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Multifaceted drivers of performance variation in animal populations: invasive predator, resource availability and hybridization

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TABLE OF CONTENTS

ABSTRACT

The biodiversity on Earth is dramatically affected by global changes. To understand biodiversity loss and determine priorities for conservation, it is important to assess whether and how individuals can adapt to a continuous and drastic environmental change. Phenotypic variation is a key process that allow populations to withstand environmental changes by increasing survival and fitness, and can be related to both adaptations and phenotypic plasticity. For this reason, studies on phenotypic variation, and its associated fitness, analysing response to different stressors are needed. This thesis investigated phenotypic variability, through the study of performance variation, in response to three of the most pressing issue of global change: alien species, resource availability and hybridization.

The first study (Chapter 2) focused on phenotypic variation of a native species in response to an alien predator. Italian agile frog embryos developed faster when exposed to the predator in laboratory condition and when collected in alien crayfish-invaded sites. This ontogenetic shift can be interpreted as a local adaptation to the alien predator and suggests that frogs are able to recognise the predatory risk. If these responses are effective, escape strategies against the invasive predator may improve the survival of frog populations.

The second study (Chapter 3) analysed how sexually selected traits vary across different degree of resource availability. We assessed the existence of condition- and context-dependent sexual dimorphism (SD) within and among populations in the Italian wall lizard. Body condition and ecosystem productivity were the main drivers of body size SD variation, and body condition was also the main driver for head shape SD. The expression of SD in the Italian wall lizard is strictly related to the changes in resource availability.

The third study (Chapter 4) evaluated how hybridization affects hybrid performance and the main drivers of performance differences between hybrid and their parents, using the meta-analytic approach to obtain generalizable conclusions. The averaged hybrid performance was similar to the

fitness of parental lineages, however both the level of genetic divergence between parental lineages and the approach used to identify hybrids affected hybrid performance. Performance was lower for hybrids between distantly related lineages and the use of imprecise approaches for hybrid identification (e.g. morphology-based) can bias assessments of performance.

Altogether, the analyses performed show how alien species, resource availability and hybridization can drastically modify the phenotypic variability of animal populations. The presence of an alien predator induced plastic and adaptive phenotypic changes in a threatened frog that could increase the survival of the populations. Resource availability determined the degree of expression of sexually selected traits among and within lizard populations. Finally, hybridization can decrease hybrid performance when epistatic interactions occurred in individuals. Here, I demonstrated that animal responses to different stressors are particularly varied and articulate. Future challenges should focus on the combined effects of several stressors on animal phenotype. The conclusions of this thesis represent a step toward a better knowledge of the impact of global changes on phenotypic traits variation related to performance in animals.

CHAPTER 1 | INTRODUCTION

1.1 | Phenotype, genotype and environment

One of the central topics of evolutionary biology is how natural and sexual selection shape phenotypes. The forces of selection vary in space and time resulting in phenotypic variation across environments. Phenotypic variation provides important information to understand the species' distributions and to predict the adaptability of species to environmental changes (Gienapp et al. 2008). Phenotypic variation (V_p), or trait variation, is determined by the joint effects of genetic sources and/or environmental sources. Genetic and environmental factors interact to drive the phenotype; however, this interaction does not fully explain phenotypic variation. Positive or negative covariance can occur between phenotypic deviations due to genotype (genotypic effects) and environment (environmental effects) across space (Cov_{GE}). Cov_{GE} shows that phenotype is contingent on the strength and direction of the relationship between genotypic and environmental sources. Hence, both G×E (genotype-environment interaction) and Cov_{GE} contribute to trait variance:

$$V_P = V_G + V_E + V_{GxE} + 2(Cov_{GE}) + V_{error}$$

V_P = phenotypic variation
V_G = genetic variance
V_E = environmental variance
V_{GxE} = the effect of GxE
Cov_{GE} = covariance between genotypic and environment effects
V_{error} = residual variation

Covariance is positive (cogradient variation) when the environmental effect on the phenotype corresponds to the effect of selection on the genotype, maximising differences across environmental gradients. Conversely, negative covariance (countergradient variation) occurs when the environmental and genotypic effects on the phenotype act opposite to one another, minimising phenotypic differences across environmental gradients. The value of G×E is inversely related to that of Cov_{GE}: the interaction is maximised when reaction norms show a "X" pattern, the covariance is maximised when reaction norms are parallel (De Jong 1990, Albecker et al. 2022) (see Box 1 for definition of reaction norm).

Box 1. Reaction norm

How a genotype gives the phenotype as function of the environment can be represented with the reaction norm. The reaction norm is a function as coded for by a genotype representing the change in phenotypic expression that occurs in response to different environmental conditions. When different genotypes of the same species have different phenotypic responses to a given environmental gradient, the species ability to be successful in different environments is higher.

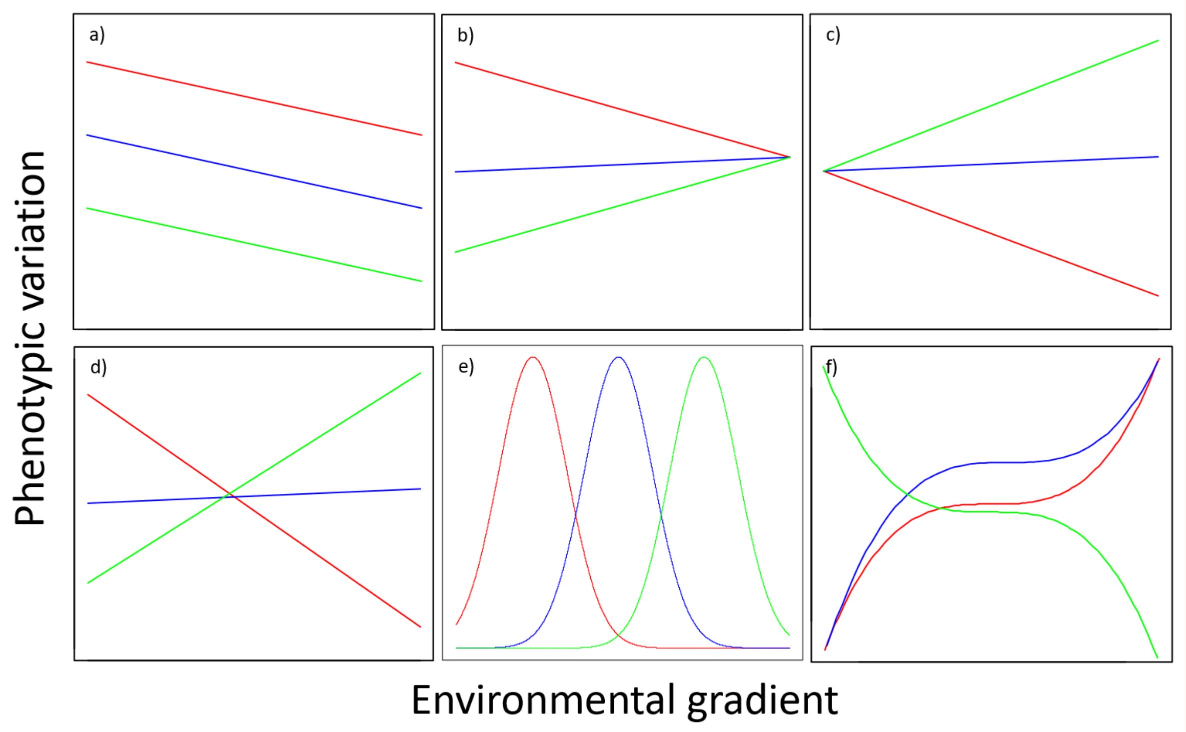

Fig. 1: Examples of norms of reaction for a given trait (y axis) and for three genotypes (red, blue, green), along an environmental gradient (x axis): genotypes can respond **(a-b)** similarly or **(c-d-e-f)** differentially to environmental gradients with **(a-b-c-d)** linear o **(e-f)** non-linear patterns.

1.1.2 | Adaptation and fitness

Genotype and environment drive the evolution of traits that improve the fitness of individuals under specific environmental conditions, independently of the consequences of these traits for individual fitness in other environmental contexts. The result is adaptation, namely genotypes in each population would show higher fitness in their habitat than genotypes arising from any other environmental context (e.g. blue genotype has the same phenotype in b, c and d panels of Fig. 1) (Williams 1996, Kawecki and Ebert 2004). The purpose of adaptation is that, as consequences of natural selection, individuals appear as if they are designed to maximise their survival and reproductive success (Darwinian fitness) and adaptation is based on the heritable variation in Darwinian fitness (Gardner 2017, Flatt 2020). Adaptation drives the phenotype to reach the fitness optimum (Fig. 2a), however the population mean phenotype typically lags behind the optimum because it has to keep pace with environmental changes. The evolution of individuals' phenotype through adaptation to environmental changes is mainly driven by natural selection. In changing environment, traits that provide an advantage for individuals in the new environment are promoted; consequently, the mean of the trait shifts in the direction driven by the new environment (Fig. 2b). This process is directional selection. In constant environment, stabilizing selection acts against extreme phenotypes, removing values further from the mean to optimize the existing trait mean. This result in a reduced range values, but without modification of the mean trait value (Fig. 2c) (Schulze-Makuch et al. 2013). Finally, disruptive selection favours extreme phenotypes as the mean phenotype experiences the lowest fitness (Fig. 2d) (Rueffler et al. 2006).

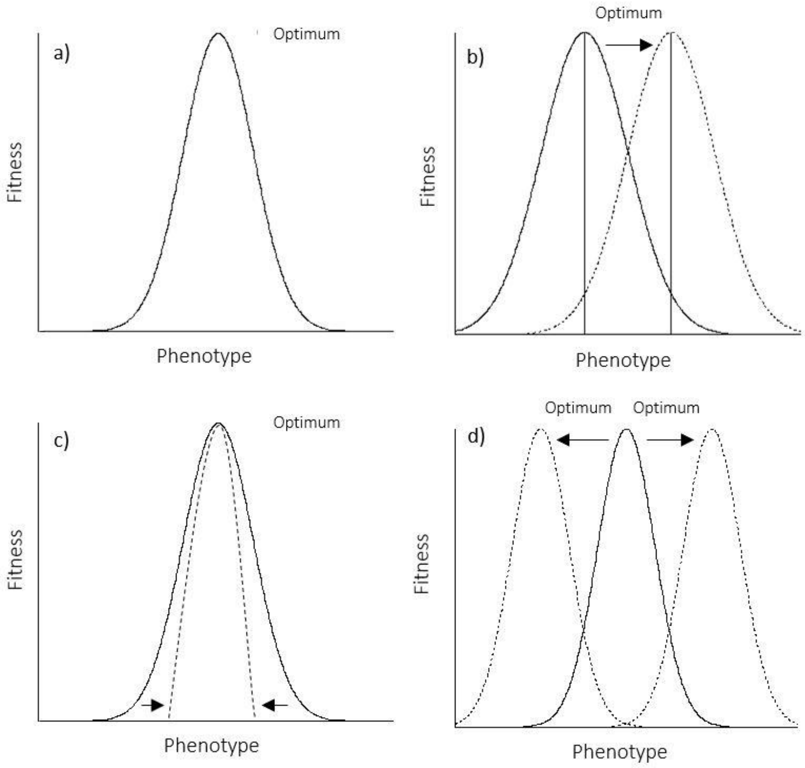

Fig. 2: Gaussian distribution of phenotype: **a)** fitness reaches the maximum level with the optimum phenotype, **b)** directional selection drives the shift of the mean optimum, **c)** stabilizing selection acts against extreme phenotypes; **d)** disruptive selection acts promoting extreme phenotypes.

Individuals pay a fitness cost for deviating from the optimal environmental phenotype (Fisher 1930, Lande and Shannon 1996, Orr 2006). Populations can adapt to a new environment by the selection of random new mutations or the selection on pre-existing genetic variation (Barrett and Schluter 2008). If random new mutations are beneficial, the mean population phenotype can move closer to the optimum. Thus, beneficial mutations are selected and their fixation can be very fast in the population. Neutral mutations are neither beneficial nor deleterious to individual fitness. Contrary, when mutations occur distant to the optimum are deleterious. Beneficial mutations increase individual's fitness, while deleterious mutations decrease it (Orr 2006). When mutations promote individual fitness, these mutations can spread in the populations. Otherwise, populations can adapt to a rapidly changing environment selecting pre-existing genetic variation. If beneficial alleles were already present in the population, adaptation to a new environment is more likely to lead to faster evolution (Barrett and Schluter 2008). Fitness can be approximated as the lifetime reproductive success of a genotype, therefore analyses on life history traits are central to understand adaptation. Among phenotypic traits, life history traits are the major determinants of fitness regarding traits as the number, size and sex ratio of offspring, reproductive effort, interbrood interval, age and size at maturity and growth rate and longevity (Stearns 1977, Stearns and Kawecki 1994, Flatt 2020). The change in life-history trait value causes a change in fitness when all other traits remain constant, together these fitness components determine the overall fitness of the individual (Flatt 2020).

Not all phenotypic changes are due to evolution, rapid responses in phenotypic traits to rapid changes in environmental conditions can be generated by phenotypic plasticity (Boutin and Lane 2014). Phenotypic plasticity is the change in phenotype expression by a given genotype in different environments (e.g. the red and green genotypes express different phenotypes in b, c and d panels of Box 1 Fig.1). Plasticity can be adaptive, when it improves the fitness of a given genotype, or non-adaptive, when unrelated to fitness, or even maladaptive, when it results in reduced fitness (Merilä and Hendry 2014, Scott et al. 2018, Arnold et al. 2019). When phenotypic plasticity allows a population to survive in a new environment by shifting the phenotypic mean to the new environmental optimum, it can increase the rate of adaptation with slow genetic assimilation of the new phenotype (Lande 2009, Fierst 2011).

1.1.3 | Ecomorphological paradigm

The ecomorphological paradigm stated that variation in phenotype determines performance, which in turn determines variations in fitness (Arnold 1983). Phenotype is shaped by a complex interplay, both parallel and opposite, between natural and sexual selection (Husak and Lailvaux 2014). The resulting capability of those phenotypes can be measured by studying performance (Arnold 1983, Husak et al. 2009b). Therefore, individual performance is a measure of the ability of an animal to conduct an ecologically relevant activity, namely any behaviour or trait that is essential for survival and reproduction, which include activities such as foraging, hunting, mating and avoiding predators. (Arnold 1983, Irschick et al. 2008, Husak et al. 2009b). Frequently, superior performers have higher fitness and animal's performance may affect many facets of its fitness. Studying factors molding individual performance is crucial to understand how animals interact and survive in their surrounding environment (Lailvaux and Husak 2014). The study of performance allows to clarify how phenotypic variation and individual fitness could be strongly linked to one another (Arnold 1983). However, individual fitness could be the result of many interactions among several performance traits, which can have independent evolutionary trajectories (Irschick 2003). Performance traits are functionally linked to different phenotypic traits (e.g. byte force to head shape / jumping to limb length) which can be under different selective pressures and the influence of all performance traits determines individual fitness (Irschick 2003). Moreover, several selective pressures can act on the same performance trait. Sexual and natural selection can often elicit opposing effect on the same performance trait, for instance female choice favours long swords in *Xiphophorus montezumae* male which, on the other hand, represent higher metabolic costs during swimming performance (Basolo and Alcaraz 2003).

Among performance traits, Husak et al. (2009) proposed two mains categories: 1) dynamic and 2) regulatory. Dynamic traits include movement of the entire or parts of animal body (e.g. sprint speed, endurance, bite force), while regulatory traits include animal physiology, or withstand environmental conditions (e.g. thermoregulation or thermal tolerance, growth, digestive capacity, immune response, production of gametes). Both categories are an integrated measure of how well individuals achieve some ecological activities and are consequently relevant to the fitness of the individual. Animal performance studies are needed to understand the functional and evolutionary significance of phenotypic patterns investigating the link between the underlying phenotypic traits and the resulting fitness (Arnold 1983, Irschick et al. 2008, Husak et al. 2009b, Kaliontzopoulou et al. 2010).

1.1.4 | The role of the environment on animal performance

Considering the ecomorphological paradigm, environmental gradients across space and time can result in phenotypic variations, and their associated performance, which confers high fitness in different environmental conditions and favours adaptation (e.g. Langerhans et al. 2003, Elstrott and Irschick 2004; Benítez et al. 2014, Pease et al. 2018). Therefore, the relationship between phenotype and environment is a central theme in evolutionary biology, to understand how individuals adapt to the environment they experience (Kaliontzopoulou et al. 2010). Almost every features of the environment, biotic and abiotic, are sources of selection on the expression of phenotype or may impact performance traits directly. Environmental factors determine higher complexity of the relationship between performance and fitness (Kingsolver and Gomulkiewicz 2003, Kingsolver and Huey 2003, Kaplan and Phillips 2006, Gienapp et al. 2008).

Among environmental variables that can affect the phenotype, resource availability, temperature and the competition for these environmental variables among individuals have particularly strong impacts on the physiology, morphology, behaviour, metabolism and survival of animals (Koenig 2002, Lailvaux and Irschick 2007, Monticelli et al. 2007, Litzgus et al. 2008, Michler et al. 2011, Nunes et al. 2019, Melotto et al. 2020, De Lisle 2022, Shu et al. 2022). Foraging success and diet are among the most important environmental features for animal performance, determining survival, growth, reproduction and other animal traits (Couret et al. 2014, Limongi et al. 2015, Shu et al. 2022). For instance, the changes in diet affect diverse performance traits across different life stages in *Drosophila suzukii* (Kaçar et al. 2016, Shu et al. 2022). Laboratory studies have better control of experimental treatment, while assessing the diet conditions experienced by individuals in natural populations can be more challenging. Nonetheless, measures of individual’s performance, such as the body condition index (a measure of animal’s physical condition based on its body size and weight), provide a good estimate of the overall foraging success and fitness of individuals in nature (Jakob et al. 1996). The body condition index (BCI) is often related to animal performance traits, for instance female in good body condition have greater reproductive output in turtles (Litzgus et al. 2008). Moreover, ecosystem productivity, the rate of whole-ecosystem biomass production, is a proxy of resource availability for field studies, which is expected to mold diet and other aspect of the food ecology of animals (Weier and Herring 2000, Evans et al. 2005, Zhou et al. 2011). Hence, changes in ecosystem productivity may affect animal performance and fitness (Monticelli et al. 2007, Ramírez et al. 2017). For instance, mongooses inhabiting areas with higher ecosystem productivity consumed different preys and showed better immunity and reproductive performance compared to mongooses inhabiting areas with low productivity (Bandeira et al. 2018).

Among the abiotic environmental features, temperature plays a crucial role in individual performance and fitness influencing everything that an organism does (Wieser 1973, Clarke 2003, Lailvaux and Irschick 2007, Aguilar and Cruz 2010). Temperature can directly or indirectly affect the development rate, survival, morphology, body size, physiology, behaviour and many other whole-animal functions as locomotion and escape (Van Damme et al. 1992, Lehmann 1999, Couret et al. 2014, Coppola et al. 2020, Islam et al. 2020). The ability to adapt to temperature is fundamental for the persistence of populations (Sinervo et al. 2010, Geerts et al. 2014, Arnold et al. 2019).

Studies on animal performance should consider the role of the environment because it is the main driver of phenotypic variation and when phenotype-environment mismatches occur, the phenotype shows poorer performance and, ultimately, has a fitness cost (DeWitt et al. 1998, Gienapp et al. 2008, Moran et al. 2010, Lazić et al. 2015).

