with a marked and concerning increase of 4.5–6% in average inbreeding. This pattern may explain their early separation from other breeds, a feature not observed in previous studies, and underscores the need for targeted breeding programs.

Ciociara Grigia, together with the Di Teramo breed, also displayed some of the greatest changes in genomic background over the past 20 years. In the IGC2 dataset, these populations included some individuals with a very distinct and uniform background, forming a breed-specific cluster, while other individuals showed a more admixed composition, mainly related to central populations, Pezzata Mochena, and the Mediterranean component. In contrast, all recent samples appeared admixed, with only minimal traces of their specific cluster. This pattern could also explain the marked reduction in F_{ROH} between historical and recent samples, particularly evident in the Di Teramo. Preservation efforts for these populations are urgently needed, especially considering their current census of about 130 registered animals, placing them at critical risk of extinction, compounded by losses resulting from earthquakes that affected their breeding range in 2009.

In Campania, two small populations are recognized: the Napoletana and the Fulva dei Monti Picentini, the latter officially recognized in 2022 but with no registered animals to date. In addition, three breeds of Cilentana are distinguished according to coat color: Nera (black), Rossa (red), and Grigia (gray). Only the Cilentana Nera was included in our dataset. This breed was considered nearly extinct in the 1980s, but subsequently increased in numbers, likely through crosses with other breeds, including Garganica [48]. Accordingly, we found that it had the lowest inbreeding values among all analyzed populations and an admixed background mainly composed of the Mediterranean component, the Fulva dei Monti Picentini, and the Garganica cluster. By contrast, both Napoletana and Fulva dei Monti Picentini showed very high inbreeding, which could further aggravate their already precarious situation and contribute to genetic erosion.

As mentioned, the majority of southern populations shared a background dominated by a common ‘Mediterranean component’, which was combined with additional ancestries shaped by geography and breed history. In particular, most Sicilian and Calabrian populations showed similar genomic profiles and comparable inbreeding values, generally low but with substantial portions of long ROH. Argentata dell’Etna and Messinese (Sicily), as well as Rustica di Calabria and Nicastrese (Calabria), exhibited admixed backgrounds with very similar patterns across time, dominated by the Mediterranean component but also including contributions from Girgentana, Comune di Sicilia, Maltese, and Sarda clusters.

Another numerous Calabrian population is the Aspromontana. It takes its name from the rugged Aspromonte massif where it originated, later spreading to surrounding provinces, and currently counts over 13,000 registered animals. Interestingly, gene flow from Girgentana was detected in several analyses.

The Girgentana is one of the most easily recognizable Sicilian goats, both genomically—due to its distinct genetic background—and phenotypically, thanks to its typical corkscrew horns resembling those of *Capra falconeri*. Because of this trait, several authors suggest an Asian origin, possibly from Himalayan *Capra prisca* or as a direct descendant of the markhor; however, these hypotheses have never been confirmed by genomic studies [72,713]. The first introduction of this breed to Italy is attributed to Arab settlers in the 9th century AD. Traditionally, it was raised in urban centers for fresh drinking milk, primarily used for feeding infants and the elderly [48,714,715]. However, the breed experienced a severe bottleneck, as also reflected by our results showing very high—and still increasing—genomic inbreeding. In 1983 about 30,000 animals were recorded, but within a decade the population had declined to only a few hundred, prompting the launch of recovery projects [714]. Recent registration trends show marked fluctuations, and currently only about 750 animals remain.

Despite sharing much of the same breeding range, the Comune di Sicilia (or Mascaruna) shows little or no introgression from the Girgentana. This population was officially recognized in 2025, following a comprehensive morphological and genomic study [407,689,716], which demonstrated its distinction from other Sicilian breeds but also revealed high variability in individual genomic backgrounds. Indeed, some individuals showed contributions from the Mediterranean ancestral pool, together with clusters from the Sarda and Maltese breeds.