1.1.5 | Methods to analyse performance

Performance can be assessed through both laboratory and field studies (Irschick 2003). Ideally, laboratory and field studies should have consistent results (Mathis et al. 2003, Hillebrand and Gurevitch 2014), however some studies revealed poor concordance between field and laboratory researches (e.g. Bezemer and Mills 2003, Irschick 2003, Joron and Brakefield 2003). Frequently, performance measured in laboratory conditions does not reflect animal performance in nature (ecological performance) (Irschick and Garland 2001, Irschick 2003). This disagreement could be caused by multiple processes, including differences of ecological context and to stressful conditions in the laboratory (Ficetola and De Bernardi 2005). On the one hand, laboratory studies have better experimental control and reduce interaction with other species / individuals that can affect the findings of experiment (Campbell et al. 2009). On the other hand, assays in captivity could induce stressful condition in animal and do not necessarily reflect the states in natural environment (Joron and Brakefield 2003, Ficetola and De Bernardi 2005, Niemelä and Dingemanse 2014). Field studies are necessary to understand how ecological performance is related to fitness and avoid artificial responses of individuals to unnatural stimulations (Fisher et al. 2015, Osborn and Briffa 2017). Nevertheless, field studies can be affected by environmental changes (Campbell et al. 2009), and can have limited replication due to their costs in terms of money and time (Campbell et al. 2009, Fisher et al. 2015). In field studies, a multi-scale approach (e.g. analysis within and among populations) can be included to assess whether the processes determining phenotypic variation are the same across space and to evaluate whether scaling issues affect the detection of the drivers of phenotypic variation. Phenotype, performance and fitness studies can be performed in both laboratory and field environment, however an inclusion of both methods can clarify the fitness consequences in nature (Irschick 2003).

Broad-range analysis can combine outcomes of field and laboratory studies and are effective to obtain general trends and conclusions on several research purposes. Systematic literature review and meta-analysis allow to assess the existing knowledge and gaps on the topic under study (Mengist et al. 2020). Meta-analyses can generate more accurate estimates on a specific issue by analyzing multiple studies with statistical techniques (Shelby and Vaske 2008, Mengist et al. 2020). The meta-analysis approach can be effective to pool very broad range performance measures and allows to gather several independent studies to obtain general trends and conclusions on performance. Meta-analysis can provide a quantitative synthesis in order to identify how the different processes can determine phenotypic, performance and fitness variations across systems and studies.

Finally, variation in fitness can be the result of variation in genetic diversity (Vrijenhoek 1994, Reed and Frankham 2003, Allentoft and O'Brien 2010). As consequence, genetic data are relevant to determine whether adaptation or phenotypic plasticity are occurring in a new environment. Moreover, identifying which traits adapt (or which alleles are fixed first) and genes involved in phenotypic traits is crucial to understand adaptive processes. The allelic substitutions can drive different evolutionary pathways, especially when these substitutions influence fitness (Weinreich et al. 2005). Moreover, first adaptation of a traits could affect the adaptive value of other traits and their evolutionary patterns. Therefore, studying loci under selection and identifying their phenotypic effects is of major importance to identify processes of adaptation (Savolainen et al. 2013, Kemper et al. 2014).

1.2 | Phenotypic variation in response to global change

In an era of drastic global changes, one of the main issues for scientific community is to understand whether and how species adapt to the rapidly changing environment. Global changes affect species distributions, abundance and composition through the alteration of abiotic and biotic environmental interactions (e.g. Ahola et al. 2007, Molnár et al. 2010, Davey et al. 2012, Falaschi et al. 2022). To understand how the environment affects populations, studies on phenotypic variation, and its associated fitness, across different environmental conditions are urgently needed. Understanding the role of phenotypic plasticity and local adaptation in population survival is central to disentangle this issue. Invasive species, variation of resource availability and hybridization are three key pressures of global change. Therefore, studying the effect of these pressures on phenotypic variation of populations is of particular scientific interest.

1.2.1 | Anti-predator strategies: prey performance

Predation is a major selective force acting on prey fitness and the ability to avoid predators is one of the relevant ecological tasks that animals have to conduct in nature (Nunes et al. 2014b). To decrease vulnerability to predators, preys can express a wide range of defences including changing in behaviour, morphology and life history (e.g. Laforsch et al. 2004, Orizaola et al. 2013, Melotto et al. 2021). These defences can be constitutive (or canalized) or induced (or plastic). Constitutive defences are spread under constant environmental conditions and high predator pressure. Inducible defences occur when predator pressure is variable in space or time and are promoted when predation cues are available. Inducible defences increase prey fitness when predators are present, but these defences are disadvantageous when predators are absent, precluding them to become constitutive (Nunes et al. 2014b). Not only the preys, but also their parents can exhibit anti-predator strategies in response to an offspring predator modulating parental care and investment (Delia et al. 2013, Shulte et al. 2020). In fact, parental investment is known to influence multiple fitness-related traits of offspring, including survival and growth rate (Ficetola and De Bernardi 2009, Ficetola et al. 2011b).

Successful predator recognition and effective anti-predator traits can determine an increase in predator avoidance performance that, in turn, could increase survival and individual fitness (Larabee and Suarez 2015; McPeek et al. 1996; Mariotto et al. 2022; Pruitt and Troupe 2010). For example, *Enallagma* damselflies that show a larger lamella have higher survival rates when exposed to dragonfly predation (McPeek et al. 1996). Conversely, absent or ineffective anti-predator responses can threat or even lead to extinction of prey species (e.g. Blackburn et al. 2004; Moseby et al. 2016; Short et al. 2002). The inability to respond with effective anti-predator responses is often associated with a lack of common evolutionary history between prey and introduced invasive predator (see Box 2 for invasive species description) (Cox and Lima 2006, Sih et al. 2010). Sometimes, invasive predators can exert an intense selective pressure on native species, which in turn can elicit a rapid expression of traits improving anti-predator performance and prey fitness. Such traits encompass those generated by phenotypic plasticity and may lead to rapid adaptations in native species (Freeman and Byers 2006, Nunes et al. 2014b, Stuart et al. 2014, Blackburn et al. 2019, Melotto et al. 2020). For instance, *Daphnia* populations co-existing with introduced fish species have rapidly evolved an adaptive decrease in body size and timing of reproduction resulting smaller but numerically abundant (Fisk et al. 2007). Moreover, some preys are able to detect alien predators and exhibit effective defences innately as a consequence of an alien predator phylogenetic or phenotypic similarity with a native predator (e.g. Epp and Gabor 2008, Rehage et al. 2009). Studies on prey adaptation or plastic responses to their predators are crucial to understand the relationship between phenotype and fitness, and phenotypic evolution in the context of anti-predatory responses and environmental changes (McPeek et al. 1996).

Box 2. Alien species

The term “alien species” refers to species introduced outside their native range due to human action. To become invasive, species (shape) pass through four stages (on the right): initial dispersal, colonization, establishment and spread. These stages are related to several filters that reduce the number of species and genetic diversity (colour) in each species. Only a small, but significant, percentage of alien species become invasive, when they are able to survive (local environmental conditions), reproduce (demographics, genetic diversity) and spread (dispersal capacities, regional environmental conditions) in a new environment (Blackburn et al. 2011).

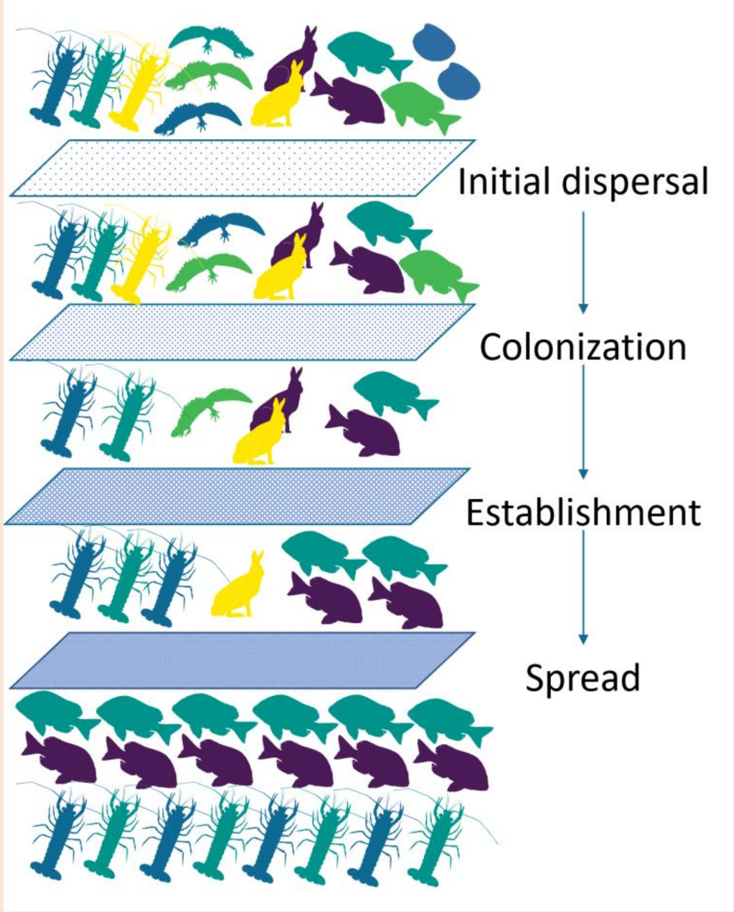

Fig. 3: Biological invasions are a multi-step processes comprising four major steps: initial dispersal, colonization, establishment and spread. Shape: different species, colour: genetic diversity

Invasive alien species (IAS) are often characterized by high grow rate, broad environmental tolerance, short generation time, prolific reproduction, good dispersal, high competitive ability, high ability to escape or survive, high plasticity and adaptive potential when faced with novel environments (Whitney and Gabler 2008). IAS are one of the key pressures of climate change and a major threat to biodiversity. More than 800 animal extinctions that have been recorded since 1500, IAS have been implicated in 33% of them (Blackburn et al. 2019). These species can affect native populations through: predation, habitat alteration, hybridization, competitive exclusion and transmission of diseases and pathogens (e.g. Damas-Moreira et al. 2020).

1.2.2 | The availability of resources can determine the variation in sexually-selected traits

Environmentally induced variation in resource availability could lead to changes in animal phenotype that may have a significant impact on individual fitness in wild populations. When resources are limited, allocating energy in a costly trait is likely to decrease the amount of resources available for other essential functions (Roff 2002). Such trade-off can shape the animal phenotype (Metcalfe and Monaghan 2001). For example, variation in food availability can affect animal's body size, reproductive success, and immune function (e.g. Brzęk and Konarzewski 2007, Wauters et al. 2007, Kahane-Rapport et al. 2022), while variation in habitat quality can affect animal behaviour, morphology, and stress responses (e.g. Morales et al. 2014, Soto-Rojas et al. 2017, Goff et al. 2020).

The effect of resource availability variation can be different among sexes, because phenotypes of males and females can respond differently to environmental gradients (Kaliontzopoulou et al. 2015). In many cases, functional and morphological traits employed to conduct an ecologically relevant activity are also important for social interactions, meaning that competition for females, natural and sexual selection are frequently the drivers of the variation of these traits across different environmental gradients (Cox et al. 2007, Kaliontzopoulou et al. 2007). Hence, the evolution of sexual dimorphism (SD) is driven by a complex interplay between sexual and natural selection. SD is widespread in the animal world and can include extensive variation in morphological, physiological and behavioral traits (Andersson 1994).

Sexual selection acts on performance of underlying phenotypic traits (e.g. head shape and byte force) that may contribute to the evolution and maintenance of SD (Husak et al. 2009a, Herrel et al. 2010). In mate choice ecology task, sexually selected traits are indicators of quality or ability of a sex to acquire and defend key limiting resources (e.g. space, food and sexual partners) (Husak et al. 2006b, 2009a, Irschick et al. 2007, Byers et al. 2010). For example, male lizards that bite particularly hard show a dominance and reproductive advantage (Husak et al. 2006a). In fact, sexually dimorphic traits are costly and their expression, exaggerated by sexual selection, should be strongly dependent on the condition of individuals (condition-dependent SD) (Bonduriansky 2007, Galeotti et al. 2011). Low value of performance corresponds to poorly adapted individuals, which would be less likely to both mate and survive. In male-biased sexual dimorphism, males' traits are more strongly affected by variation in conditions than females' traits, and choosing male with better performance allows females to choose a mate directly assessing adaptation (Snowberg and Benkman 2009). Moreover, individual condition is strictly related to the environment where individuals live and the individual's efficiency at translating resources into fitness (context-dependent SD) (Bonduriansky 2007). As a consequence, the expression of sexually selected traits is tightly linked to several environmental variable, such as resource availability and temperature (Andersson 1994, Bonduriansky and Rowe 2005, Cothran and Jeyasingh 2010, García-Roa et al. 2020). For example, the degree of sexual dimorphism could be higher in larger islands, as larger islands offer more resources than small ones and populations in large islands experience less demographic stochasticity (Sacchi et al. 2015). Consequently, the environment can determine the variation in fitness-related traits, thus triggering variation of sexual dimorphism (Cox et al. 2007). Sexually dimorphic traits often show strong variability across gradients of resource availability, and the patterns of variation within and between sexes are caused by both genetic and developmental processes (Bonduriansky 2007).

1.2.3 | The effect of hybridization on performance

Many species have responded to the current climate change by modifying their geographic range and this modification considerably increases the probability of sympatry between divergent species. In turn, sympatry may increase the potential for hybridization and this phenomenon is accentuated by the introduction of alien species in a new environment (Mainka and Howard 2010, Taylor et al. 2015). Hybridization is pervasive in the animal world and pivotal for our understanding of evolutionary processes (Ficetola and Stöck 2016, Atsumi et al. 2021, Thompson et al. 2021). Hybridization can play a crucial role in speciation, extinction, and adaptive radiations (Sakai et al. 2001, Seehausen 2004, Mallet 2005, Capblancq et al. 2015, Kagawa and Takimoto 2018). In fact, hybridization causes large phenotypic variation in hybrid, which may result in fitness and adaptation alteration (Mallet 2007, Atsumi et al. 2021). Hybrids are not uniformly disadvantaged compared to parents, as was historically hypnotized. Hybridization may lead to either decreased, increased, or similar fitness compared to parental lineages (Arnold and Hodges 1995, Lohr and Haag 2015, Atsumi et al. 2021).

Many biological processes can potentially determine the performance outcomes of hybridization, such as genetic distance between parental lineages (Coyne and Orr 1998, Stelkens and Seehausen 2009, Atsumi et al. 2021, Coughlan et al. 2021), hybrid generations of a single cross (Dobzhansky 1970, Burton 1990, Ellison et al. 2008, Šimková et al. 2021), or hybridization between native and invasive populations (Huxel 1999, Dlugosch et al. 2015, Grabenstein and Taylor 2018). Among these, the role of genetic distances between parent species have been elicit great interest in evolutionary studies. A large genetic distance between parents may increase hybrid performance (e.g. Shikano and Taniguchi 2003, Gao et al. 2014), but long-diverged genomes of parents can cause problems when pooled in a single individual because "intrinsic postzygotic isolation" (Dobzhansky 1937, Matute et al. 2010). Thus, the hybrid performance is expected to be highest with average values of genetic distance between parents (Wei and Zhang 2018). However, many studies are still in contrast and this issue is largely uncertain.

In addition to the biological effects, the methods used in studies assessing hybrid performance can affect the results of analyses. Several methods can be assed in hybrid studies as the setting in which hybrids are measured (e.g. field or laboratory studies) (e.g. Ficetola and De Bernardi 2005), hybrid identification (morphology or molecular approach) (e.g. Vanhaecke et al. 2012) and the trait considered for the analysis (e.g. breeding success, morphology, behavior) (e.g.

Casas et al. 2012). For example, it is known that laboratory and field studies could have poor agreement between their conclusions (e.g. Bezemer and Mills 2003, Joron and Brakefield 2003). However, methodological studies on hybrid performance are rare in the literature. It is known that hybridization is a driver of phenotype and fitness of animals (e.g. Arnold and Hodges 1995, Dufresnes et al. 2016), but broad-scale analyses of factors shaping hybrid performance are still missing and are needed to understand how biological and methodological processes can influence hybrid performance variation in animal world.

1.3 | Aims and structure of the thesis

Studying variation in the performance of animal populations allows better understanding the adaptive or plastic modulation of phenotype in response to the local environment that individuals experience. This thesis investigates animal phenotypic variability through the study of performance variation in response to three key pressures of global change: alien species, resource availability and hybridization. Phenotypic variability is assed studying: i) anti-predator responses to an alien species in amphibians, through a common rearing experiment, ii) condition- and context- dependent sexual dimorphism in lizards, analysing field observations, and iii) hybridization across the animals, using meta-analysis approach.

The first study (Chapter 2) focuses on phenotypic variation of induced anti-predator strategies in a threatened frog (*Rana latastei*). I tested whether variability in parental investment among frog populations is related to an invasive crayfish presence (*Procambarus clarkii*) as a modulation of maternal investment in response to an offspring predator. Furthermore, I tested whether, under experimental rearing conditions, the rate of embryo development is faster in populations invaded by the crayfish (potential local adaptations) or when exposed to the crayfish (potential phenotypic plasticity).

The aim of the second study (Chapter 3) is to assess whether resource availability determines phenotypic variation in the Italian wall lizard (*Podarcis siculus*) across Aeolian islands. In particular, I focused on sexually selected traits (body size and head shape) whose variation can be different among sexes. I assessed three predictor variables representing resource availability on the degree of sexual dimorphism (SD): (i) individual body condition to investigate condition-dependent SD, (ii) ecosystem productivity and (iii) land surface temperature, that may affect context-dependent SD. I performed the analysis at two different spatial scales, to evaluate whether scaling issues affect the detection of the drivers of SD.

Finally, the third study (Chapter 4) is a meta-analysis evaluating differences in performance between hybrids and their parental lineages in animal world, and investigating some of the possible predictors of these patterns. Specifically, three potential biological processes were tested: 1) genetic distance between parental lineages, 2) hybrid generations (i.e. F_1 vs. backcrosses or other crosses), 3) effects of invasive species. Moreover, other three potential processes related to study design and approaches were studied: 4) laboratory vs. field studies, 5) hybrid identification method 6) traits considered for analyses.

CHAPTER 2 | INVASIVE PREDATORS INDUCE PLASTIC AND ADAPTIVE RESPONSES DURING EMBRYO DEVELOPMENT IN A THREATENED FROG

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Abstract

Invasive predators can strongly affect native populations. If alien predator pressure is strong enough, it can induce anti-predator responses, including phenotypic plasticity of exposed individuals and local adaptations of impacted populations. Furthermore, maternal investment is an additional pathway that could provide resources and improve performance in the presence of alien predators. We investigated the potential responses to an alien predator crayfish (*Procambarus clarkii)* in a threatened frog (*Rana latastei*) by combining field observations with laboratory measurements of embryo development rate, to assess the importance of parental investment, origin and exposure to the crayfish cues. We detected a strong variation in parental investment amongst frog populations, but this variation was not related to the invasion status of the site of origin, suggesting that mothers did not modulate parental investment in relation to the presence of alien predators. However, cues of the invasive crayfish elicited plastic responses in clutches and tadpole's development: embryos developed faster when exposed to the predator. Furthermore, embryos from invaded sites reached Gosner's development stage 25 faster than those from non-invaded sites. This ontogenetic shift can be interpreted as a local adaptation to the alien predator and suggests that frogs are able to recognise the predatory risk. If these plastic responses and local adaptation are effective escape strategies against the invasive predator, they may improve the persistence of native frog populations.

Introduction

Biological invasions are a major threat to biodiversity and exert multiple impacts on the ecosystems on a global scale (Bellard et al. 2016, Nentwig et al. 2018). Invasive predators often cause declines and extinctions of native species (Mooney and Cleland 2001). These negative impacts have been often explained by the lack of common evolutionary history, which can hamper predator recognition in native prey and can limit the expression of effective anti-predator responses (Sih et al. 2010). However, there are several mechanisms that allow native species to implement effective responses against invasive predators (e.g. Freeman and Byers 2006, Weis and Sol 2016, Falaschi et al. 2020).