Maltese and Rossa Mediterranea are widely valued for their excellent dairy aptitude, which enabled them to spread from Sicily—where they were originally imported from the Middle-eastern Mediterranean basin—to the rest of southern Italy [48]. However, both breeds have shown a consistently negative trend in registrations over the last 15 years. Temporal comparison of Rossa Mediterranea highlighted higher admixture in recent samples, particularly with the Mediterranean component, as well as Nicastrese and Maltese clusters. For the Maltese, instead, we observed a clear substructure not related to temporal change but to sampling location: one cluster prevailed in goats sampled in Sardinia and Rome; the other in those sampled in Sicily and Calabria; an intermediate background was present in goats sampled in Basilicata. This latter cluster was also more evident in recent Sarda samples compared to those from IGC2, although only in limited proportions. As the name suggests, the Sarda is native to Sardinia, where it represents the predominant goat breed, especially in harsh and marginal mountainous areas unsuitable for sheep grazing. Given the strong caprine vocation of the island, the Sarda is the most numerous Italian goat breed (about 15,000 animals) and was included among breeds under selection programs. However, in recent years only a very limited number of bucks have been registered (36 in 2024), which qualifies it as vulnerable according to FAO classification. For this reason, since 2025, it has been reclassified among breeds under conservation programs.

Overall, our genomic and demographic analyses reveal a highly variable picture of Italian goat biodiversity, with breed-specific differences shaped by recent management practices, policy support, and historical trajectories. Beyond providing high-quality products, local goat populations sustain micro-economies and maintain marginal landscapes that might otherwise be abandoned. They also constitute a form of intangible heritage, reflecting centuries of traditional breeding, cultural practices, and adaptation to diverse environments. Integrating genomic tools with morphological, phenotypic, and breeder knowledge enables more accurate breed characterization and recognition, while guiding population management that preserves genetic diversity, maintains breed typicality, and valorizes resilient, well-adapted animals. Supporting these practices can prevent depopulation of internal areas and reinforce the vital role of goat farms in safeguarding both the territory and its cultural and biological legacy.

4.4.5.2 Landscape genomics

The genomes of populations are shaped by mutation, genetic drift, gene flow, and both natural and artificial selection. Among these forces, spatially heterogeneous environmental pressures can drive local adaptation, favoring genetic variants that increase fitness in specific habitats and generating distinctive allele frequency patterns across populations [175,177]. Understanding the genomic basis of such adaptation has become increasingly important for sustaining agricultural systems under climate change [26]. Local breeds are particularly valuable in this context, as they are often well adapted to challenging environments and tend to be resilient, hardy, and tolerant to stressors, pathogens, and parasites [12]. Italy, despite its modest size, provides an ideal system for studying goats, thanks to its environmental heterogeneity and the richness of local breeds shaped over centuries by traditional husbandry, extensive farming, and diverse environmental pressures.

Recent methodological advances have made it possible to study genomic–environment associations (GEAs) more effectively. Both univariate approaches, which test each SNP against environmental variables individually, and multivariate approaches, which account for the polygenic nature of adaptation, are now commonly used. Because neutral genomic structure can produce signals that mimic adaptation, it is critical to account for it in GEA analyses. For these reasons, we applied two complementary approaches in this study: a univariate method (LFMM) and a multivariate method (pRDA), correcting both for neutral genetic structure to reduce false-positive signals [179].

Only three genes were found by both pRDA and LFMM analyses, all associated with axis 1—which was mainly driven by BIO4, tmax_summer, and BIO15—and BIO8: *LRP8*, *KPNA1*, and *PARP9*. In addition, a SNP associated with three variables in LFMM (BIO8, BIO4, and tmax_summer) and RDA1 was located in an intergenic region, but very close to *MC4R* (melanocortin-4 receptor), a melanocortin involved in the leptin pathway, well known for its role in appetite regulation, food intake, meat production and quality, obesity predisposition, energy balance, and thermogenesis [717–719].

KPNA1 (Karyopherin Subunit Alpha 1) is part of the importin alpha family, which mediates nuclear protein import. This gene is abundantly expressed in the mammalian brain, where it regulates neuronal differentiation and synaptic function. Variants in this gene have been associated with schizophrenia development in humans, while knockout mice showed behavioral alterations such as reduced anxiety and impaired learning, memory, and sensorimotor gating [720–722]. One of the main interacting genes with *KPNA1* is *RAG1*, which is involved in the activation of immunoglobulin V(D)J recombination, a process that allows the somatic rearrangements of antigen receptor genes during lymphocyte development [723,724]. Another crucial role of *KPNA1* in the immune system is the nuclear translocation of *STAT1*, *STAT2*, and *STAT3*, transcription factors that regulate inflammation and immunity, especially during viral infections, including small ruminant lentiviruses (SRLV) [725–727]. In addition, *STAT1* and *STAT3* are well known to be involved in cattle mastitis, to influence milk production traits in domestic ruminants [728], and to be important in the response to heat stress through several pathways, including reproductive function and immunity [729–732].

PARP9 (Poly(ADP-Ribose) Polymerase Family Member 9) also interacts with *STAT1*, regulating macrophage activation [733]. In general, the *PARP* family targets proteins for ADP-ribosylation and plays a fundamental role in innate immune signaling pathways as well as in the regulation of interferon-I responses during viral infections and antiviral response [734–736]. Additionally, *PARP9* is involved in fat metabolism through the expression of low-density lipoprotein (LDL) receptors and apolipoproteins in macrophages [737].

This is especially interesting when considering that the third identified gene is *LRP8* (LDL Receptor Related Protein 8), a LDL receptor crucial in lipid metabolism and cholesterol uptake [738]. Several studies on cattle species pointed out that this gene is highly expressed in granulosa cells, being involved in cholesterol transportation and the response to reactive oxygen species (ROS), with differential expression during heat stress conditions [739–741]. In addition, the protein encoded by this gene plays a critical role in the migration of neurons during development by mediating Reelin signaling [597], controls osteoblast differentiation through the Wnt/β-catenin pathway [742,743], and allows selenium uptake, through selenoprotein P transportation, into several organs, such as brain, testis, and placenta [744,745]. Selenoproteins are especially important for antioxidant defense and ROS regulation, reproductive function, immune response, and thyroid function [746,747]. Since selenium concentration and bioaccessibility are highly variable worldwide, and within Italy as well, depending on soil properties and pedoclimatic factors—in particular rainfall—[748,749], the regulation of its receptors could represent a genomic adaptation to maintain selenium homeostasis in the organism. Moreover, the antioxidant properties of selenium help mitigate the detrimental effects of heat stress in several species [750].

When considering all the genes identified by either LFMM or pRDA, we observed that many have already been associated with environmental adaptation in goats (*KSR2*, *PPM1K*, *MAPK14*, *SPATA16*, *SASH1*, *JAK2*) [510,751,752], sheep (*MCPH1*, *NBEA*, *PKP2*, *PKP4*, *ANO2*) [648,753], or across multiple ruminant species (e.g. *ENOX1*, *CCSER1*) [510,659,751,753]. This overlap supports the robustness of our signals and indicates that Italian goat populations share adaptive responses with other livestock. Notably, some genes (*DCLK1*, *HDAC*, *NBEA*, *RNF150*, and *NLN*) were already detected in our previous landscape genomic study on IGC2 Italian goats [74], possibly reflecting stable adaptation mechanisms specific to these populations.

More specifically, we found that *DLG2* was associated with day length adaptation in sheep [648], an important factor influencing reproductive regulation in small ruminants [754]. In addition, we identified GO terms linked to rhythmic behaviour and ultradian rhythms, as well as *NPAS2*, a well-known circadian clock regulator [755].

Genes linked adaptation to high-altitude environments included *EXTL1*, *PARP2*, *ACCS2*, *PPP1R12A*, *DCLK1* [659,756–758] and, in particular, *FGF5* [573,659], which was consistently found across species. Animals living at high elevations must cope with hypoxia, which matches the enriched GO terms related to oxygen transport and erythrocyte differentiation that we observed.

High altitudes also expose animals to intense UV radiation. Among our candidates, *LEF1* was previously associated with UV tolerance in sheep [581] and with coat colour in goats

[759,760]. Since pigmentation is one of the most visible adaptive traits, it being related to UV radiation absorption/reflection and thus thermoregulation [82,684], it is not surprising that we also identified other pigmentation-related genes such as *OCA2* [761,762], *RALY* [763], *SLC2A1* [764], *WNT6* [765], and *GLI3* [766,767].

Cold adaptation was reflected in several candidates, including *MYH2* [573], *IFNGR1* [768], and *PRDM16*, a well-known regulator of fat browning and thermogenesis [769,770]. *FGF5* again emerged [573], reinforcing its pleiotropic role in regulating hair length and goat fiber traits [771–773]. Supporting this, we found a significant GO term related to hair follicle morphogenesis and additional fiber-related genes such as *CCSER1* [629], *DUOX1* [763], *LEF1* [774–776], and four keratins (*KRT25*, *KRT26*, *KRT27*, and *KRT28*) [777–780]. Together, these results suggest that hair length and texture, like coat colour, are important targets of both anthropic and natural selection.