First, prey can display plastic responses to predator selective pressures acting on morphological, life history, physiological and behavioural features (Peacor et al. 2006, Nunes et al. 2014a, Melotto et al. 2021a). Prey generally express phenotypic plasticity when they are able to recognise the alien predator, for instance, because it shares cues with a native predator or is phylogenetically similar to it (Ferrari et al. 2007). Furthermore, aliens can drive strong selective pressures that induce genetic changes in native populations and may determine an evolutionary response of the prey (Cousyn et al. 2001, Nunes et al. 2014a, Ortega et al. 2017, Melotto et al. 2020). If responses to invasive predators are effective, they may increase prey fitness and, ultimately, can allow long-term persistence of native populations. In addition, in some cases, parents can improve the fitness of their offspring through the modulation of parental investment (Ghalambor and Martin 2000). Parental investment allows modulating the phenotype of offspring on the basis of the conditions experienced by parents (Pick et al. 2019). In many oviparous taxa, egg volume is a major form of parental investment affecting key traits of offspring's fitness, such as survival, morphology, stress tolerance, growth and development rate (Mousseau and Fox 1998). Nevertheless, the actual usefulness of a parental investment can be highly context dependent and the increase in parental investment is not necessarily associated with a rise in offspring fitness.

Indeed, females may not be able to predict the environment in which their offspring will grow, thus the invested resources would be not appropriate to it (Kaplan 1992). While the selective pressures exerted by invasive species can promote the quick evolution of behavioural and morphological traits limiting exposure to predation (Skelly and Freidenburg 2000, Melotto et al. 2020), there is little information available on the role played by parental investment. Importantly, the effectiveness of parental investments in allowing native prey species persistence remains an open question.

Amphibians are an excellent model system to assess plastic and evolutionary responses and to evaluate the role of parental investment, as they show a broad diversity of phenotypic plasticity, they can rapidly adapt to strong selective pressures and many species are easy to handle under experimental rearing conditions (Kaplan 1998, Relyea 2001, Beebee 2005, McCartney-Melstad and Shaffer 2015). For instance, frogs lay clutches that display a strong variation in number of eggs and egg size and these traits are commonly used to determine the parental investment (Kaplan and King 1997); clutch features are closely related to female body condition and to the environment experienced by mothers (e.g. Dziminski and Ross 2005, Sinsch et al. 2015). Populations of native amphibians exposed to invasive predators often show strong variation in developmental rate. For instance, populations of the Italian agile frog (*Rana latastei*) came into contact with the American red swamp crayfish (*Procambarus clarkii*) in northern Italy approximately 20 years ago (Lo Parrino et al. 2020). The red swamp crayfish exerts a strong predator pressure on amphibians, inasmuch as it is a voracious predator on larvae of amphibians (Cruz et al. 2006, Ficetola et al. 2011c). This invasive crayfish also feeds on amphibian eggs and is able to separate eggs from their protective jelly (Gherardi et al. 2001, Renai and Gherardi 2004). This alien predator is listed amongst the “100 worst” invasive alien species in the world (Cruz et al. 2008, Nentwig et al. 2018). Recent research showed that the Italian agile frog tadpoles are able to metamorphose earlier when exposed to the red swamp crayfish in experimental conditions, especially when belonging to populations already invaded (Melotto et al. 2020). This suggests that both phenotypic plasticity and local adaption can accelerate tadpole development in order to limit exposure to invasive predators (Melotto et al. 2020). However, in invaded populations, the faster development is expected to require higher energetic investments for growth and anti-predator behaviours (Burraco et al. 2020, Melotto et al. 2020). Parental investments might allow parents to partially counteract the constraints posed by the red swamp crayfish. Furthermore, we do not have information on potential responses of early development stages, even though the rate of embryo development can be modulated to reduce predation pressure (Warkentin 2005).

The aim of this study is to test the role of parental investment, phenotypic plasticity and adaptations in the interactions between alien predator and native populations and to evaluate whether parents are able to modulate their investment in response to the presence of an alien predator in *Rana latastei*. We first tested: i) whether variability in parental investment exists amongst frog populations and ii) if this could be related to the invasive crayfish presence as a modulation of maternal investment. Furthermore, iii) we tested whether, under controlled conditions, the rate of embryo development is related to differences in parental investment, whether it is faster in populations invaded by the crayfish (potential local adaptations) or when exposed to the crayfish (potential phenotypic plasticity).

To test these hypotheses, we measured several features of egg clutches to evaluate the variability in parental investment between frog populations invaded and not invaded by the crayfish. Subsequently, we used a common rearing experiment to measure differences in development rates across clutches and tadpoles with different origin, parental investment or exposed/unexposed to the red swamp crayfish. As different climate conditions seem to affect clutch laying in anurans and to avoid differences in parental investment and development time amongst populations living in different climatic conditions (Ficetola and Bernardi 2005), we selected multiple populations from the same altitude and with similar local climate. Our study underlines the importance of phenotypic plasticity and rapid adaptation for anti-predator responses during biological invasions.

Methods

Study system

The target species of this study is the Italian agile frog (*Rana latastei*), which lives from the sea level up to 500–700 m a.s.l. This frog is endemic of northern Italy and nearby areas and is listed by IUCN as vulnerable due to habitat reduction and fragmentation, pollution and alien species introduction of breeding sites (Schmidt et al. 2020). *Rana latastei* females produce their globular clutches from February to mid-April. Each *Rana latastei* female lay a single egg mass that displays strong variability, with the number of eggs per clutch ranging from 300 to > 2700 eggs (Bernini et al. 2007, Ambrogio and Mezzadri 2018). Clutches are laid in ponds and ditches surrounded by woods and they hatch in 12–15 days, while tadpoles metamorphose in about 3 months. Variation in parental investment (egg size) is known to influence multiple fitness-related traits of larvae, including survival and growth rate (Ficetola and De Bernardi 2009, Ficetola et al. 2011b).

The red swamp crayfish, *Procambarus clarkii,* is native from eastern North America and Mexico, but has been introduced worldwide (except in Australia and Antarctica). This crayfish was introduced in Italy thirty years ago and, since then, its range showed an impressive expansion. Nowadays *Procambarus clarkii* is widespread in Italy and it invaded the study area between 2005 and 2009 (Lo Parrino et al. 2020, Melotto et al. 2020). Landscape-level analyses have shown that the crayfish has strong effects on frog populations, reducing tadpole survival in the wild and affecting the dynamics of population networks (Ficetola et al. 2012, Manenti et al. 2020, Falaschi et al. 2021).

We studied frog populations living in the foothills of the Lombardy Region (north-western Italy). To avoid differences amongst populations living in different microclimatic conditions (Morrison and Hero 2003, Ficetola and Bernardi 2005), we focused on foothill populations living at an altitude of 177–295 m above sea level (a.s.l) within the Monza-Brianza, Como and Lecco

Provinces (Suppl. material 1: Table S1). To confirm that the variability amongst clutches in parental investment is unrelated to variation of climatic conditions, we downloaded the ChelsaClim maps at 30-arc second resolution (Karger et al. 2017) and analysed them with QGIS 3.4.13 (https://qgis.org/). We considered two key climatic parameters, known to affect frog fitness and phenology: monthly precipitation and annual mean temperature (Ficetola and Maiorano 2016). The study area is heavily populated, but this region also hosts several fragmented broadleaved forests and wetlands. Within the study area, we sampled eight breeding sites. Each site was represented by a wetland (either a pond or a ditch); all clutches from the same wetland were < 50 m from each other. Four of the eight sites considered are colonised by *Procambarus clarkii*, while four are crayfish-free.

Clutch sampling and measurement of parental investment.

Field activities were performed in February 2020, at the beginning of the breeding season of the Italian agile frog. Sites were monitored daily to collect egg clutches laid during the night before. Newly-laid clutches were photographed in the field to obtain two measures of maternal investment: number of eggs and egg volume. To take pictures, egg masses were removed from the ponds and gently divided in smaller fragments (4 ± 1.5 SD, fragments per clutch) to make eggs individually distinguishable. Clutch fragments were positioned on a white support (mobile table top) wet with the water of the breeding sites. A ruler was placed above the support and photographs were taken with the help of a camera, equipped with a macro lens. The clutch fragments were returned to the breeding sites, except for two small fragments per clutch, which were brought to the laboratory for the common rearing experiment. Overall, we obtained pictures from 50 clutches (total number of pictures processed: 223). We then used ImageJ (Schindelin et al. 2015) to measure the number of eggs and to estimate egg volume (cm^3). To count the number of eggs, the photographs were taken in black and white because the Analyze Particles function requires binarised photographs (Moraga and

Pervin 2018). We then used the Threshold function to adjust the photos contrast and the Analyze Particles function to automatically count them and to calculate the average diameter of eggs. Egg volume was then calculated assuming a spherical shape. Finally, the value of total parental investment of each clutch was calculated as total number of eggs × average egg volume.

Development and survival under common rearing conditions

We set up a common rearing experiment to measure differences in development and survival across clutches with different origin, parental investment or exposed/unexposed to the crayfish. We used the same experimental set elaborated by Melotto et al (2020). Two small fragments (40 ± 12 eggs) from each of the 50 sampled clutches were transported in the laboratory the day after deposition. The two fragments from the same clutch were randomly assigned to one of two treatments: absence of the crayfish or non-lethal presence of the crayfish. The fragments of the clutches were housed in containers and containers were stored in six 70 x 48 cm blocks filled with aged tap water. Clutches belonging to the same blocks were arranged nearby the same central compartment. In half of the blocks, we placed one adult crayfish in the central compartment (predator treatment), while in the remaining blocks, the central compartment was empty (controls). The containers hosting the clutches were separated from the crayfish area by a fine wire mesh that allowed the flow of chemical and visual cues by the crayfish. Control and experimental blocks were maintained under identical outdoor conditions; the three crayfishes were randomly re-assigned to the experimental blocks every 7 days. Half of the water in the blocks was changed weekly and crayfish were fed with flaked fish food and rabbit pellets. The containers were monitored daily to record the timing of hatching (as average time elapsed between the hatching of the first and last tadpoles of the fragment) and the reaching of Gosner's stage 25 (i.e. free-swimming tadpole) (Gosner 1960).

Due to the COVID-19 pandemic, it was not possible to complete the research as originally conceived, because a total local lockdown, starting on 9 March 2020, caused the interruption of laboratory activities. However, despite that, we collected 100 fragments; it was possible to measure hatching time for 42 fragments only (18 collected from sites crayfish-free and 24 colonised by the predator), taken from five ponds. Finally, we determined the time to reach Gosner's stage 25 in 239 larvae: 116 developed with *Procambarus clarkii* non-lethal presence and 123 without crayfish in the rearing experiment.

Data analyses

A linear mixed effects model was used to determine if there was a correlation between number of eggs and egg volume within populations; site of origin was included as the random effect. Adding population as the random factor allowed us to consider differences between populations when analysing variation within populations. We used Pearson's Correlation test to analyse whether there is a covariation between the average number of eggs and the average egg volume of each population. The inclusion of random effect was not necessary when assessing the relationship across populations, as in this case, we only considered one value (average across all the egg masses) for each population.

To assess differences amongst populations in parental investment (egg volume, number of eggs and total parental investment), we used three generalised linear models, one for each parameter describing parental investment, including site of origin as the fixed factor. Subsequently, we used linear mixed effects models (LMMs) to determine the factors related to parental investment across populations (egg volume, number of eggs and total parental investment). Invasion status (invaded/non-invaded by the crayfish), monthly precipitation and annual mean temperature were used as candidate fixed factors with site of origin as the random factor. We then calculated Akaike's Information Criterion (AIC) for all the combinations of fixed independent variables (invasion status and climatic parameters). The model with the lowest AIC value is the one that explains the most variation with the fewest variables and is considered to be the “best model” (Burnham and Anderson 2002). In all models, number of eggs and total investment were log-transformed to improve normality.

LMMs were also used to test factors affecting average hatching time and the time required to reach Gosner’s stage 25 (free-swimming tadpole). Average egg volume of the fragment, invasion status and treatment (non-lethal exposure to the crayfish vs. no exposure) were the independent variables. In preliminary tests, we also evaluated statistical interactions between invasion status and treatment. However, these interactions were not significant ($p > 0.3$); consequentially, we excluded them from the analyses. All LLMs included site of origin and rearing block as random factors. We also used the DHARMa R package to assess the residuals of mixed models (Hartig 2021); in all of the analyses, the residuals showed no significant deviations from expectations (for all the models: KS test: $p > 0.43$, dispersion test: $p > 0.79$). We performed all the statistical analyses in R environment, version 3.4.2, (http://www.r-project.org). We used the lme4, lmerTest, car and MuMIn packages for linear mixed models (Kuznetsova et al. 2017) and visreg package (Breheny and Burchett 2017) to generate conditional regression plots. Furthermore, we calculated marginal and conditional R^2 as a measure of effect size in LMMs (Nakagawa and Schielzeth 2013, Johnson 2014).

Results

Parental investment

Fifty newly-laid clutches were collected and photographed to determine the parental investment for each population. We found a strong variation of parental investment across clutches and populations. The number of eggs per clutch ranged from 500 to 2500, while the average egg volume ranged between 0.003 and 0.008 cm^3 (Fig. 1a). The total number of eggs, egg volume and total parental investment showed strong and significant differences amongst frog populations (number of eggs: $F_{7,39} = 6.474$, $p < 0.001$; egg volume: $F_{7,39} = 4.652$, $p < 0.001$; total investment: $F_{7,39} = 6.136$, $p < 0.001$; Figs. 1a, b, c). There was no correlation between number of eggs and egg volume within population ($F_{1,43.6} =-0.247$, $p = 0.812$). Across populations, there was a positive correlation between average number of eggs per clutch and egg volume, but the correlation was not significant at $\alpha = 0.05$ ($r = 0.681$, N = 8, $p = 0.063$).

When we assessed the relationship amongst the three parameters representing parental investment and population features (climate and presence of the crayfish), the null-model always showed lower AIC values, compared to the models including independent variables (Table 1). This suggests that the number of eggs, egg volume and total investment were not related to either climatic parameters or to the presence of the crayfish in the site. Furthermore, none of the independent variables was significantly related to any of the parameters representing maternal investment (Suppl. material 1: Table S2).

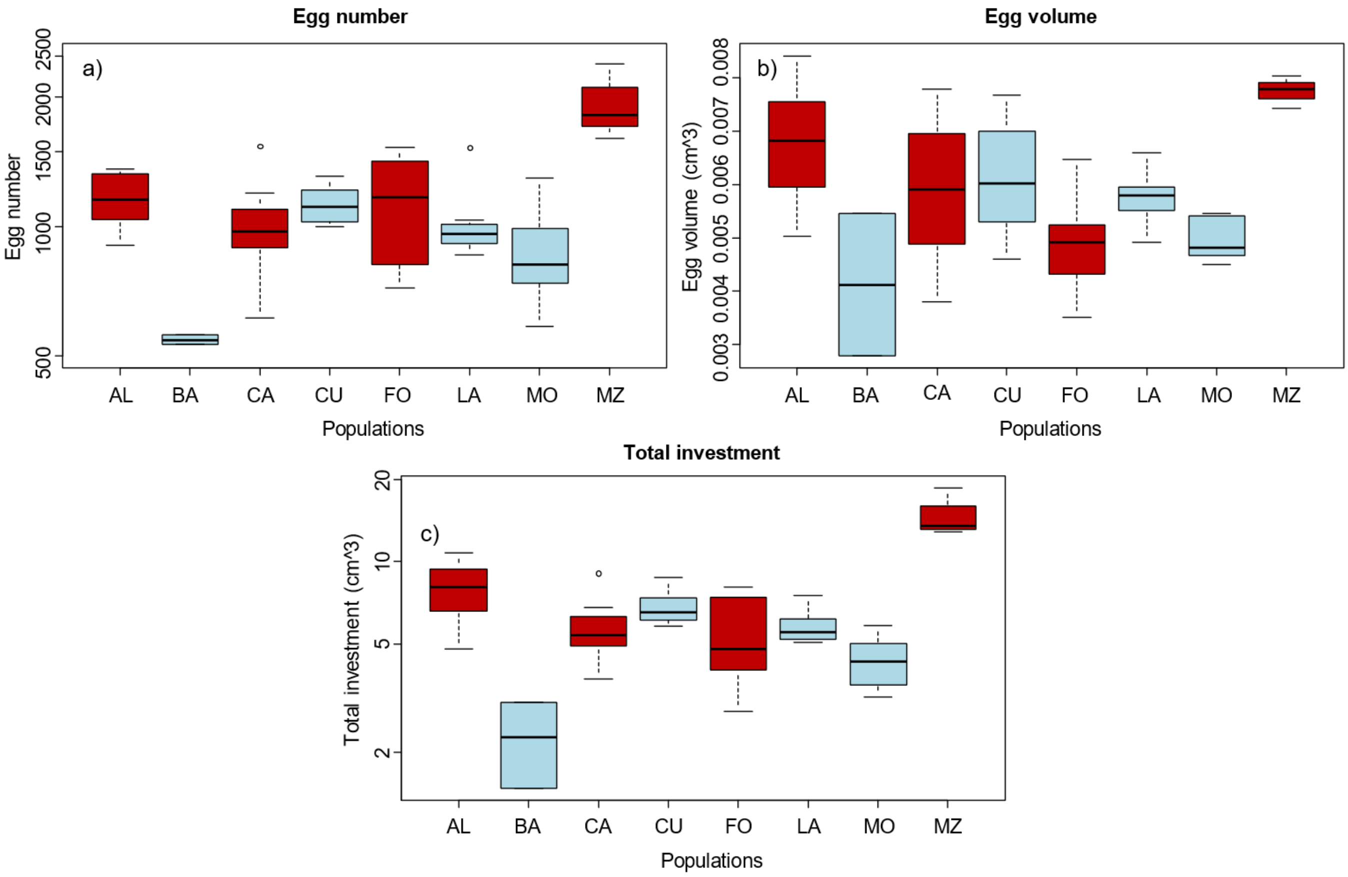

Fig 1. Variability of the three parameters considered describing parental investment amongst populations: **a** egg number **b** egg volume; c total investment (i.e. egg number × egg volume). Red boxplots represent crayfish presence in the original ponds, while blue boxplots represent crayfish-free sites. N = 50 clutches. For data analysis, the number of eggs and total investment were logarithm transformed to improve normality; therefore, we show the log-transformed y-axis.

	AIC	Random factor	Variables
A) Egg number	22.3	Site	-
	23.4	Site	*Procambarus clarkii* (+)
	25.3	Site	Annual mean temperature (+)
	25.7	Site	Monthly precipitation (-)
	27.3	Site	*Procambarus clarkii* (+), Monthly precipitation (+)
	27.8	Site	*Procambarus clarkii* (+), Annual mean temperature (+)
	29.5	Site	Montlhy precipitation (-), Annual mean temperature (+)
	31.4	Site	*Procambarus clarkii* (+), Monthly precipitation (+), Annual mean temperature (+)
B) Egg volume	-479	Site	-
	-465.6	Site	*Procambarus clarkii* (+)
	-464.6	Site	Monthly precipitation (-)
	-463.1	Site	Annual mean temperature (+)
	-450.6	Site	*Procambarus clarkii* (+), Monthly precipitation (-)
	-449.4	Site	Procambarus clarkii (+), Annual mean temperature (+)
	-448.4	Site	Monthly precipitation (-), Annual mean temperature (+)
	-434.5	Site	*Procambarus clarkii* (+), Monthly precipitation (-), Annual mean temperature (-)
C) Total investment	42.9	Site	-
	43.3	Site	*Procambarus clarkii* (+)
	45.3	Site	Monthly precipitation (-)
	45.7	Site	Annual mean temperature (+)
	46.2	Site	*Procambarus clarkii* (+), Monthly precipitation (+)
	47	Site	*Procambarus clarkii* (+), Annual mean temperature (+)
	48.6	Site	Monthly precipitation (-), Annual mean temperature (+)
	49.7	Site	*Procambarus clarkii* (+), Monthly precipitation (+), Annual mean temperature (+)

Tab 1. Candidate mixed models assessing the factors related to variation in parental investment across populations. Models are ranked according to their AIC values; models with lower AIC values are the most supported ones by the data. For all the parameters considered, the null model showed the lowest AIC values, suggesting that none of the variables has relevant support. The dependent variables of models are: **a** egg number; **b** egg volume; **c** total investment. The sign of the relationship between parental investment and variables is in parentheses. In Suppl. material 1: Table S1, we also report the significance of the variables in the three mixed models, including all the independent variables.