Lastly, since climatic conditions are expected to lead to hotter and drier environments [74,490], goat adaptation to these conditions will be increasingly important. In this context, we found *CACNA2D1*, previously associated with adaptation to arid environments in sheep [581]. Water balance is a major challenge in such conditions, consistent with the significant GO terms we observed for kidney and urinary system development. In addition, heat-tolerance genes emerged, including *KSR2* and *SLC22A8* [507,781]. We also detected *INSR*, whose signaling is disrupted under heat stress [782–784] and which is linked to feed efficiency [785], highlighting its dual role in stress response and productivity.

Beyond overlaps with earlier studies, our analyses highlighted broader adaptive pathways. Oxidative stress is a common challenge, as both hypoxia and heat stress increase ROS [786]. Several antioxidant-related ontologies were enriched, supporting their role in resilience under harsh environments. Autophagy also emerged as a key process. This catabolic and cytoprotective pathway is activated under conditions such as hypoxia, nutrient shortage, and infection to provide nutrients for vital cell functions and remove damaged cytosolic material [787]. Several of our candidate genes (*DAPK2*, *OPTN*, *AMBRA1*, *ATG10*) [788,789] are directly involved in autophagy, consistent with its central role in maintaining cell homeostasis. Moreover, autophagy contributes to pathogen defense, as also demonstrated in domestic ruminants [788].

Immune response is a particularly relevant adaptive context, as climate change can impair immune systems while simultaneously altering the distribution of pathogens and vectors [679,790]. Consistently, we identified several immune-related genes, including loci associated with mastitis (e.g. *CACNA2D1*, *KCNQ1*, *SIRT7*) [791–793], *PARP14* and *TMEM232* linked to SRLV [794,795], *NEDD4L* related to *peste des petits ruminants* [796], and *IFNGR1*, a receptor mediating interferon-γ responses to multiple infections including brucellosis in goats [797]. Interestingly, four genes (*DLG2*, *EPYC*, *GPHN*, and *DLNOL4LL*) were associated with foot and claw health in cattle [798–801], a relevant trait for goats grazing in rugged terrains where mobility is essential.

Parasitic infestations also represent a major constraint for extensive systems. We found the previously mentioned *LRP8* as well as *DUOX1* and *GATA3* [802,803], all related to resistance against gastrointestinal nematodes. Supporting this, we identified GO terms linked to digestive function, stomach development, and gastric acid secretion, which are commonly impaired during parasitic infections [804]. Efficient digestion and feed utilization are especially critical under harsh conditions. In this context, it is interesting to note that *CTBP1* and *LRPAP1* are associated with tail fat deposition in sheep [805–807], a strategy evolved to cope with food scarcity [808]. We also identified *ACSS2*, associated with methane emissions in cattle [809], a trait relevant for mitigation of livestock's contribution to climate change. Feed efficiency is equally important for productivity, particularly in meat production when goats are raised under extensive management. Accordingly, several genes were linked to muscle development or meat quality (e.g., *FGF5*, *TEAD1*) [810,811] and to growth and body size (e.g., *CCSER1*, *JAK2*, *GLI3*, *STC2*) [812–814]. Genes related to milk production and quality were also found (e.g., *ABCG2*, *CCSER1*, *ELF5*, *JAK2*) [625,815–818], including *ACSS2* and *DPYD*, which are associated with the development of typical organoleptic traits of goat milk [559,819]. These morphological and production-related loci highlight how natural selection shapes traits that are simultaneously adaptive and economically relevant; nevertheless, it remains crucial to validate these candidate regions and genes through complementary approaches, such as expression analyses, functional assays, ad hoc experiments, or association studies in independent populations [179]. Taken together, the genes and GO terms identified here illustrate a multifaceted adaptive landscape, where metabolic, immune, developmental, and morphological traits interact to confer resilience. This complexity reflects how livestock must respond simultaneously to climatic pressures, pathogen load, and production demands in heterogeneous environments such as Italy. Future studies could refine these analyses by narrowing the focus to specific areas or populations while enhancing the resolution of both environmental and genomic data. For instance, tracking seasonal movements such as goat transhumance would make it possible to capture the full range of climatic conditions experienced throughout the year—particularly in mountainous regions, where sharp environmental contrasts can occur over short distances. Beyond commonly used climatic variables, additional data on vegetation, landscape features, lithomorphology, and land management could be integrated to provide a richer context. Moreover, combining high-resolution environmental information from farm- and pasture-level sensors with more detailed genomic datasets would further strengthen landscape genomic approaches, offering deeper insights into the genetic basis of adaptation in goat populations.

4.4.5.3 Adaptive vulnerability

Local goat breeds in Italy are predominantly managed under extensive farming systems. Such conditions have historically promoted long-term adaptation to the specific ecological features of their native environments, allowing these populations to persist and remain productive even under challenging circumstances [12,490]. At the same time, their strong dependence on local environmental conditions makes them particularly sensitive to ecological shifts [26]. Given that environmental changes are increasingly recognized as rapid and profound at the global scale in recent decades [75,321,490], it becomes essential to identify which breeds may face heightened risk of maladaptation in the future, and which breeds, conversely, could serve as valuable reservoirs of adaptive variation.

Within this framework, the concept of genomic offset provides a quantitative approach to estimate the degree of mismatch between the current genetic composition of a population and the genetic composition expected to be required under future environmental conditions, such as those projected under climate change scenarios [198–200]. Nevertheless, genomic offset is not without limitations [198,199]. First, it inherently relies on a spatial-to-temporal pattern substitution when used to infer future vulnerability. Second, it assumes that the present genetic composition of a population reflects a perfect adaptation and optimal fitness to the current environment—an assumption that is unlikely to be universally valid. Third, it does not explicitly consider other evolutionary processes, such as genetic drift, gene flow, or artificial selection, which can strongly influence genomic variation in domestic species alongside environmental adaptation. Despite these caveats, genomic offset remains a useful proxy to approximate the relative vulnerability of different populations to environmental change [199,200]. By highlighting contrasts in adaptive potential, it can provide valuable guidance for prioritizing conservation efforts and developing management strategies tailored to safeguard genetic resources under future climate scenarios.

Our results highlight a heterogeneous landscape of future vulnerability for Italian goat breeds, with clear differences between regions and adaptive groups. Although the present adaptive index divided Italy into two main clusters, with one group covering northern and central-eastern areas and the other the southern, central-western, and insular regions, the projected shifts in g.o. suggest that vulnerability is not evenly distributed within or between these groups. Under moderate scenarios, the highest g.o. values were concentrated along Alpine fringes, the eastern Po Valley, and other northern areas, while under severe scenarios, vulnerability hotspots expanded further into the Ligurian and Tosco-Emilian Apennines, the Murgia-Gargano area, and parts of Sardinia and Sicily. Importantly, however, few goat populations were actually sampled in the northern areas with the highest g.o. values, such as the eastern Po Valley. This reflects the fact that farms raising local goat breeds are sparse in these areas, despite the region's strong agricultural potential, as livestock production is dominated by other species and more intensive farming systems. As a consequence, with the exception of Bionda dell'Adamello, northern goats in our dataset mostly fell into low–low clusters, giving the impression of resilience, while in fact the absence of populations in vulnerable areas may conceal substantial risks for livestock more broadly. By contrast, southern and insular breeds consistently emerged as most vulnerable, both in their average g.o. values and through their frequent assignment to high–high clusters. Breeds such as Garganica appear to have limited adaptive margins and face compounding exposure to hotter and drier conditions.

These results align with previous analyses showing that northern Italy, especially the Po Valley and Alpine arc, is undergoing the fastest warming in summer temperature–related variables, while southern Italy already endures high baseline heat stress, marked precipitation variability, and more frequent droughts [321]. Climate projections further suggest that some of the coldest regions today—including parts of the Alpine arc and the Adriatic coast—are likely to shift toward hotter and drier climate classes in the coming decades [337,490], reinforcing the hotspot patterns we observed.

An additional layer to these patterns is the different genetic background of the populations: central–southern breeds tend to be more admixed with one another, whereas northern populations generally show more clearly defined genomic backgrounds. While admixture may increase standing variation and thus adaptive potential, it may also dilute fine-tuned local adaptations [820,821]. Conversely, the more homogeneous northern breeds might preserve stronger signatures of past adaptation but could be less flexible when facing the rapid climatic changes projected for their regions [822,823].