Common rearing experiment

The average hatching time of embryos (± SD) was 10.18 ± 0.83 days. Hatching time was not related to the average egg volume (mixed model: $F_{1,\ 32.5} = 0.029$, $p = 0.867$; Fig. 2c) or to the occurrence of crayfish in the site ($F_{1,\ 18} = 2.721$, $p = 0.116$, Fig. 2a). Clutches reared with the crayfish in the block hatched faster than controls ($F_{1, 14.6} = 10.786$, $p = 0.005$, Fig. 2b; no significant interaction between invasion status and treatment: $p = 0.227$). The model explained a good amount of variation (marginal $R^2 = 0.17$; conditional $R^2 = 0.69$).

The average time required for reaching Gosner's stage 25 (free-swimming tadpole) (± SD) was 16 ± 1 days. Tadpoles from clutches with smaller egg volumes tended to reach Gosner's stage 25 faster than those with a larger volume one ($F_{1, 24} = 7.138$, $p = 0.013$; Fig. 2f) and we detected significant differences between clutches from invaded vs. non-invaded sites, as tadpoles from invaded sites reached stage 25 significantly earlier ($F_{1, 13.45} = 5.017$, $p = 0.04$; Fig. 2d). The model explained a good amount of variation (marginal $R^2 = 0.47$; conditional $R^2 = 0.78$). However, these results were strongly dependent on the time to reach Gosner's stage 25 of tadpoles hatched from one single clutch fragment with very short time of development. If this fragment was removed from the dataset, the relationship between hatching time and both egg volume and crayfish presence in the ponds of origin become non-significant (egg volume = $F_{1, 23} = 3.442$, $p = 0.077$, crayfish presence = $F_{1, 13} = 3.9$, $p = 0.069$). Additionally, in this analysis, tadpoles exposed to the crayfish treatment reached Gosner's stage 25 more quickly than those not exposed to the crayfish ($F_{1, 13.5} = 20.893$, $p < 0.001$; Fig. 2e).

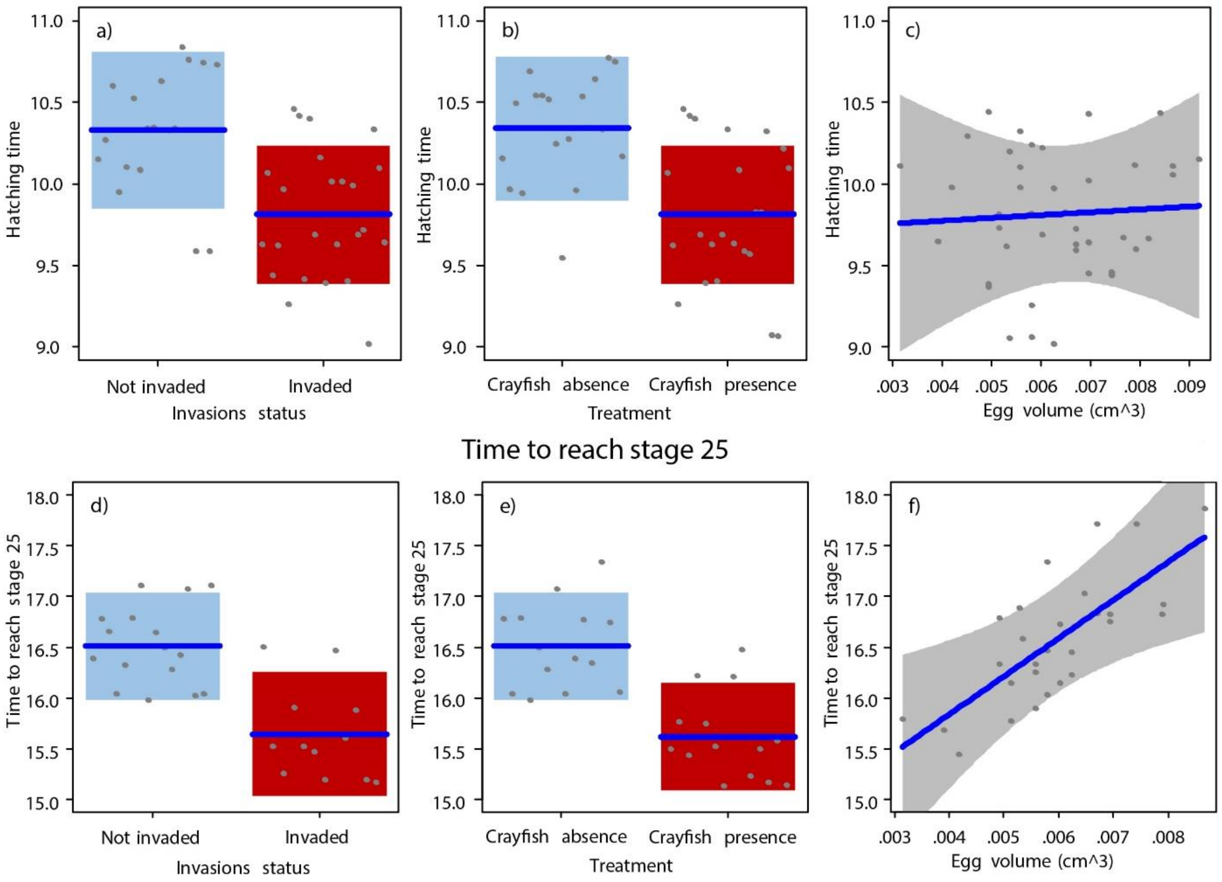

Fig 2. Hatching time and time to reach Gosner's stage 25 of *Rana latastei* in relation to **a–d** the invasion status of populations **b–e** treatment during the rearing experiment and **c–f** egg volume as parameter of parental investment. Red plots represent crayfish presence in the original ponds **a–d** or in the treatment **b, e** and blue plots represent crayfish-free sites **a–d** or the rearing experiment controls (**b, e**). Line shows mean correlation and coloured shaded area shows the 95% confidence interval. N = 42 clutch fragments, N = 239 larvae.

Discussion

Phenotypic plasticity, local adaptation and maternal investment are key mechanisms that can allow withstanding alien predators through the modulation of phenotype. Our study detected strong differences in parental investment across frog populations, even though this variation was unrelated to the presence of the crayfish in the site. We evidenced that tadpole origin and exposure to the crayfish affected the development of frog embryos and larvae, suggesting that plasticity and local adaptations can play a role.

Variation of maternal investment across populations

Very limited information exists about variation of parental investment in *R. latastei*. In literature, just a few counts of the number of eggs are available, with values consistent with our study (Bernini et al. 2004, Ambrogio and Mezzadri 2018). We detected a very strong variation for both number of eggs and egg volume across females of different populations. Several explanations to this great variability exist, inasmuch as different selective forces and resource availability jointly act on parental investment (Roff 2002). In many cases, the variation in female conditions is a key driver of maternal investment. In amphibians, female body conditions and body size are frequently related to clutch features (Prado and Haddad 2003, Tessa et al. 2009, Chen et al. 2012, Sinsch et al. 2015). In turn, variation in size and conditions can be caused by differences in lifespan, food availability, climatic conditions and other environmental features (e.g. Reim et al. 2006, Roitberg et al. 2013). For instance, previous studies showed that females of the Monza (MZ) population are significantly larger than the ones of other populations from the study area, perhaps because of higher food availability or longevity (Ficetola et al. 2006) and this might allow them to provide better parental investments. Indeed, this hypothesis aligns with our data as Monza females have a significantly higher parental investment for all the parameters considered, if compared to the other populations (Fig. 1).

Previous studies showed that differences in parental investment could provide differential fitness advantages under specific environmental conditions in amphibians (e.g. predator pressure, climate, environmental stress) (Dziminski and Ross 2005) and that females can accordingly modulate their investment (Räsänen et al. 2005). For instance, in amphibians, mothers can modify the composition of their egg coats to improve tolerance to acidic conditions in embryos (Shu et al. 2016). We did not detect relationships between maternal investment and climate, as we selected a homogeneous pool of populations to better assess the impact of the alien crayfish, but it will be interesting to assess patterns over a broader climatic gradient. Similarly, variation in maternal investment was unrelated to predator pressure. Other studies demonstrated that amphibians, depending on their life history, modulate parental care in response to geographic differences in climate and in presence of an offspring predator (Delia et al. 2013, Shulte et al. 2020). However, the crayfish has invaded the study area only recently, thus it is possible that the populations have had only a limited time to adapt to this predator (Lo Parrino et al. 2020).

Relationships amongst invasive crayfish, maternal investment and embryo development

Multiple factors affected development rate of *Rana latastei* embryos and tadpoles and crayfish presence in the pond of origin and the non-lethal exposure to the crayfish caused developmental acceleration. Egg provisioning is a key driver of the development rate in frog populations (Dziminski and Ross 2005, Ficetola et al. 2011b). In our study, there were no differences in hatching time related to the average egg volume. Even though hatchlings from clutches with smaller egg volume tended to develop faster than those with a smaller one, this relationship was affected by one single clutch with very short development time. A faster development of embryos hatched from smaller eggs is inconsistent with previous studies (Nussbaum 1985, Berven and Chadra 1988, Dziminski and Ross 2005). In fact, a rapid larval development is often assumed to be positively related to fitness and a larger parental investment is expected to provide resources that can allow embryos to reach faster large size and/or late development stages (Kaplan 1992, Warkentin 1999, Capellán and Nicieza 2006, Ficetola et al. 2011b). However, during the embryo development, the cell cleavage may need more time for larger cells than smaller ones and this could explain the faster development time of small eggs. Further studies, involving analyses of a large sample size, are required to better understand the multifaceted relationships between egg size and time of development.

We observed a significant plastic response in embryos and tadpoles reared in presence of the crayfish; individuals hatched and reached Gosner's stage 25 earlier than those unexposed to the predator. This development acceleration confirms that embryos and larvae can recognise the crayfish cues as a risk. This is the first evidence that *Rana latastei* is able to modify hatching phenology in response to the presence of predators and, thus, the crayfish pressure is strong enough to elicit plasticity in hatching. The coevolutionary history of species may influence the recognition of a novel predator and, therefore the expression of phenotypic plasticity. Before the crayfish invasion, the Italian agile frog was often syntopic with a native predator that is rather similar to the red swamp crayfish, i.e. the European white-clawed crayfish (*Austropotamobius pallipes*). In turn, the long evolutionary history with a similar native predator can facilitate responses against non-native predators. In fact, a recent study demonstrated that tadpoles of species that co-evolved with the native crayfish are able to recognise the alien crayfish and to better modulate anti-predator strategies when facing the invasive crayfish (Melotto et al. 2021b). Rapid development can be particularly important in the period from hatching to stage 25, when tadpoles are highly vulnerable, given their limited escape ability. The faster development rate can reduce the exposure to this predator, even though tadpoles may suffer costs for other fitness-related traits (Melotto et al. 2020).

Furthermore, we detected differences between colonised and crayfish-free populations in development time. In this case, the effect of *Procambarus clarkii* was only evident after hatching, as tadpoles from invaded sites reached stage 25 significantly earlier, while no differences in hatching time existed. We also acknowledge that the effect of *Procambarus clarkii* after hatching was affected by one single clutch with particularly rapid development, highlighting the importance of additional tests. However, our findings are consistent with the conclusions of Melotto et al. (2020), despite the fact that we tested different populations in different years. This can be interpreted as local adaptation which can limit mortality and suggests that the adaptation to the crayfish occurs in invaded populations. Finally, plasticity and local adaptation often induce coherent phenotypic variation, jointly determining fitness variation across populations (Levis et al. 2018, Stamp and Hadfield 2020). Our study contributes to the growing evidence that invasive predators, as a novel selective pressure, can induce rapid evolutionary changes in native populations (Langklide 2009, Moran and Alexander 2014). Studying potential evolutionary outcomes of native prey is also important to understand the impact of invasive species and to predict potential long-term effects (Sih et al. 2010, Nunes et al. 2014b).

Despite the strong predatory pressure imposed by *Procambarus clarkii*, so far, the total abundance of clutches in invaded populations by the crayfish does not seem to have undergone a significant decrease (Manenti et al. 2020). Frog persistence is certainly due to the immigration of frog individuals from source populations (Manenti et al. 2020, Falaschi et al. 2021), but it is also possible that plastic and adaptive responses to this predator help to counteract the heavy predator pressure, allowing a sufficient number of tadpoles to attain metamorphosis.

In conclusion, we did not observe a significant relationship between variation in parental investment and the occurrence of a major invasive predator, nor did we detect evidence that parental investment improves development rate in this system. Nevertheless, anti-predator strategies, such as phenotypic plasticity or adaptive variations, can help native populations to reduce the impact of an alien predator. So far, most of the studies analysed variation of amphibian performance under laboratory conditions and more studies are needed to understand how these processes act in the wild. Furthermore, in a world where invasive species are increasingly widespread and abundant, continuous monitoring is required to evaluate whether these responses will allow the long-term persistence of native species.

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Supporting Information

Supporting Information is available at: https://neobiota.pensoft.net/article/65454/

Supplementary material

Table S1. Geographic coordinates of the monitored sites. To avoid poaching on the threatened frog, we approximated the coordinates (Lunghi et al. 2019)

ID site	Site name	Latitude	Longitude
AL	Alserio	45.47	9.14
BA	Bassone	45.46	9.6
CA	Carpanea	45.44	9.15
CU	Curone	45.42	9.23
FO	Fornacetta	45.43	9.15
LA	Lavatoio	45.45	9.15
MO	Molera	45.42	9.15
MZ	Monza	45.36	9.17

Table S2. The significance of the independent variables in the three parental investment mixed models.

	Monthly precipitation			**Annual mean temperature**			**Crayfish presence**		
	F	df	p	F	df	p	F	df	p
Egg number	0.169	1, 3.7	0.704	0.587	1, 3.7	0.489	0.884	1, 3.8	0.402
Egg volume	0.113	1, 3.7	0.755	0.0003	1, 3.7	0.986	0.193	1, 3.8	0.684
Total investment	0.053	1, 3.8	0.83	0.224	1, 3.8	0.662	0.668	1, 3.9	0.454

CHAPTER 3 | CONDITION- AND CONTEXT- DEPENDENT VARIATION OF SEXUAL DIMORPHISM ACROSS LIZARD POPULATIONS AT DIFFERENT SPATIAL SCALES

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Abstract

The evolution of sexual dimorphism (SD) is driven by intricate interplays between sexual and natural selection. When it comes to SD variation within populations, however, environmental factors play a major role. Sexually selected traits are expected to be strongly dependent on individual body condition, which is influenced by the local environment that individuals experience. As a consequence, the degree of SD may also depend on resource availability. Here, we investigated the potential drivers of SD expression at two sexually dimorphic morphometric traits, body size (snout vent length) and head shape (head geometric morphometrics), in the Italian wall lizard (*Podarcis siculus)*. We assessed the existence of condition- and context-dependent SD across ten islands of the Aeolian archipelago (southern Italy), at within- and among-population scales. We observed strong geographical variation of SD among islands, and tested three potential SD predictors related to resource availability (individual body condition, ecosystem productivity, temperature). Body condition and ecosystem productivity were the main drivers of body size SD variation, and body condition was also the main driver for head shape SD. Our results highlight that the expression of SD in the Italian wall lizard is both condition- and context-dependent. These results are congruent at within- and among-populations scales highlighting that spatial multi-scale analysis represents a useful approach to understand patterns of SD expression.

Introduction

Sexual dimorphism (SD) is widespread in the animal world and can involve extensive variation in morphological, physiological and behavioural traits (Andersson 1994). Understanding the processes leading to SD has been a central topic of evolutionary studies since Darwin's work (Darwin 1871); these studies have shown that the evolution of SD is driven by a complex interplay between sexual and natural selection. Understanding the variation of sexually dimorphic traits is complicated by the fact that many traits are dependent on individual condition (Bonduriansky 2007), which is in turn a product of resource availability and the individual's efficiency at translating the available resources into fitness (Bonduriansky and Rowe 2005). Consequently, sexually dimorphic traits often show strong phenotypic plasticity across gradients of resource availability, and the patterns of variation within and between sexes are caused by both genetic and developmental processes (Bonduriansky 2007). During organism development, the allocation of resources to sexual traits can be costly and their expression is tightly linked to the availability of resources (Andersson 1994, Godin and McDonough 2003, Emlen et al. 2012). For instance, in *Hyalella* amphipods, male gnathopod size (a sexually selected trait) is more susceptible to resource stress (food availability) than non-sexual or female traits (Cothran and Jeyasingh 2010).

Given the costs of sexually dimorphic traits, theory predicts that the expression of traits exaggerated by sexual selection should be strongly dependent on the condition of individuals (Bonduriansky 2007). As a result, sexual selection acting on male traits should lead to condition-dependent sexual dimorphism, where differences between males and females are stronger in individuals in better condition, and males' traits are more strongly affected by variation in conditions than female traits (Bonduriansky 2007). Assessing the conditions experienced by individuals in natural populations can be challenging, but measures such as the body condition index (BCI, obtained from the residuals of the relationship between body mass and body length), can provide a good estimate of the overall foraging success and fitness of individuals (Jakob et al.

1996). The expression of SD can thus correlate with BCI, as evidenced for the sex-specific coloration (yellow cheek-parches) at population level in the Hermann's tortoise (Bonduriansky 2007, Galeotti et al. 2011).

The individual condition of animals is strongly affected by the environment, hence the degree of SD is also expected to depend on resource availability (context-dependent SD) (Sacchi et al. 2015). Several environmental features can be used as proxies of resource availability or can determine variation in fitness-related traits, thus triggering variation of sexual dimorphism. Environmental variables that can affect SD include ecosystem productivity (e.g. Greenberg and Olsen 2010) and temperature, the latter having particularly strong impacts on the physiology, morphology, behavior and metabolism of ectotherms (Clarke 2003, Ficetola et al. 2010, García-Roa et al. 2020). The effect of environmental features on SD can be assessed at different spatial scales. Some studies focused on differences between individuals within population, while others used an eco-geographical approach, evaluating broad scale drivers of differences among populations (Ficetola et al. 2010, Sacchi et al. 2015, Simmons et al. 2017, Avramo et al. 2021). While both scales can provide useful information on the potential drivers of SD, multi-scale studies are required to assess whether the processes determining the variation in SD between individuals within a population are the same across spatially isolated populations.

Lizards are a good model for studying the degree of SD because they show strong variation in direction and magnitude of SD at multiple traits, across and within species (Cox et al. 2003). The family Lacertidae generally shows a male-biased SD, with larger male body size and head dimensions driven by sexual selection (combat and mating performances), while females usually have larger abdomen length (a trait related to fecundity) (Olsson et al. 2002, Kaliontzopoulou et al. 2007). Such considerable SD is also found in the Italian wall lizard, *Podarcis siculus* (Zuffi et al. 2012). This species, widespread in the Mediterranean basin (Corti et al. 2011a, Silva-Rocha et al. 2019), has a broad ecological tolerance and shows variation of SD across populations (Avramo et al. 2021).