Overall, our results indicate that future vulnerability is not confined to a single region but distributed in different forms across Italy. Southern and insular populations face greater exposure due to current climatic constraints, while northern populations may encounter sharper challenges if rapid warming undermines their historically stable adaptive backgrounds. Conservation and management strategies should therefore consider both dimensions—environmental exposure and genetic history—recognizing that adaptive capacity is shaped by the interplay between baseline conditions, future climatic trajectories, and the genetic structure of each population.

4.4.6 Supplementary material

The supplementary material is available at: https://doi.org/10.13130/RD_UNIMI/YBPDPJ

Supplementary Table S4.4.1 Composition of genomic datasets used for different analyses, after quality control, removal of duplicates and relatives, and breed size balancing (7-27 individuals).

Supplementary Table S4.4.2 Description of all environmental variables retrieved from WorldClim 2.1.

Supplementary Table S4.4.3 Significant *f3* tests representing gene flow from populations B and C to target population A.

Supplementary Table S4.4.4 Significant SNPs identified by LFMM and/or pRDA analyses.

Supplementary Table S4.4.5 Genes located near significant SNPs identified by LFMM and/or pRDA analyses.

Supplementary Table S4.4.6 Significant GO terms associated with genes involved in gene-environment association analyses (see Supplementary Table S4.4.5).

Supplementary Figure S4.4.1 Yearly trends in animal and farm registrations per breed from 2010 to 2024.

Supplementary Figure S4.4.2 TreeMix analysis with one migration event.

Supplementary Figure S4.4.3 Admixture analysis for cluster numbers (K) ranging from 2 to 25.

Supplementary Figure S4.4.4 Five-fold cross-validation (cv) error for the admixture analysis. For each tested number of clusters (K), a violin plot shows the distribution of cv values across all runs, with the black dot representing the median cv.

Supplementary Figure S4.4.5 Line plot of the logarithmic values of genomic effective population size (Ne) over the last 100 generations, as estimated by the GONE software.

Supplementary Figure S4.4.6 Admixture analysis for cluster numbers (K) ranging from 17 to 27, including both recent and historical samples (codes including "old").

Supplementary Figure S4.4.7 Population structure of samples used for landscape genomic analyses.

A) First two components (PCs) from the multidimensional scaling analysis.
B) Cross-entropy (ce) values from the sNMF analysis. For each tested number of clusters (K), a violin plot shows the distribution of ce values across all runs, with the black dot representing the median ce. The horizontal gray line indicates the ce threshold defined by the third-quartile cutoff of a derivative-based criterion, where ce was modeled as a function of K, and the most parsimonious K was identified as the lowest K with a derivative close to that of the best-fitting K.

C) Best-fitting sNMF model (K = 13).

D) Most parsimonious sNMF model (K = 7).

Supplementary Figure S4.4.8 Correlation matrix of all analyzed environmental variables. The number of asterisks indicates the significance level (* for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$), while the size and color of the circles represent the correlation strength and direction.

Variables retained after collinearity reduction and used for subsequent analyses are shown in red.

Supplementary Figure S4.4.9 Manhattan plots for each environmental variable analyzed with LFMM. Q-values for all SNPs are shown on a –log10 scale. Horizontal red lines indicate the significance threshold ($q < 0.05$), and red points mark SNPs significantly associated with the tested environmental variable.

Supplementary Figure S4.4.10 Current adaptive index (AI) calculated for each significant RDA axis.

Supplementary Figure S4.4.11 Pixel-wise differences (Delta) between present and future projected values of the analyzed environmental variables under the SSP2-4.5 scenario.

Supplementary Figure S4.4.12 Pixel-wise differences (Delta) between present and future projected values of the analyzed environmental variables under the SSP5-8.5 scenario.

Supplementary Figure S4.4.13 Cluster maps from local spatial autocorrelation analyses performed on individual genomic offset values (mean across the two scenarios considered), for each tested number of k-nearest neighbours (from 5 to 100, at 5-lag intervals).

5 CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis provides a comprehensive exploration of sheep and goat biodiversity by integrating historical, phenotypic, genomic, environmental, and landscape data. Across multiple scales—from individual breeds to international populations—it demonstrates how domestication, breeding practices, and socio-cultural management, together with past and current environmental conditions, have shaped the genomes, adaptation, and vulnerability of small ruminants.