The aim of this study is to test whether resource availability determines variation of SD within and among populations of *P. siculus* on the Aeolian archipelago (Southern Italy). To this end we measured SD in body size and head shape, and tested if the expression of SD at these traits shows the same response to environmental variation within and across islands (Butler and Losos 2002, Avramo et al. 2021). We evaluated the effect of three predictor variables representing resource availability on the degree of SD: (i) individual body condition (BCI) to investigate condition-dependent SD, (ii) ecosystem productivity (estimated through the Normalized Difference Vegetation Index, NDVI) and (iii) land surface temperature, that may affect context-dependent SD not accounted for by BCI. We predict that SD should be more pronounced in individuals showing better body conditions, and/or in environments with more resources (higher productivity or warmer temperature). Furthermore, we assessed the effects of these predictors at two spatial scales: the individual scale (male-female differences within populations) and the island scale (SD degree among spatially isolated populations), to evaluate whether scaling issues affect the detection of the drivers of SD.

Material and methods

Data collection

The Aeolian archipelago is composed of seven main islands and several islets, and includes active, dormant and extinct volcanoes. The island volcanic landforms, characterized by altitude from the sea level up to 962 m above sea level (a.s.l), provide a variety of climatic conditions and a high environmental heterogeneity (Lucchi et al. 2013). In September 2021, we sampled lizards from the seven main islands of the Aeolian archipelago (Alicudi, Filicudi, Salina, Panarea, Stromboli, Lipari, Vulcano) and from three islets (Bottaro, Lisca Bianca and Basiluzzo) (Fig. 1). Sampling design covered the whole altitudinal range of each island. Lizards were sexed, measured, and weighed (accuracy: 0.1 g). The head of each individual was photographed in dorsal view using Olympus TG-

5 or TG-6 cameras in a photo light box. The pictures were used to perform head geometric morphometrics. We also recorded the GPS coordinates of each captured individual (accuracy: 3m).

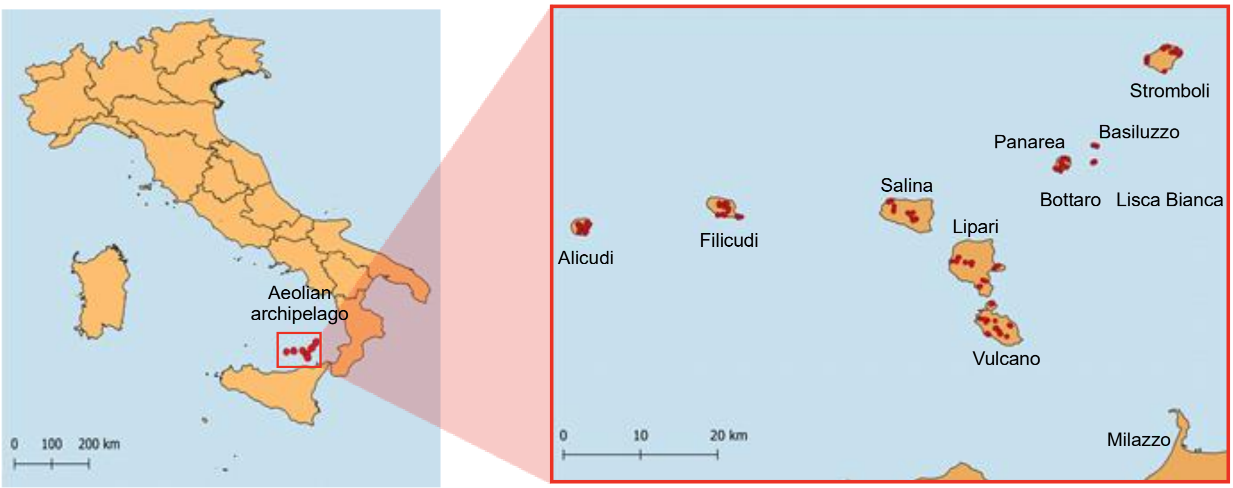

Fig 1. Study area in the Aeolian archipelago and sampling locations of the 408 sampled individuals (red dots). Lisca Bianca and Bottaro islets are geographically close and points are partially overlapped. The map was created using QGIS (QGIS.org 2022).

Phenotypic traits and measures of sexual dimorphism

To analyze sexual dimorphism, we considered three phenotypic traits: 1) snout-vent length (SVL), 2) head size and 3) head shape. SVL was measured with a Vernier caliper (Meiri 2008) and log-transformed to improve normality. Head size and shape variables were obtained using landmark-based geometric morphometrics. Head geometric morphometrics was performed using 28 landmarks located at intersections and borders of cephalic scales (Fig. 2a; see Kaliontzopoulou et al. 2007) for a description of landmarks). Individuals for which any of the landmarks could not be defined properly were excluded from the study. A TPS file with all the individuals was created using tpsUtil version 1.87 (Rohlf 2021) and landmarks were digitized using tpsDig2 version 2.31 (Rohlf 2018). Variation due to scale, orientation, and position was removed by applying a Procrustes superimposition using the IMP software CoordGen8 (Sheets 2014a). We applied a principal component analysis (PCA) on Procrustes-aligned head shape coordinates using the software PCAGen8 (Sheets 2014b) and the resulting individual scores on each PC were used as shape variables. Head size was determined as the log-transformed centroid size, but was strongly correlated to SVL (Pearson's $r = 0.89$, $p < 0.001$, Fig. S2) and was not considered for subsequent analyses.

Sexual dimorphism was thus quantified for body size (SVL) and head shape (scores on PC1). We estimated the size dimorphism with the Lovich and Gibbons index (Lovich and Gibbons 1992):

$$\mathrm{SDI} = \frac{\text{mean SVL of larger sex(males)}}{\text{mean SVL of smaller sex (females)}} - 1$$

The head shape dimorphism was calculated as the average of Euclidean distances among all male-female pairs on PC1 shape variable using the usedist R package (Bittinger 2020).

Potential drivers of sexual dimorphism

We focused on three variables that can represent condition and context variation, and may affect phenotypic traits and sexual dimorphism: body condition of individuals, ecosystem productivity (NDVI), and land surface temperature. Body condition is a fitness-related parameter providing an overall index of animal conditions and foraging success (Jakob et al. 1996). The body condition index (BCI) was calculated as the residuals from the regression of body mass on SVL. This regression included individuals from both sexes and all the islands, and sex as independent variable to discriminate between males and females. Body mass and SVL were log-transformed (Fig. S3) (Schulte-Hostedde et al. 2005).

Proxies of productivity and temperatures were obtained from remote-sensing data. As a measure of productivity/peak greenness, we used the Normalized Difference Vegetation Index (NDVI). NDVI is a proxy of photosynthetic activity and green biomass; it can represent resource availability and resource partitioning (Weier and Herring 2000, Evans et al. 2005). All the Landsat-8 TOA images (LANDSAT/LC08/C01/T1_TOA - 30m resolution) available for the time frame April 1st - September 30th (year 2015 to 2020) were processed, and the seasonal NDVI maximum, averaged over the years of interest, was calculated. April 1st – September 30th represents the period with highest activity of the study species (Corti et al. 2011b). Land surface temperatures were retrieved for the same period, following the approach detailed in Ermida et al. (2020). Being ectotherms, lizards are strongly affected by the abiotic environment. Environmental temperature, in particular, affects performance of many biochemical processes and can determine variation at fitness-related traits (e.g. survival rate and fecundity) (Porter 1993, Angilletta et al. 2002). The Landsat-8 TOA collection was used to retrieve brightness temperature, while the Landsat-8 SR collection (LANDSAT/LC08/C01/T1_SR) for computing fractional vegetation cover (FVC), using standard NDVI thresholds ($NDVI_{bare} = 0.2$ and $NDVI_{veg} = 0.86$). Landsat emissivity was obtained by correcting ASTER GEDv3 (NASA/ASTER_GED/AG100_003) surface emissivity for bare ground, using Landsat-8 FVC. Temperature was measured as land surface temperatures. These temperatures were calculated by applying the Statistical Mono-Window algorithm to the Thermal infrared band of Landsat-8 TOA, and implementing information from atmospheric water content (TCWV) from the NCEP/NCAR reanalysis (NCEP_RE/surface_wv). The obtained bi-monthly surface temperatures were averaged over the period April 1st – September 30th. A total of 199 images were used (tile ids: LC08_188033, LC08_188034, LC08_189033), all collected between 09:34 and 09:42 am. All the analyses of satellite imagery were run using the cloud service Google Earth Engine (GEE) and the R package *rgee* (Aybar et al. 2020).

Island area can be a further measure of resource productivity in insular environments, as larger islands often have more resources (Sacchi et al. 2015). The area of each island was obtained from the ReptIsland database (Bonardi et al. 2022), available at https://doi.org/10.6084/m9.figshare.14346416). However, island area was strongly correlated with the NDVI averaged across all the sampled points of each island (Pearson's $r = 0.90$, $p < 0.001$; Fig. S2), thus island area was not considered in further analyses. Differences in SD were unrelated to geographic distances between islands (Mantel's tests, 9999 permutations; SDI: $r = -0.099$, $P = 0.646$; head shape dimorphism: $r = -0.068$, $P = 0.608$).

Statistical analyses

The analysis of sexual dimorphism was repeated at the i) individual and ii) island scales. We first tested the differences between sexes in body size and head shape among islands using analysis of variance (ANOVA). Then, we built a series of linear mixed effects models (LMMs) (individual scale) and linear models (LMs) (island scale) testing the effect of resource availability (BCI, NDVI, temperature) on body size and head shape dimorphism. At the individual scale, the effect of BCI, NDVI and temperature on body size and head shape was tested as the statistical interaction between sex (M/F) and each predictor (Bonduriansky 2007). Interactions between sex and predictors account for different responses between males and females to that variables. For individual-scale analyses, we used NDVI and soil temperature of the capture location of each individual and the BCI of each individual. For island-scale analyses, these three variables were averaged among all the sampling points of each island. We included islands as a random factor to consider the non-independence of lizards collected on the same island.

We built models with all the potential combinations of predictors, and calculated the corrected Akaike's Information Criterion (AICc) for each model. The model with the lowest AICc value was considered to be the "best model" (Burnham and Anderson 2002) (see Tab. S2). AICc can select excessively complex models, consequently we considered a complex model as a candidate model only when it had a lower AICc than the AICc of all its simpler nested models (Richards et al. 2011). Moreover, we only considered models with AICc values lower than the null model. For each model, we calculated the AIC weight, which represents the support of the model, given the data and the set of candidate models. Finally, we determined the evidence ratios E = w_i/w_j to compare the relative support of the models (Lukacs et al. 2007). We used the marginal and conditional R^2 (R^2_M and R^2_C, respectively), as measures of the variation explained by each model (Nakagawa and Schielzeth 2013). Three islets (Basiluzzo, Bottaro and Lisca Bianca) had smaller sample size than larger islands. To confirm the robustness of our conclusions, we re-run the best-AICc models after removing these three islets.

We used the lme4, lmerTest, car and MuMIn packages for ANOVA, LLMs and LMs (Kuznetsova et al. 2017) and visreg package (Breheny and Burchett 2017) to produce conditional regression plots. All statistical analyses were performed in R 3.4.2 (R Core Team 2021, http://www.r-project.org).

Ethics statement

Capture and manipulation of lizards, and all the experimental protocols were authorized by the Italian Ministry for the Environment (prot. 0037921.13-04-2021). Lizards were collected by noosing and immediately released in the site where they had been captured after measurements, as specified in the permits of the Ministry for the Environment. All methods were carried out in accordance with relevant guidelines and regulations.

Results

We captured 408 adult lizards (239 males and 169 females) from the seven main islands of the Aeolian archipelago (Alicudi, Filicudi, Salina, Panarea, Stromboli, Lipari, Vulcano) and from three islets around Panarea (Bottaro, Lisca Bianca, Basiluzzo) (Fig. 1). The number of individuals per island ranged between 48 and 59 in large islands and between 7 and 12 in the islets (Tab. S1). Snout-vent length (SVL) was significantly different across islands and between sexes, males being longer (68.34 ± 9.69mm) than females (58.82 ± 9.71mm) (ANOVA: effect of sex: $F_{1, 397}=225.32$, $p < 0.001$; island: $F_{9, 397}=4.12$, $p < 0.001$; Fig. S1a).

Head shape variation (landmark-based geometric morphometrics, Fig. 2a) was assessed using individual scores (PC) of a principal component analysis on head shape coordinates of a subset of 302 individuals (Tab. S1). Since we were interested in head shape dimorphism, we tested the effect of sex on each PC using linear mixed effects models (LMMs), including island of origin as random factor and body size as covariate. PC1 showed strong differentiation between sexes ($F_{1,}$

$_{294.02} = 64.24$, $p < 0.001$, marginal $R^2=0.54$, conditional $R^2 = 0.61$), while differences between sexes for PC2 were very small ($F_{1,\ 292.08} = 0.866$, $p = 0.35$; marginal $R^2 = 0.04$, conditional $R^2 = 0.44$, Fig. 2b). We therefore focused analyses of head shape dimorphism on PC1. Head shape variation on PC1 accounted for 21.6% of total variation (Fig. 2b). The two sexes differed mainly in the shape of the area around the back of the head, with males having longer parietal scales compared to females (Fig. 2b). We found significant variation of head shape between sexes and across the ten islands (ANOVA: effect of sex: $F_{1,\ 290} = 230.09$, $p < 0.001$; effect of island: $F_{9,\ 290} = 5.07$, $p < 0.001$; Fig. S1b).

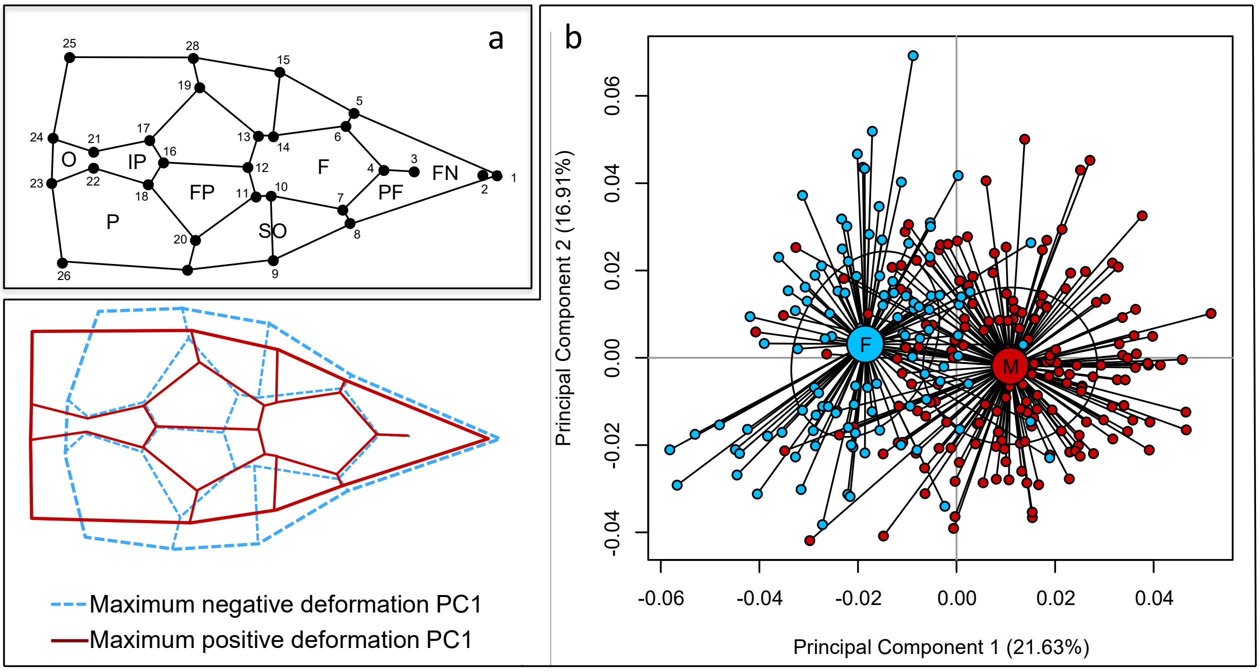

Fig 2. Head shape sexual dimorphism. **a)** Localization of the 28 landmarks recorded on the head of *Podarcis siculus*. Solid black lines: mean shape among all individuals. Nomenclature of scales: F: frontal, FN: frontonasal, FP: frontoparietal, IP: interparietal, N: nasal, O: occipital, P: parietal, PF, prefrontal, SO: supraocular. **b)** Principal Component Analysis (PCA) on Procrustes shape coordinates, with 38% of total variance accounted by the first two PCs. Schematic representation of landmark deformations on the first PC reconstructed from grids and vectors (exaggeration factor of 2). Solid red lines: male maximum deformation, dashed blue lines: female maximum deformation.

Individual scale (within populations)

We assessed the effect of environmental variables (BCI, NDVI, temperature) on body size and head shape differences between males and females at the individual scale using LMMs, testing the significance of interactions between sex and each environmental variable and including island as a random factor to take into account the non-independence of lizards collected on the same island. The best-AICc model explaining the SVL of individuals included BCI, NDVI, sex, the interaction between sex and BCI, and the interaction between sex and NDVI. Males were consistently larger than females ($F_{1,\ 401.4} = 8.34$, $p = 0.004$; Fig. 3). A strong effect of the interaction between sex and BCI showed that body size scaled differently with body condition in each sex ($F_{1,\ 404.8} = 121.56$, $p < 0.001$; Fig. 3a). Furthermore, lizards from localities with high NDVI were larger ($F_{1,\ 382.6} = 19.64$, $p < 0.001$), and the difference between males and females slightly increased in sites with high NDVI, even though the effect of the interaction between sex and NDVI was not significant at the 0.05 level ($F_{1,\ 401}= 2.87$, $p = 0.09$; Fig. 3b) (Tab. 1).

For head shape, AICc identified three models showing similar AICc values (Tab. 1). All models suggested that head shape was different between males and females ($F_{1,\ 294.7} = 5.654$, $p = 0.018$ in the best-AICc model). Furthermore, the model with the lowest AICc value suggested that head shape differences between males and females were stronger in individuals with better body condition ($F_{1,\ 294.6} = 4.32$, $p = 0.038$; Fig. 3c), and in individuals found in sites with high NDVI, although the latter effect was not significant ($F_{1,\ 294.2} = 1.943$, $p = 0.164$; Fig. 3d). Head shape was unrelated to the NDVI of the location where individuals were captured, nor to the BCI of individuals (in both cases, $p > 0.3$). However, for head shape, there was uncertainty in model selection. The competing models included two simpler models, the first model included neither NDVI nor the interaction between NDVI and sex, while the second model, which showed a slightly higher AICc, included neither BCI nor the interaction between sex and BCI, but included NDVI, soil temperature, and the respective interactions (Tab. 1). All the results remained consistent after the removal of the islets with the smallest sample size (Tab. S3a).

Dependent	Predictors	AICc	Δ AICc	*w*	R^2_M	R^2_C
Individual scale						
SVL	BCI (-), NDVI (+), Sex, Sex*BCI, Sex*NDVI	2561.6	-	>0.999	0.51	0.55
Head shape	BCI (-), NDVI (+), Sex, Sex*BCI, Sex*NDVI	-1618.6	-	0.36	0.44	0.49
	BCI (-), Sex, Sex*BCI	-1618.4	0.2	0.33	0.42	0.49
	NDVI (+), Temp (+), Sex, Sex*NDVI, Sex*Temp	-1617.4	1.1	0.2	0.44	0.48
Island scale						
Body size SD	BCI (+), NDVI (+)	-30.9	-	0.45	0.68	
	BCI (+)	-30.8	0.1	0.43	0.48	
	NDVI (+)	-28.2	2.7	0.12	0.34	
Head shape SD	BCI (+)	-67.6	-	>0.999	0.43	

Tab. 1. Best models assessing the effect of environmental predictors on sexual dimorphism (SD) at individual and island scales. The dependent variables of models are: SVL; head shape; body size SD and head shape SD. Models are ranked according to their AICc values. Only models with *w* > 0.02 and with AICc lower than the null model are shown (see Tab. S2). The sign of the relationship is in parentheses.