The work highlights the complexity of breed formation and evolution, showing that genetic diversity and adaptive potential cannot be fully understood from a single perspective. Analyses of population structure, genetic diversity, and genomic background, combined with temporal comparisons using historical samples, reveal recent evolutionary shifts and provide insights into the dynamics of local breeds over the last decades. Demographic information complements these genomic findings, offering practical insights into population viability and extinction risk. By integrating a broad characterisation of the environmental and climatic conditions in which populations live and have historically adapted, it becomes possible to implement landscape genomic analyses that link genetic patterns to ecological gradients and pressures. Interpreted within the broader historical, management, and socio-cultural context, these results reveal how past and contemporary forces together shape breed variability, adaptation, and potential vulnerability.

Italy provides a particularly informative case study, as its complex geography, climatic heterogeneity, and long-standing agricultural traditions created diverse ecological pressures and management systems. This variability exposes different breeds to a range of vulnerabilities while offering opportunities to identify resilient populations and understand the mechanisms of adaptation, with lessons applicable to livestock systems worldwide. The combination of evolutionary history, environmental characterisation, and human management practices highlights that safeguarding livestock biodiversity requires multidisciplinary approaches and the active engagement of breeders, associations, and policymakers to translate knowledge into effective conservation and management strategies.

Looking forward, continued monitoring of genetic, demographic, and environmental dynamics will be essential to support sustainable management and conservation planning. Equally important is the development of tools and approaches that are not only robust from a scientific perspective but also easily applicable on a broader scale, routinely implementable, and interpretable by breeders and associations. This would allow genomic knowledge to be translated into practical decisions, strengthening the role of local stakeholders in safeguarding biodiversity. At the landscape level, progress in the characterisation of ecosystems, environmental variables, and seasonal movements of animals will allow more accurate and powerful applications of landscape genomic approaches, further clarifying how breeds interact with and adapt to their environments. Additionally, the validation of results through functional analyses and complementary methodologies will be crucial to refine our understanding of the genomic regions involved in adaptation, ultimately enabling the development of breeding indices that enhance resilience. Finally, rapidly evolving genomic technologies—including sequencing, pangenomics, epigenomics, and transcriptomics—promise to uncover previously inaccessible layers of variation, opening new perspectives on adaptation, population dynamics, and evolutionary processes. Together, these future directions will strengthen our capacity to safeguard small ruminant biodiversity, making conservation strategies more effective, inclusive, and adaptive to the challenges posed by climate change and socio-economic transformations.

6 WORKS PUBLISHED AND PRESENTED DURING THE PHD PROGRAM

6.1 STUDIES INCLUDED IN THE PRESENT THESIS

6.1.1 Published articles

Crepaldi P, Bionda A, Colli L. **Where Do Goats Come From?** In: Mattiello S, Battini M, editors. *The Welfare of Goats*, Springer Nature Switzerland AG; 2024, p. 1–19. https://doi.org/10.1007/978-3-031-62182-6_1

Bionda A, Negro A, Grande S, Crepaldi P. **Mapping risks and landscapes: conservation insights for Italian small ruminant populations**. *Pastor Res Policy Pract* 2025;15:14997. https://doi.org/10.3389/past.2025.14997. (Role: first and corresponding author)

Bionda A, Cortellari M, Negro A, Crepaldi P. **70 years of heat waves and summer climate change affecting Italian small ruminant populations**. *Pastor Res Policy Pract* 2024;14:12848. https://doi.org/10.3389/past.2024.12848. (Role: first and corresponding author)

Bionda A, Lopreiato V, Amato A, Cortellari M, Cavallo C, Chiofalo V, Crepaldi P, Liotta L. **Phenotypic and Genomic Characterization of the Comune di Sicilia Goat: Towards the Conservation of an Endangered Local Breed**. *Animals* 2023;13:3207. https://doi.org/10.3390/ani13203207. (Role: first author)

Floridia V, Bionda A, Arias KD, Amato A, Cavallo C, Chiofalo V, et al. **Genomic insights on the history and selection trajectories of the Comune di Sicilia goat**. *BMC Genomic Data* 2025;26:78. https://doi.org/10.1186/s12863-025-01368-5. (Contribution: writing and formal analysis)

Bionda A, Negro A, Floridia V, Sarti MF, Grande S, Crepaldi P. **Genomic insights into the recent evolution and biodiversity of Italian sheep breeds**. *Mamm Genome*. 2026;37:5. https://doi.org/10.1007/s00335-025-10170-8. (Role: first and corresponding author)

6.1.2 Submitted articles

Bionda A, Negro A, Barbato M, Liotta L, Grande S, Crepaldi P. **Spatio-temporal genomics of Italian goats: recent evolution, adaptation, and future vulnerability**. (Role: first and corresponding author)

6.1.3 Congress posters and presentations

Bionda A, Cortellari M, Negro A, Rahnaward Ghulami H, Grande S, Crepaldi P. **Italian small ruminant geolocalization to monitor inbreeding and climate threats**. Int. Work. Integr. Genomic Geogr. Inf. Syst. data Wildl. Conserv., 2022, p. 21–2. (Oral presentation, WIGGIS Workshop, Piacenza; role: presenting author)

Cortellari M, Bionda A, Negro A, Grande S, Crepaldi P. **Mapping Italian caprine genetic resources: local distribution and environmental risk**. In: European Federation of Animal Science (EAAP), editor. B. Abstr. 73rd Annu. Meet. Eur. Fed. Anim. Sci., The Netherlands: Wageningen Academic Publishers; 2022, p. 565. https://doi.org/10.3920/978-90-8686-937-4. (Poster, 73rd EAAP Annual Meeting, Porto)

Bionda A, Cortellari M, Lopreiato V, Crepaldi P, Chiofalo V, Liotta L. **Genomic characterization of the Comune di Sicilia goat, a local Sicilian genetic resource**. Ital J Anim Sci 2023;22:98–9. https://doi.org/10.1080/1828051X.2023.2210877. (Oral presentation, 25th ASPA Congress, Monopoli; role: presenting author)

Cortellari M, Negro A, Bionda A, Fresi P, Grande S, Crepaldi P. **Genomic inbreeding distribution in Italian dairy goat farms**. Ital J Anim Sci 2023;22:186–7. https://doi.org/10.1080/1828051X.2023.2210877. (Poster, 25th ASPA Congress, Monopoli)

Liotta L, Lopreiato V, Amato A, Cavallo C, Tomasella C, Bionda A, *et al.* **Phenotypic characterization of an endangered indigenous goat: Comune di Sicilia ecotype**. Ital J Anim Sci 2023;22:229. https://doi.org/10.1080/1828051X.2023.2210877. (Poster, 25th ASPA Congress, Monopoli)

Bionda A, Negro A, Cortellari M, Fresi P, Grande S, Crepaldi P. **Different exposure to heat waves in Italian small ruminant populations**. Ital J Anim Sci 2023;22:233–4. https://doi.org/10.1080/1828051X.2023.2210877. (Poster, 25th ASPA Congress, Monopoli)

Bionda A, Cortellari M, Negro A, Grande S, Crepaldi P. **Landscape characterization of Italian small ruminant populations**. In: European Federation of Animal Science (EAAP), editor. B. Abstr. 75th Annu. Meet. Eur. Fed. Anim. Sci., European Federation of Animal Science (EAAP); 2024, p. 628. (Poster, 75th EAAP Annual Meeting, Florence)

Bionda A, Negro A, Cortellari M, Fresi P, Grande S, and Crepaldi P. **SHEEP&GOAT biodiversity: una fotografia genomica del panorama ovino e caprino italiano** (Oral Presentation, XXV Congresso Nazionale SIPAOC, Sassari; role: presenthing author)

Bionda A, Cortellari M, Negro A, and Crepaldi P. **Caratterizzazione climatica, ambientale e paesaggistica delle popolazioni ovine e caprine italiane**. 2024 (Oral Presentation, XXV Congresso Nazionale SIPAOC, Sassari; role: presenting author)

Floridia V, Cortellari M, Bionda A, Arias K. D, Virga A. N, Crepaldi P, Liotta L, and Barbato M. **Approfondimenti genomici sulla storia e sulle traiettorie di selezione della capra Comune di Sicilia**. 2024 (Oral Presentation, XXV Congresso Nazionale SIPAOC, Sassari)

Floridia V, Bionda A, Arias K D, Virga A N, Crepaldi P, Liotta L, Barbato M **Genomic insights on the history and selection trajectories of the local goat breed Comune di Sicilia**. 2025 (Poster, 26th ASPA Congress, Turin)

6.2 GENOMIC STUDIES IN SHEEP AND GOAT SPECIES

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