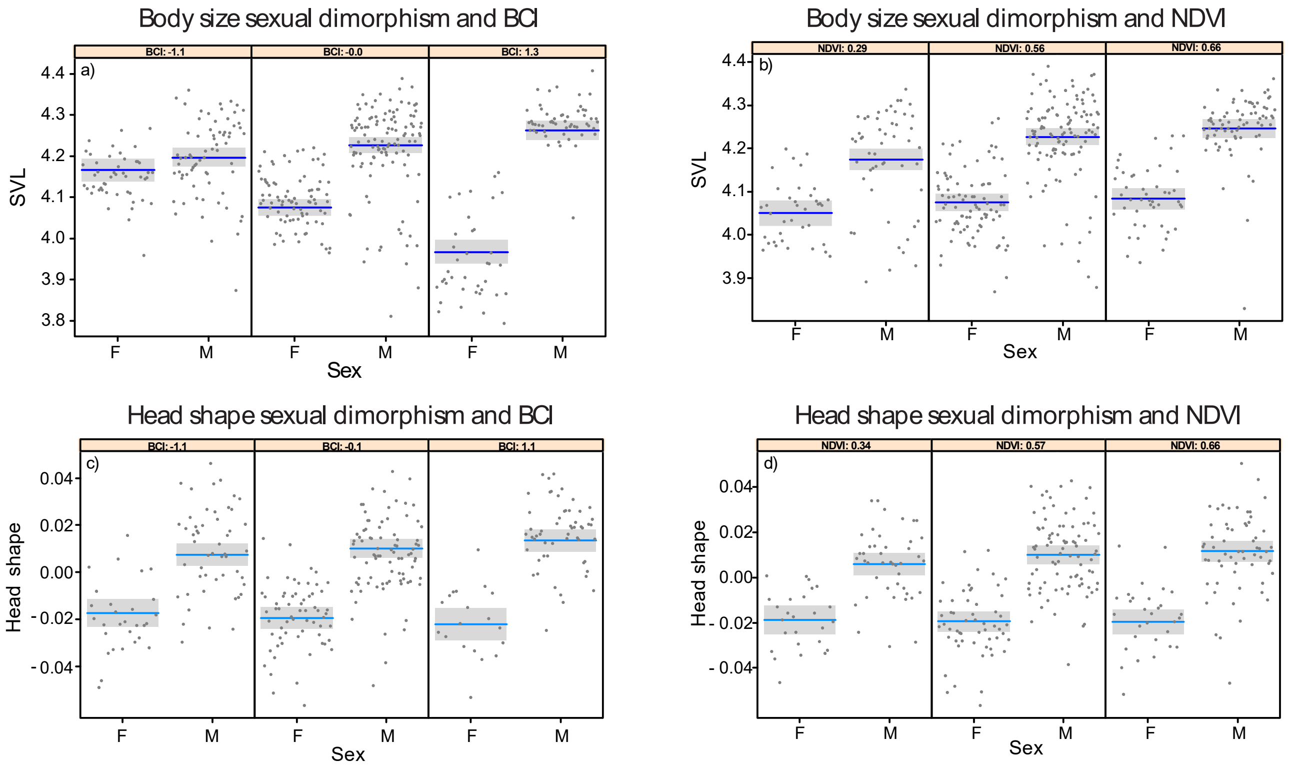

Fig. 3. Conditional plots showing the relationship between sexual dimorphism and the environmental drivers included in the best AICc models at the individual scale (N=408). Phenotypic traits: **(a-b)** SVL, **(c-d)** head shape; drivers: interactions between **(a, c)** sex and BCI and **(b, d)** sex and NDVI. Male–female differences are divided in three categories of environmental drivers: low (10th quantile), intermediate (median), and high (90th quantile) of BCI **(a, c)** and NDVI **(b, d)**.

Island scale (among populations)

The effect of environmental variables on body size SD (Lovich and Gibbons index) and head shape SD (average Euclidean distance among all male-female pairs on PC1 shape variable) at the island scale was assessed using linear models (LMs), testing the significance of each environmental variable averaged among all the sampling points of each island. The best-AICc model suggested that body size dimorphism is higher in islands where the average BCI is largest ($F_{1,7}$= 13.5, p = 0.008; Fig. 4a) and with highest NDVI ($F_{1,7}$= 5.87, p = 0.046; Fig. 4b). Simpler models, only including one of these variables, showed slightly higher AICc values (Tab. 1). For head shape dimorphism, the best-AICc model included the average BCI of the island, indicating that sexual dimorphism is higher in islands where individuals have better BCI ($F_{1,8}$= 6.684, p = 0.032; Fig. 4c) (Tab. 1). All the results remained consistent after the removal of the islets with the smallest sample size (Tab. S3b).

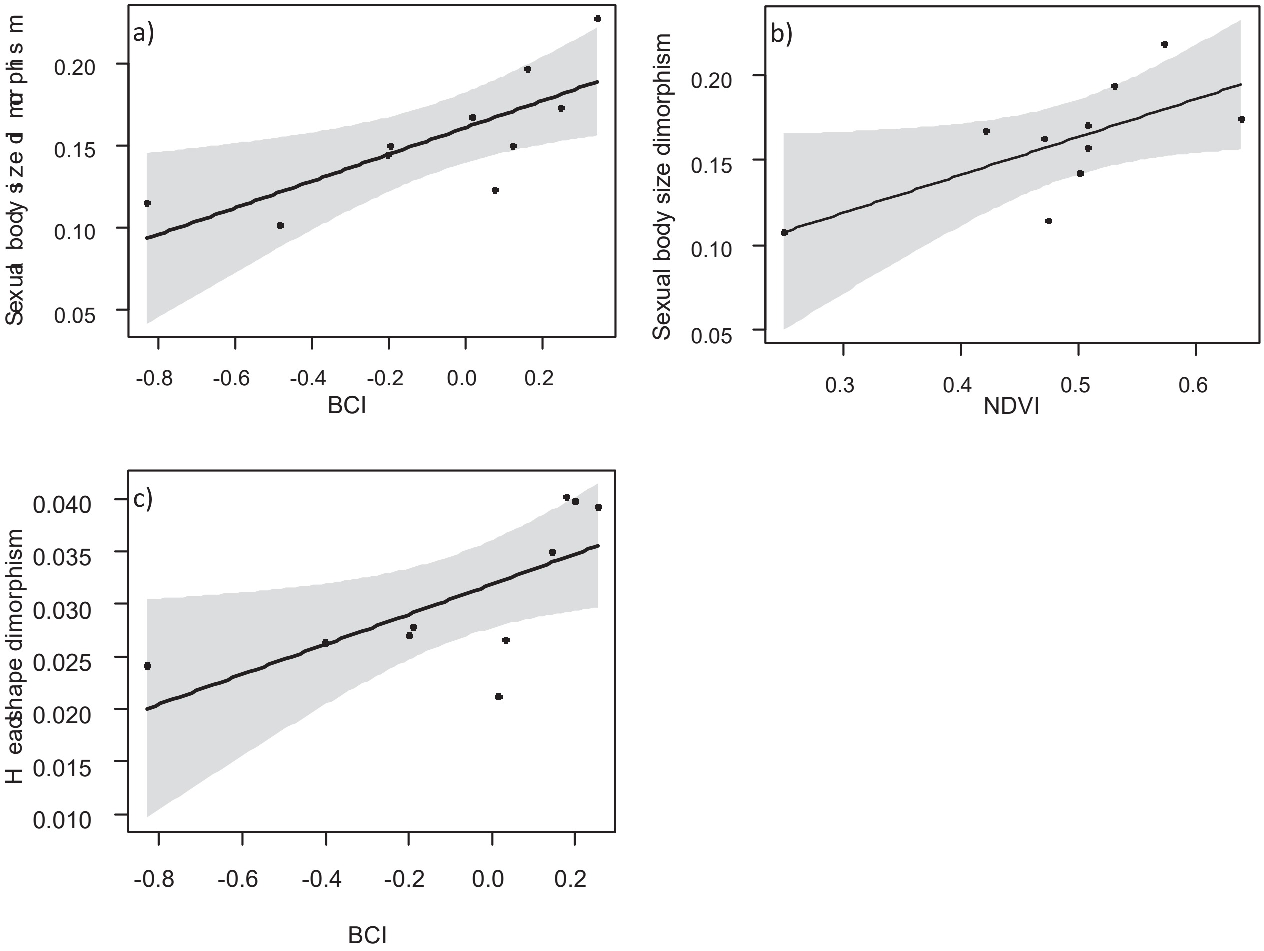

Fig. 4. Conditional plots showing the relationship between sexual dimorphism and the environmental drivers included in the best AICc models at the island scale (N=10). Sexual dimorphism: **a-b)** body size dimorphism, **c)** head shape dimorphism; drivers: **a-c)** BCI, **b)** NDVI. Black line: regression line, grey shaded area: 95% confidence interval.

Discussion

The degree of sexual dimorphism can be influenced by individual condition and environmental resource availability (Bonduriansky 2007, Sacchi et al. 2015). By assessing the drivers of SD at both the individual and island scales, we demonstrate that (i) the difference in body size and head shape between males and females in Italian wall lizards varies across individuals and islands, and (ii) the pattern of variation of SD is consistent at different geographical scales corresponding to within- and among-population levels. Despite the limited number of sampled islands, our results all support the hypothesis that body condition and context-dependent factors (i.e. ecosystem productivity) concur to drive SD degree in *P. siculus*.

Sexual dimorphism is widespread in lizards, body size and head shape being the main dimorphic characters (Butler and Losos 2002, Olsson et al. 2002, Kaliontzopoulou et al. 2008). In *P. siculus* both of these characters strongly differ between sexes. Sexual dimorphism of these traits is related to two behaviors widespread in lacertid lizards: male-male fights and forced copulation (Olsson et al. 2002). Head shape is an extremely good predictor of bite force, which in lizards is fundamental for territory defense, female accession and copula (Herrel et al. 2001).

Several studies demonstrated geographic variation of sex-related traits, with significant relationships with island features (e.g. Lomolino 2005, Sacchi et al. 2015). Morphological changes can occur rapidly on islands, providing different contexts of resource availability and environmental features (Madsen and Shine 1993, Millien 2006, de Amorim et al. 2017). We show that body size and head shape in *P. siculus* strongly vary across the different islands of the Aeolian archipelago. The variation across islands can be the result of phenotypic plasticity and/or local adaptation (Levis et al. 2018), and multiple processes can contribute to this variation. Sexual selection generally favors large male size, and the extent of differences between sexes can be affected by environmental features, for instance because when more resources are available phenotypic plasticity allows maximal divergence of growth trajectories. Furthermore, we cannot exclude that variation of resources determines variation of sexual and / or natural selection, for instance if more resources relax natural selection. Distinguishing between these hypotheses is challenging, and long-term common rearing environments would be required to fully tease apart the role of these processes. Nevertheless, the positive relationship between SD and resource availability was clearly observed across individuals within the population, supporting the hypothesis of an important role of phenotypic plasticity (Cox et al. 2006, 2008, Cox and Calsbeek 2010).

The incredible diversity of sexually selected traits has been of particular interest to evolutionary biologists. However, the intraspecific variation in these traits often remains unexplained (Wiens and Tuschhoff 2020) and is rarely investigated at multiple spatial scales. Our analyses show that differences between males and females are related to both the condition of individuals and the resource availability in the environment at the individual and island scales (Bonduriansky 2007), body condition having the strongest effect on the expression of sexual dimorphism. Populations with the largest average BCI values also showed the strongest SD (Fig. 4 a-c). Furthermore, within-population differences between males and females were exacerbated for the individuals with the highest BCI (Fig. 3), and this condition-related pattern of SD expression was confirmed in different traits (body size and head shape). Males in better condition can allocate more resources to sexually selected traits; which are costly and are tightly linked with body condition. For example, in the crowned leafnose snake, better body condition of males determines longer tails, which are important for mating (Sivan et al. 2020).

Nevertheless, body condition alone is not enough to fully explain the variation of SD. The individual condition is strongly affected by the context in which individuals live and, therefore, to the availability of resources (Andersson 1994, Godin and McDonough 2003, Emlen et al. 2012), and BCI variation is unable to fully capture the environmental variation experienced by individuals. Among the environmental features that can be used as a proxy of resource availability in the wild and determine variation of SD, we found support for a role of ecosystem productivity (here measured on the basis of NDVI, which is a proxy of plant productivity and / or biomass (Weier and Herring 2000, Evans et al. 2005) at two different spatial scales. Primary productivity strongly determines the amount of available resources and often affects size SD expression. For instance, the positive relationship between size SD and primary productivity in the Iberian newt (body size) and in tidal-marsh sparrows (bill size) suggests that abundant productivity may relax natural selection (Greenberg and Olsen 2010, Peñalver-Alcázar et al. 2019). The two morphometric traits studied here do not follow the same context-dependent pattern of SD expression, as the positive relationship between ecosystem productivity and SD was only observed for body size but not head shape SD. It is possible that size SD shows a stronger plastic response in relation to resource availability compared to head shape. Indeed, several studies have shown that head shape may vary with other environmental features such as altitude, urbanization, island area and food niche breadth (Thorpe and Baez 1987, Sagonas et al. 2014, Lazić et al. 2015). The size of an island is an alternative proxy for resource availability as well as for population size, because larger islands offer more resources than small ones and populations in large islands experience less demographic stochasticity. Island size has therefore also been related to variation in SD (MacArthur and Wilson 1967, Sacchi et al. 2015, Alzate et al. 2019). Island area and ecosystem productivity are collinear in our system (see method), and we focused on productivity because of its more direct link with the resources available for resources. The strongest importance of productivity is also supported by our data, as alternative models considering island area instead of productivity did not show any significant relationship between island area and degree of SD (Tab S4).

Temperature played a minor role on SD expression compared to body condition and NDVI. This result partially contrasts previous studies. Indeed, temperature plays a crucial role in the fitness of ectotherms, affecting organism physiology, morphology, behavior and metabolism (e.g. Wieser 1973, Clarke 2003), and can determine the intensity of sexual selection and the degree of SD (García-Roa et al. 2020). For example, the SD of a seed beetle population decreases in extreme temperature conditions compared to an intermediate temperature in common garden experiments, suggesting a better allocation of resources when individuals experience an optimal thermal environment (Stillwell and Fox 2009). In our study system, the variation of thermal conditions across the study area was strong. In the morning, average surface temperature during the warmest semester of the year ranged from 21°C (mostly at high elevations and in north-facing slopes) to 43°C. Despite this substantial variation, no population experienced very cold conditions that could strongly limit lizard activity. This suggests that, under the relatively benign conditions of the study area, the high thermoregulation capacity of lizards may allow to buffer environmental variation.

In conclusion, our results highlight that better body condition and higher primary productivity, two proxies for resource availability, can increase the expression of SD. The expression of SD in the Italian wall lizard is both condition- and context-dependent, with context-dependent effects being mostly evident for body size. Abundant accessible resources can determine strong phenotypic plasticity, but might also relax natural selection and lead to increased selection for sexual traits (Greenberg and Olsen 2010, Peñalver-Alcázar et al. 2019). In high-resource habitats, males have longer head parietal scales and body size compared to low-resource habitats, while female head shape and body size do not change in relation to resource availability (Fig. 5), possibly because they invest more in other traits not measured here, e.g. interlimb length (Olsson et al. 2002). It is also possible that the strength of sexual selection is similar across islands, but variation in energy availability determines different potential for males to achieve the largest body sizes. In other words, variation in environmental conditions can determine a broad range of sexual dimorphism even if sexual selection remains constant. The agreement between our results and what already observed for different species in other geographic areas suggests that spatial variation of SD could be a general pattern for lizard populations facing environmental stressors in resource-constrained habitats (Sacchi et al. 2015). Nevertheless, the joint evaluation of direct measures of individual condition and habitat resource availability will allow a better identification of mechanisms that affecting sexual dimorphism at multiple traits.

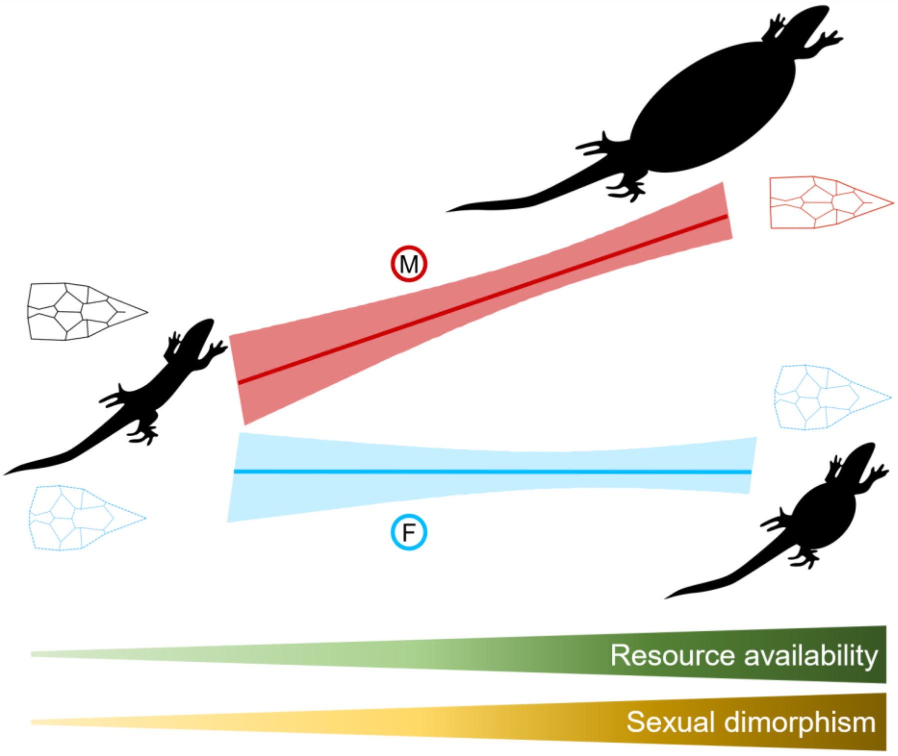

Fig. 5. Condition and context-dependent expression of sexual dimorphism in *Podarcis siculus*. General trends of body size and head shape expression according to resource availability in males (M, red) and females (F, blue).

Acknowledgements

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Supporting Information

Supporting Information is available at: https://www.nature.com/articles/s41598-022-21358-2

Supplementary material

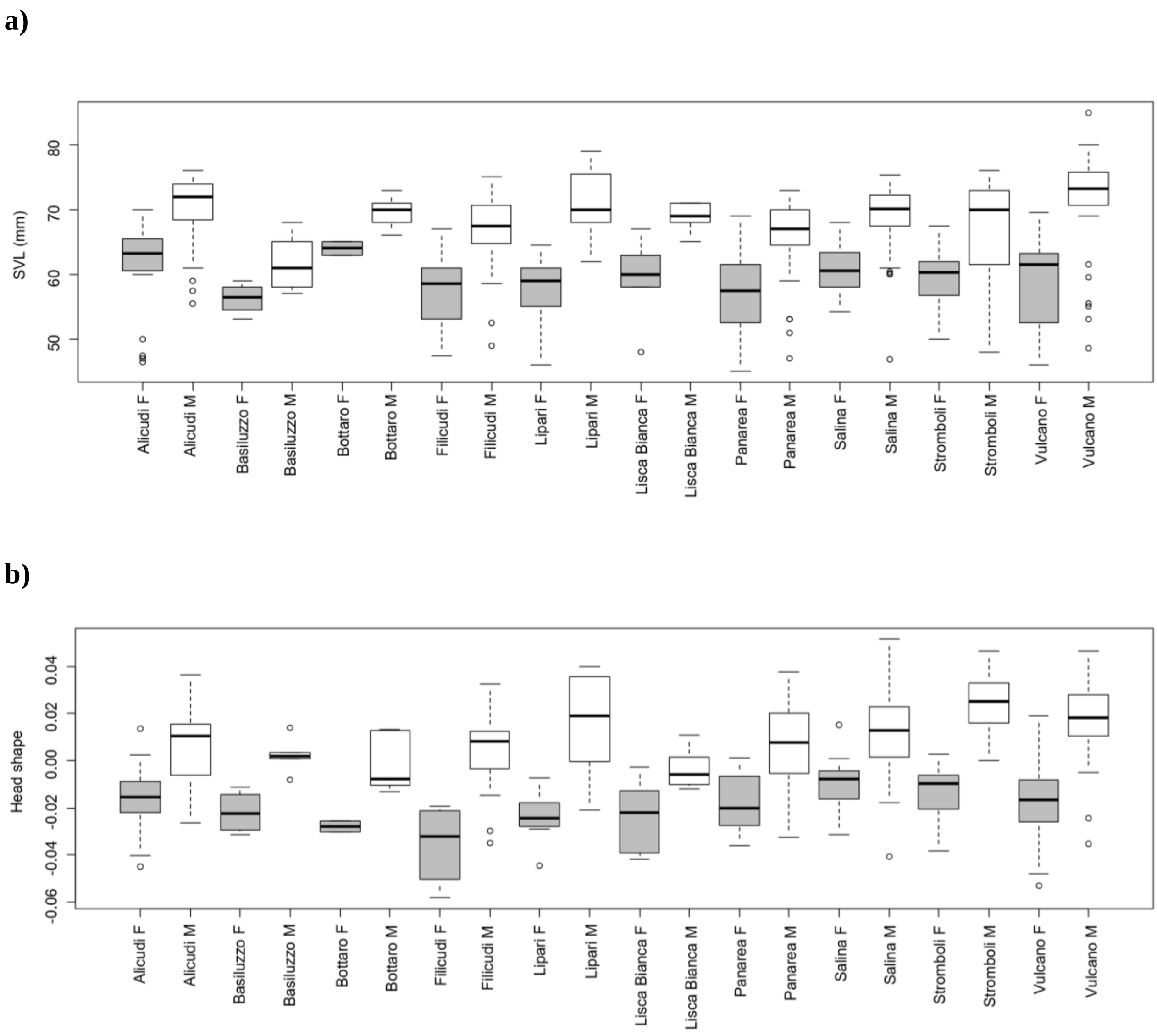

Figure S1. Variation of phenotypic traits of *Podarcis siculus* among the 10 islands: **a)** SVL, **b)** head shape. Female (grey) and males (white) are shown separately.

Figure S2. Pearson's correlation coefficients at different scales (ind= individual scale, isl= island scale) for: a) phenotypic traits (SVL, Hsize=head size, Hshape= head shape), b) resource variables (BCI= Body Condition Index, NDVI= Normalized Difference Vegetation Index, TEMP=temperature).

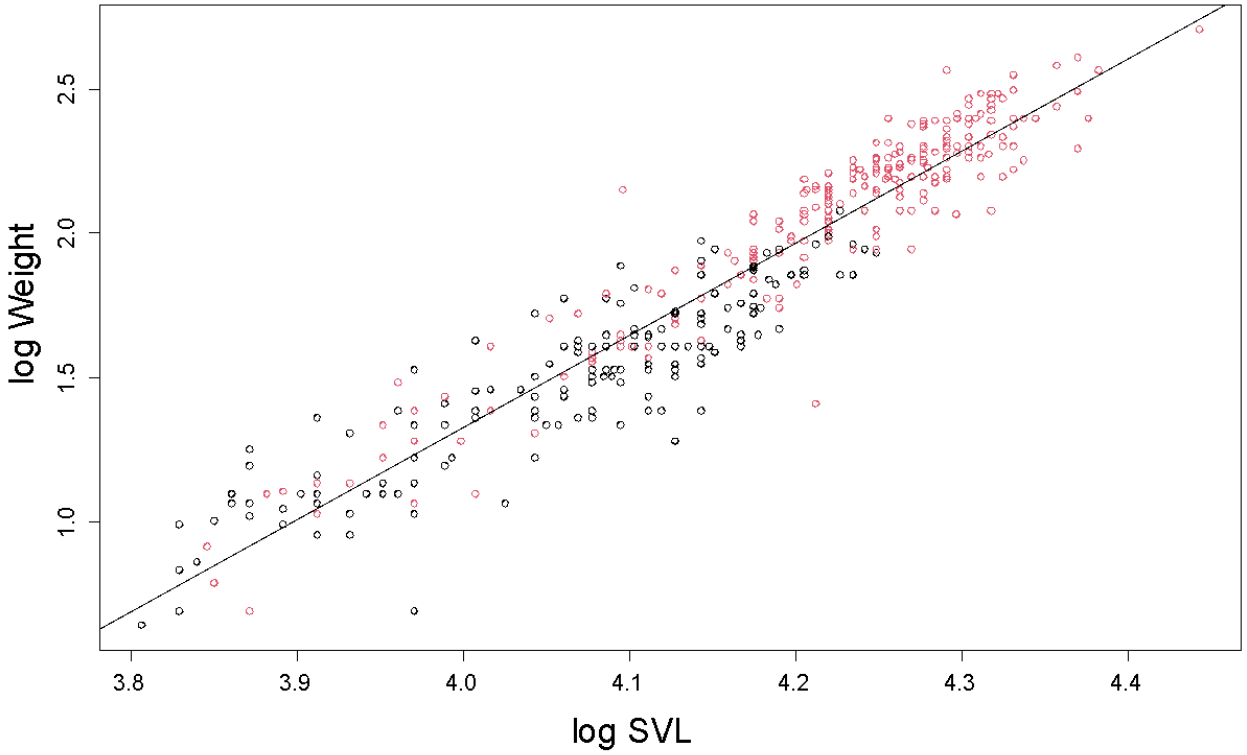

Figure S3. Regression plot of weight on SVL. The residuals of this model were used as body condition index. Male: red dots, female: black dots.

Table S1. Sample size of *Podarcis siculus* populations. Data: SVL = snout-vent length, and GM = geometric morphometrics. The individuals were collected in the seven main islands of the Aeolian archipelago and in the three islets around Panarea. M = males, F = females, TOT = total

Island	SVL_M	SVL_F	SVL_{TOT}	GM_M	GM_F	GM_{TOT}
Alicudi	33	22	55	22	13	35
Filicudi	27	21	48	21	8	29
Salina	34	21	55	26	16	42
Panarea	35	24	59	33	24	57
Stromboli	35	24	59	21	10	31
Lipari	26	21	47	21	11	32
Vulcano	32	24	56	29	18	47
Islet	SVL_M	SVL_F	SVL_{TOT}	GM_M	GM_F	GM_{TOT}
Bottaro	5	2	7	5	2	7
Lisca Bianca	6	6	12	6	6	12
Basiluzzo	6	4	10	6	4	10
TOT	239	169	408	190	112	302

Table S2. Candidate models assessing the effect of environmental predictors on phenotypic differences between females and males at different scales: **a)** individual, and **b)** island. The dependent variables of models are: snout-vent length (SVL); head shape; body size dimorphism, and head shape dimorphism. Models are ranked according to their AICc values. ΔAICc = difference between the AICc of a model and the best AICc; *w* = Akaike's weight of the model; R^2_{M} and R^2_{C} marginal and conditional R^2 respectively. The sign of the relationship is in parentheses. BCI = body condition index, NDVI = Normalized Difference Vegetation Index, Sex = sex of the lizards, Temp = land surface temperature.

a) Individual-level analysis

Dependent	**Predictors**	**AICc**	**Δ AICc**	***w***	R^2_{M}	R^2_{C}
SVL	BCI (-), NDVI (+), Sex, Sex*BCI, Sex*NDVI	-797.44	-	>0.99	0.51	0.55
	BCI (-), NDVI (+), Temp (-), Sex, Sex*BCI, Sex*NDVI, Sex*Temp	-793.32	4.12	-	-	-
	BCI (-), Sex*BCI	-774.57	22.87	≤0.02	-	-
	BCI (-), Temp (-), Sex, Sex*BCI, Sex*Temp	-772.13	25.31	-	-	-
	NDVI (+), Sex, Sex*NDVI	-693.55	103.90	≤0.02	-	-
	NDVI (+), Temp (-), Sex, Sex*NDVI, Sex*Temp	-692.47	104.97	-	-	-
	Sex	-675.51	121.94	-	-	-
	Temp (-), Sex, Sex*Temp	-675.24	122.21	-	-	-
Head shape	BCI (-), NDVI (+), Sex, Sex*BCI, Sex*NDVI	-1618.57	-	0.36	0.44	0.49
	BCI (-), Sex, Sex*BCI	-1618.40	0.17	0.33	0.42	0.49
	BCI (-), NDVI (+), Temp (+), Sex, Sex*BCI, Sex*NDVI, Sex*Temp	-1618.39	0.18	-	-	-
	NDVI (+), Temp (+), Sex, Sex*NDVI, Sex*Temp	-1617.44	1.13	0.20	0.44	0.48
	BCI (-), Temp (+), Sex, Sex*BCI, Sex*Temp	-1616.82	1.75	-	-	-
	NDVI (+), Sex, Sex*NDVI	-1616.21	2.36	≤0.02	-	-
	Sex	-1615.75	2.82	-	-	-
	Temp (+), Sex, Sex*Temp	-1615.36	3.21	-	-	-

b) Island-level analysis

Dependent	**Predictors**	**AICc**	**Δ AICc**	***w***	R^2	R^2_{C}
Body size dimorphism	BCI (+), NDVI (+)	-30.9	-	0.45	0.68	-
	BCI (+)	-30.8	0.1	0.43	0.48	-
	NDVI (+)	-28.2	2.7	0.12	0.34	-
	-	-27.89	2.99	-	-	-
	Temp (+)	-26.49	4.38	-	-	-
	BCI (+), Temp (+)	-26.19	4.68	-	-	-
	NDVI (+), Temp (+)	-22.98	7.89	-	-	-
	BCI (+), NDVI (+), Temp (+)	-21.90	8.97	-	-	-
Head shape dimorphism	BCI (+)	-67.60	-	>0.99	0.43	-
	-	-65.81	1.79	-	-	-
	Temp (+)	-65.04	2.57	-	-	-
	BCI (+), Temp (+)	-63.78	3.83	-	-	-
	NDVI (+)	-62.71	4.89	-	-	-
	BCI (+), NDVI (+)	-62.06	5.54	-	-	-
	NDVI (+), Temp (+)	-59.05	8.55	-	-	-
	BCI (+), NDVI (+), Temp (+)	-54.78	12.82	-	-	-

Table S3. The best-AICc models after removing three islets (Bottaro, Basiluzzo, Lisca Bianca) showed smaller sample size than larger islands, at different scales: **a)** individual, and **b)** island**.** The dependent variables of models are: snout-vent length (SVL); head shape; body size dimorphism, and head shape dimorphism. BCI = body condition index, NDVI = Normalized Difference Vegetation Index, Sex = sex of the lizards.

a) Individual scale	**Sex*BCI**			**Sex*NDVI**			**Sex**		
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
SVL	1, 378.24	111.659	<0.001	1, 373.9	3.493	0.062	1, 373.81	5.89	0.016
Head shape	1, 267.17	4.393	0.037	1, 265.68	1.579	0.21	1, 265.77	4.565	0.034

b) Island scale	**BCI**			**NDVI**		
	df	**F**	***p***	**df**	**F**	***p***
Body size SD	1, 4	7.199	0.055	1, 4	6.257	0.067
Head shape SD	1, 5	11.772	0.019	-	-	-

Table S4. The best-AICc models after replacing ecosystem productivity (NDVI = Normalized Difference Vegetation) with island area, at different scales: **a)** individual, and **b)** island**.** The dependent variables of models are: snout-vent length (SVL); head shape; body size dimorphism, and head shape dimorphism. BCI = body condition index, area = island area, Sex = sex of the lizards.

A) Individual scale	**Sex*BCI**			**Sex*Area**			**Sex**		
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
SVL	1, 406.35	117.118	<0.001	1, 399.17	0.808	0.369	1, 398.68	270.238	<0.001
Head shape	1, 295.678	4.272	0.04	1, 293.232	1.795	0.181	1, 293.172	240.184	<0.001

B) Island scale	**BCI**			**Area**		
	df	**F**	***p***	**df**	**F**	***p***
Body size SD	1, 7	12.843	0.009	1, 7	5.227	0.056
Head shape SD	1, 7	11.772	0.019	-	-	-

CHAPTER 4 | PATTERNS OF PERFORMANCE VARIATION BETWEEN ANIMAL HYBRIDS AND THEIR PARENTS: A META-ANALYSIS

Martina Muraro*, Mattia Falaschi and Gentile Francesco Ficetola

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Abstract

Hybridization is a widespread phenomenon in animals, and hybrid heterosis / breakdown could be key processes determining the evolutionary dynamics of hybrids. Indeed, hybrids are not consistently disadvantaged compared to the parental lineages, as was historically assumed. Multiple processes could lead to performance differences between parental lineages and their hybrids. Despite many studies evaluated the performance of hybrids, a quantitative synthesis is required to assess the general pattern. Here we used meta-analytic and meta-regression approaches to quantify the fitness differences between parental lineages and their hybrids, and to identify possible processes that could lead to these differences. Specifically, we tested biological and methodological parameters that could determine differences in performance between hybrids and parental lineages. Hybrid performance was extremely variable across studies, being often significantly higher or lower compared to the mean performance of their parents. Nevertheless, the averaged hybrid performance was similar to the fitness of parental lineages, with differences across studies related to how performance was assessed. Genetic divergence between parental lineages, and the approach used to identify hybrids were the parameters most strongly related to variation in hybrid performance. Performance was lower for hybrids between distantly related lineages. Furthermore, study settings and the use of imprecise approaches for hybrid identification (e.g. morphology-based) can bias assessments of performance. Studies performed on wild populations and using genetic approaches for hybrid identification detected more often a decreased hybrid performance, compared to laboratory studies. We highlight the importance of appropriate settings for a realistic understanding of the evolutionary impacts of hybridization.

Introduction

Historically, animal hybridization was often considered of limited importance because uncommon or restricted to sympatric areas where distinct genetics lineages come into secondary contact (Schwenk et al., 2008, Duckworth & Semenov 2017). Nevertheless, genomic evidences are increasingly showing that hybridization and introgression are widespread phenomena that can play a crucial role in speciation, extinction, and adaptive radiations (Sakai et al., 2001, Seehausen 2004, Mallet 2005, Capblancq et al., 2015, Bay & Ruegg 2017, Kagawa &Takimoto 2018). Hybridization is now considered to be pervasive in animals, with major consequences on evolutionary processes (Ficetola & Stöck 2016, Atsumi et al., 2021, Thompson et al., 2021). It was often hypothesized that hybrids are generally disadvantaged compared to parental lineages (Barton & Hewitt 1985). However, the growing evidence of a major role of hybridization for evolutionary outcomes suggests that hybrids are not uniformly disadvantaged compared to parents (Arnold and Hodges 1995). In fact, hybridization may lead to either decreased, increased, or similar fitness compared to parental lineages (Lohr & Haag 2015, Atsumi et al., 2021). For example, hybridization can lead to an increase in F1 fitness compared to the fitness of parents and F2, termed hybrid vigor or heterosis (Chen 2013, Chan et al. 2018), while hybrid breakdown can occur when hybridization results in a decrease in fitness from F1 to F2 or backcross generation, because of genetic incompatibility or for limited performance of hybrids in the environment (Allendorf et al., 2001, Barreto et al., 2015). Overall, the performance of hybrids compared to their parents can show multiple patterns, with multiple studies showing heterogeneous outcomes (e.g.Casas et al. 2012, Barreto et al. 2015, Walsh et al. 2016, Gélin et al. 2019).

There are several processes that can potentially determine the differences observed in performance between hybrids and their parental lineages, including true biological effects, and processes related to the methods used in studies. Among the biological effects, 1) the genetic distance between parental lineages probably plays a key role in hybrid performance (Coyne and Orr

1998, Stelkens and Seehausen 2009, Atsumi et al. 2021, Coughlan et al. 2021). An increase in genetic distance could increase heterosis, but too large genetic distances determine genetic incompatibility and can cause hybrid breakdown (Dobzhansky 1937, Matute et al. 2010). Thus, the hybrid performance is expected to be highest when the genetic distance between parents is neither too small nor too large (Wei and Zhang 2018). However, this issue is still largely uncertain, and a recent meta-analysis suggest that genetic divergence between parental species increases the probability of hybrids to have smaller traits size than both parents (Atsumi et al. 2021). 2) Different generations of a single cross can show different performance (Rhode and Cruzan 2005). For instance, it is possible that first generation hybrids are characterized by heterozygote advantage, while later generations could suffer of hybrid breakdown (Dobzhansky 1970, Burton 1990, Ellison et al. 2008, Šimková et al. 2021). Nevertheless, there are many factors that determine performance differences among the different generations of the same cross. 3) The hybridization between native and invasive species can be a major mechanism in accelerating the speed of biological invasions (Huxel 1999, Dlugosch et al., 2015, Grabenstein & Taylor 2018), thus, it is possible that in systems involving successful invaders, hybridization with native lineage could lead to offspring with better performance (Huxel 1999).

In addition to the biological effects, the methods used in studies assessing hybrid performance can influence the results of analyses. 4) Even though laboratory and field studies should ideally lead to consistent results (Mathis et al., 2003, Hillebrand & Gurevitch 2014), some studies revealed poor agreement between field and laboratory researches (e.g. Bezemer & Mills 2003, Joron & Brakefield 2003). This discordance could be caused by multiple processes, including differences of ecological context and to stressful condition in the laboratory (Ficetola and De Bernardi 2005). 5) Hybrids are often identified through characteristic morphological traits, but molecular analysis can better detect hybrid and introgression avoiding classification errors (e.g. Vanhaecke et al., 2012). 6) Hybrid performance can be assessed on the basis of a variety of traits (e.g. breeding success, morphology, behavior), and the same hybrid can have poorer, better, or similar performance compared to parental parents, depending on the considered traits. For example, hybrid partridges can lay larger clutches than their parental lineages, but also suffer a higher predation rate (Casas et al. 2012). Broad-scale analyses, assessing performance variation across multiple systems are needed to evaluate how these processes can influence the observed variation of performance between hybrids and their parental lineages.

In this study, we used meta-analytic and meta-regression approaches (Arnqvist and Wooster 1995, Nakagawa and Santos 2012) to evaluate differences in performance between hybrids and their parental lineages in animals, and investigate some of the possible predictors of these patterns. In fact, there are many studies using experimental data on hybrid performance relative to specific cross between populations or species in animals, but literature syntheses are required to identify the general effects of these factors. The meta-analytic approach allows us to gather several independent studies to obtain general trends and conclusions on the animal hybrid performance. The aim of our study was to provide a quantitative synthesis on the hybrid performance compared to parental lineages, in order to identify how the different processes can determine variation across systems and studies. Specifically, we tested if differences in performance between hybrids and parental lineages are related to three potential biological processes: 1) genetic distance between parental lineages, 2) hybrid generations (i.e. F_1 vs. backcrosses or other crosses), 3) effects of invasive species, and to three potential processes related to study design and approaches: 4) lab vs. field studies, 5) hybrid identification method 6) traits considered for analyses.

Methods

Literature search and selection criteria

To obtain journal articles reporting hybrid performance, we performed a systematic literature research in Web of Science database using the key words “hybrid” and “fitness” with no restriction on publication year. Even though the term “fitness” refers to the breeding success of individuals, many ecological and evolutionary studies use this term when assessing differences for a very broad range of performance measures. Because these terms have a broad meaning and can be used in different contexts, we refined the research selecting Web of Science categories particularly relevant for evolutionary or ecological studies: ecology, evolutionary biology, genetics heredity, computer sciences interdisciplinary applications, multidisciplinary sciences, biology, zoology, entomology, marine freshwater biology, environmental sciences, fisheries, biodiversity conservation, forestry, behavioural sciences, ornithology, oceanography, water resources, physiology, reproductive biology, developmental biology. The literature research was performed on May 4th 2020 and produced 1595 journal articles which were screened in several steps (Fig. 1a). We examined each article to verify eligibility to the selection criteria for inclusion in the meta-analysis (Fig. 1b). The following criteria for data inclusion were adopted:

1. Only studies focusing on animal hybrids were included
2. We selected studies that report at least one quantitative comparison between one hybrid and one parental population, obtained with a statistical analysis that can be converted into an effect size. If no effect sizes were available, but raw data were obtainable, we extracted data directly from text, plots, or tables (average ± the amount of variation or dispersion, and sample size) and subsequently converted into an effect size. We used the ImageJ software to extract data from the plots (Schindelin et al. 2015).
3. We only used comparisons of traits representing hybrid performance. Morphological and behavioural characters were considered when they could be interpreted in terms of performance (e.g. differences in body condition, growth rate, foraging ability).
4. We exclude studies about parasitism, which were analysed in a dedicated review (Theodosopoulos et al. 2019).

The comparison between the performance of hybrids can be performed using different approaches, each of which has its own merit and limitations. In the mid-parent approach, the performance of hybrids is compared with the average value of parental lineages (Atsumi et al. 2021, Thompson et al. 2021). This approach is used to test the null-hypothesis that hybrids have intermediate performance compared to the parental species. The mid-parent approach maximizes the probability of detecting additive or non-additive genetic effects determining whether hybrid traits are intermediate, biased toward one parent (dominance) or a novelty compared to those of their parents. Conversely, other studies compared hybrid performance with the performance of parental species separately (either with the performance of each parent, either with only one parent) (hereafter: separate-parent approach; e.g.: Debes et al. 2013; Duckworth and Semenov 2017; Good et al. 2000; Liss et al. 2016). This approach does not test explicitly whether hybrid performance mismatches or matches with the mid-value of parental lineages, but has larger power at detecting general patterns of variation in hybrid performance compared to parental one and the main drivers of these patterns (e.g. Kleindorfer et al. 2014; Walsh et al. 2018). Furthermore, the majority of studies retrieved by the literature analysis used the separate-parent approach (see results), thus considering this approach allowed to include a larger number of tests in the meta-analysis, increasing statistical power.

For each study, if possible we extracted the effect size of the difference in performance between the hybrid and the mid-point between parental lineages (mid-parent approach), and of the difference in performance between the hybrid and each parental lineage (separate-parent approach).

We also extracted information about six biological and methodological parameters that could determine differences in performance between hybrids and parental populations from the collected journal articles. 1) To analyse genetic distance between parental lineages, we used two partially overlapping approaches. First, we discriminated between intra-specific and inter-specific crosses. Furthermore, to estimate the genetic distance between parental lineages we also used TIMETREE, which calculates the divergence time for a pair of taxa (http://www.timetree.org)

(Kumar et al., 2017). Unfortunately, TIMETREE information was only available for a limited subset of species, and was unavailable for intraspecific crosses. 2) As hybrid generations, we considered the generations belonging to: F1, first generation of backcross (BC), and hybrid above F1 and BC1 (e.g. F2, BC2, hereafter F>1). In some studies, F>1 included multiple generations that were pooled as a single type of hybrid by the authors. 3) We determined whether crosses occurred between natives or between one native and one invasive population. As for methodological parameters, we distinguished between: 4) field or laboratory study (the setting in which hybrid were measured), 5) genetic and morphological hybrid identification and 6) trait category considered for comparisons. Many different traits were used for the comparisons between hybrid and parental lineages in studies, and thus traits were pooled in larger traits category: fitness (e.g. clutch size, survival, development success), morphological (e.g., fluctuating asymmetry, wing length, fin height), and behavioural (e.g. total duration of suckling, foraging technique, arrival rank for reproductive season).

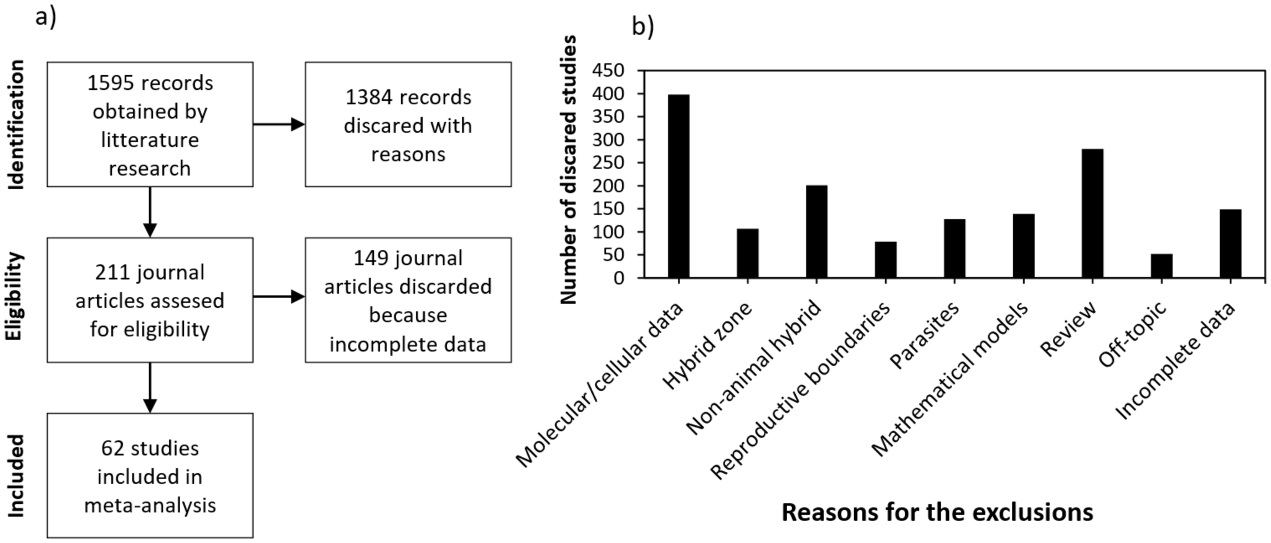

Fig. 1 Excluded studies. **a** Studies inclusion and exclusion steps, **b** Number of discarded studies and reasons for the exclusion

Extraction of effect size measures

For each comparison between hybrid and parental populations, we calculated the effect size as the difference in performance between hybrid and parental population. As effect size we used Fisher's *z*; the more the Fisher's *z* value was greater or lesser than zero, the greater the extent of differences between hybrid and parental lineages. Comparisons where hybrids showed lower performance compared to the parental lineages were coded as negative Fisher's *z* value, and vice-versa. All the analyses were repeated considering both the mid-parent and the separate-parent comparisons between hybrids and parental species.

The mid-parent value was calculated as the average performance of the two parental lineages; furthermore, we calculated their combined standard deviations. We transformed the obtained mean and standard deviation in Fisher's *z* and its variance (*z*-var) and we extracted one effect size for each comparison between hybrid and mid-parent value. For separate-parent approach, we transformed the statistics values reported in studies (F, t, R^2, χ^2, means, and standard deviation of populations) in Fisher's *z* and its variance (*z*-var) using the compute.es package in R (Del Re 2013). When the statistic reported was *Z*-value, we directly calculated Fisher's *z* and its variance as: Fisher's $z = Z/\sqrt{(n-3)}$ and $z\text{-var} = 1/(n-3)$ (Hartung et al., 2008). For one study, we converted *d*-value to Pearson's correlation coefficient *r* and then we extracted the Fisher's *z* and its variance using the compute.es package. For studies that did not report test statistics, we calculated the effect size from *P*-values. In many cases, one hybrid group was compared to two parental lineages. In these cases, we extracted one effect size for each comparison. Different comparison between the same hybrid group and the two parental lineages were then identified by the same identity (hereafter: hybrid ID).

Finally, we recorded whether each comparison showed statistical differences between hybrids and parental lineages.

Statistical analyses

For each comparison approach (mid-parent and separate-parent), we calculated the Rosenberg's fail-safe number to evaluate file-drawer bias. Rosenberg's fail-safe number establishes the studies that should be added to the meta-analysis to make the difference between observed and expected no longer significant and it estimates the strength of the results of sampling bias meta-analysis. We used Egger's regression test and Begg's rank test to evaluate the occurrence of publication bias in the dataset as procedure to implement the funnel plot (Begg and Mazumdar 1994, Egger et al. 1997). Finally, we quantified heterogeneity using I^2 (Nakagawa & Santos 2012).

Factors potentially affecting the significance of comparisons

We used a χ^2 test to assess if the studies detected significant differences between hybrids and the mid-parent values more frequent than expected by chance. Subsequently, we ran two generalized linear mixed-effects models (GLMM) to analyse the factors related to the frequency of significant comparisons. First, we evaluated if the sign of the comparisons was different between significant and non-significant comparisons. The positive sign represented a better hybrid performance compared to the average of parental groups, while the negative sign represents the opposite. We thus fitted a binomial GLMM to assess if significant positive results were more frequent than negative ones, by including taxonomic group (genus), study identity and hybrid ID as random factors. A second binominal GLMM assessed whether the frequency of significant effect sizes was related to: relationships between parents, hybrid generations, alien populations in parental cross, laboratory or field study, hybrid identification method, and trait category as fixed factors. Also in this case, we included taxonomic genus, identity of the study and hybrid ID as random factors. Binomial GLMMs were run using the lme4 package in R; we used a likelihood-ratio test to assess the significance of fixed factors.

Meta-analysis

We implemented meta-analysis and meta-regression approaches in a Bayesian framework using generalized linear mixed models (MCMCglmm package in R) (Hadfield 2010, Nakagawa & Santos 2012). We fitted different mixed models with different aims. All MCMC models were run with 60,000 iterations, discarding the first 10,000 iterations as a burn-in and with a thinning interval of 24. We used the *mev* argument in the MCMCglmm function to consider 1/*z*-variance as a weight for the records (Hadfield & Nakagawa 2010).

Overall meta-analysis: model of the mean

For each comparison approach, first, in order to analyse the mean performance value of hybrid relative to their parents, we ran a model of the mean considering the effect sizes of all different comparisons of collected studies. This analysis allowed us to assess whether the average fitness of hybrids was higher or lower relative to their parents. The effect sizes of the comparisons (Fisher's *z*) were used as dependent variables, no fixed effect was included, and three random factors were added: taxonomic genus, identity of the study, and hybrid ID.

Average performance for different categories

In order to discriminate factors that may determine differences in hybrid performance relative to their parents, we categorized comparisons by different author methods, hybrid features, and parental cross characteristics. The same categories were used for both mid-parent and separate- parent approaches. We performed several models of the mean to test the mean value of the effect sizes in different subsets of data. The following subsets were considered: 1) relationships between parents (intraspecific vs. interspecific crosses, and genetic distance between parental species), 2) hybrid generations (F1, F>1, and backcrosses), 3) presence of native vs. invasive populations in parental cross, 4) laboratory or field study, 5) hybrid identification method (genetics vs. morphology), 6) trait category used for comparisons. In addition, we run a separate model for each taxonomic group (class) of parental lineages for which we obtained effect sizes from at least three different genera. For hybrid identification, laboratory crosses between morphologically identified parents are expected to be more accurate than the morphological of hybrids, even though without genetic data on parental lineages collected in the field also laboratory crosses could be imprecise, for example because of an unknown amount of introgression. Therefore, we re-run the analysis of hybrid identification method, by splitting morphological identification in two different categories: controlled crosses conducted in laboratory without genetic identification vs. morphological recognition of hybrids. All mixed-effects models included only the intercept and three random factors: taxonomic genus, identity of the study and hybrid ID.

Meta-regression for divergence between parental lineages

To visualize how the divergence between parental lineages can affect the hybrid performance, we ran two meta-regressions for the mid-parent approach, and four meta-regressions for the separate-parent approach. In all the models, hybrid performance (Fisher’s *z*) was the dependent variable. In the two models of mid-parent approach we used as predictors the relationships between parents (expressed as intra or inter-specific cross), and the genetic distance between parents obtained with TIMETREE, respectively. For the separate-parent approach, in two models we used relationships between parents (intra or inter-specific cross) as predictor. In the first model we considered all the effect sizes of the comparisons as dependent variable and in the second one we only considered the effect sizes of genetically identified hybrids as dependent variable. These models were also re-run after excluding four articles where hybrids were compared with only one parent; results were nearly identical to the analysis including all the studies (Tab. S1a). These two analyses were then repeated considering the genetic distance between parents obtained with TIMETREE as predictor; this analysis was limited to interspecific crosses for which divergence time was available on the basis of TIMETREE data. In the third model, we used all the effect sizes as dependent variable and in the fourth model we considered the effect sizes of only genetically identified hybrids as dependent variable. Due to small sample size, for the mid-parent approach it was not possible analysing separately the hybrids identified with genetic tools.

Meta-regression: factors potentially affecting hybrid performance

To determine the factors related to the variation of hybrid performance compared to parents, we run two multivariable generalized linear mixed models one for each comparison approach. In these analyses, we used relationships between parents (intra or inter-specific) as an estimate of the divergence between parents, inasmuch there was available data for all the comparisons included. Contrary, TIMETREE database had a limited sample size for the divergence times. We used as dependent variable all the effect sizes of the comparisons and six parameters as fixed effects: relationships between parents, hybrid generations, invasive species cross, field vs lab studies, hybrid identification method, trait category used for comparisons; taxonomic genus, identity of the study and hybrid ID were added as random factors.

Moreover, we run a third model using separate-parent approach considering only the effect sizes obtained from studies that used genetic hybrid identification methods. We used five parameters as fixed effects: relationships between parents, hybrid generations, invasive species cross, lab vs field studies and trait category used for comparisons. Taxonomic genus, identity of the study and hybrid ID were added as random factors. We also we re-run the analysis excluding four articles in which hybrids were compared with only one parent, and obtained identical results (Tab. S1b).

Results

We retained 33 studies (Appendix S1) assessing hybrid performance with comparisons between hybrid and mid-parent value. These studies included 357 comparisons and 32 different animal species belonging to 9 taxonomic classes. For the separate-parent value approach, we retained 60 articles including 982 comparisons between hybrid and each parental lineage separately (Appendix S1). Studies focused on 94 different animal species belonging to 11 taxonomic classes. Overall, 66.7% of the collected studies compared hybrid to each parent separately, 12% compared hybrid with mid-parent value, 22% used both methods and 7% compared hybrid with only one parent.

Frequency of significant comparisons

Among the 357 comparisons between hybrids and the mean performance value of both parental populations, in 125 cases hybrid showed performance significantly different from parents ($P < 0.05$), and in 232 cases there were no significant differences ($P > 0.05$). The frequency of significant comparisons was much greater than expected under randomness ($\chi^2 = 67.7$, df = 1, p << 0.001; number of significant comparisons expected under randomness: 17.85). The frequency of studies showing a positive significant effect was similar to the frequency of studies showing a negative significant effect ($\chi^2 = 0.971$, df = 1, p = 0.615). For the mid-parent approach, both Egger's regression test ($b = -0.022$, 95% CI = -0.094/-0.05) and Begg's rank test (Kendall's τ coefficient = 0.0123, p = 0.0014) suggested some publication bias. Furthermore, we detected a strong heterogeneity of performance differences between hybrid and their mid-parent value across studies (total $I^2 = 96.91\%$). Nevertheless, the file drawer analysis suggested that 3622 unpublished, non-significant comparisons between parental and hybrids would be required to reduce the frequency of significant relationships to values similar to what is expected under randomness.

For the separate-parent approach, in 465 out of 982 comparisons hybrids showed performance significantly different from a parental species, while in 517 cases the authors did not detect significant differences. For the separate-parent approach, neither Egger's regression test (*b* = -0.037, 95% CI = -0.108/-0.034) nor Begg's rank test (Kendall's τ coefficient = -0.027, p = 0.241) suggested publication bias. Also in this case, we found strong heterogeneity across studies (total I^2 = 98.13%).

Factors potentially affecting the significance of comparisons

Within the 125 significant comparisons between hybrid and mid-parent performance, in 48 cases hybrids showed a lower performance, while in 77 comparisons hybrids showed better performance than parental lineages. Hybrids originating from intraspecific comparisons were more frequently different from the mid-parent value, compared to hybrids originating from interspecific comparisons (binomial generalized linear mixed model: χ^2 = 3.86, df = 1, p = 0.049). The frequency of significant studies was similar between studies considering: different hybrid generations (χ^2 = 3.283, df = 2, p = 0.194), alien populations in parental cross (mid-parent: χ^2 = 2.019, df = 1, p = 0.156), hybrid identification method (mid-parent: χ^2 = 0.886, df = 1, p = 0.347) and trait category (mid-parent: χ^2 = 3.49, df = 2, p = 0.174). Finally, the mid- parent approach detected more often significant differences between hybrids and parental lineages in field studies, compared to laboratory studies (χ^2 = 6.957, df = 1, p = 0.008).

Average difference in performance between hybrid and parental lineages

Using the mid-parent approach, the meta-analytical models calculating the average effect size across all the studies (model of the mean) suggested that the average performance of hybrids was slightly higher than the performance of the respective parental lineages, while the separate-parent approach suggested a slightly lower value. However, for both approaches the credible intervals overlapped zero, indicating that the average differences in performance were extremely limited (mid-parent approach: *z* = 0.027, 95% CI= -0.05/0.113; separate-parent approach: mean *z* = -0.052, 95% CI= -0.136/0.031).

Meta-analysis for subsets of comparisons

Using the mid-parent approach, we did not detect significant differences between the considered subsets of data. The effect size of performance differences between hybrids and mid-parent performance overlapped zero for all the categories: intra-specific vs inter-specific parental lineage crosses, all the different hybrid generations, crosses involving only-native vs non-native parental lineages, all the systematic classes of parental lineages, field and laboratory studies, morphological vs. genetic hybrid identification methods, and the species traits measured for the comparisons (Tab S2).

When we re-ran the meta-analytic models for different subset of our data using separate-parent approach, we obtained, considering the relationships between parents, that the credible interval of the effect size of performance differences between hybrids and parental lineages overlapped zero for both intra-specific and for inter-specific crosses (Fig. 2a). When considering the hybrid generations, the credible interval of effect size overlapped zero for F1 crosses and for backcrosses, while was slightly more negative for crosses of subsequent generations (F>1) (mean z = -0.125, 95% CI = -0.209/-0.042; Fig. 2b). Moreover, the credible interval of the effect size overlapped zero for: both crossing involving only native and crosses between non-native parental lineages (Fig. 2c), all the systematic classes of parental lineages and (Fig. 2d) field and laboratory studies (Fig. 2e). The mean effect size was significantly smaller than zero for hybrids identified through genetic approaches (mean z = -0.116, 95% CI = -0.219/-0.015), while for hybrids identified through morphology the mean effect overlapped zero (Fig. 2f). The credible interval overlapped zero also for hybrids generated from controlled crosses conducted in laboratory without genetic identification of parental lineages which, in the previous analysis, were attributed to the “identified through morphology” group (Fig. S2). Finally, average effect size was not affected by methodological differences, as the credible interval of the effect size overlapped zero for studies considering fitness, morphological, and behavioural traits (Fig. 2g). The frequencies of the subset categories used in the collected studies and the means with 95% confidence interval of the subsets effect size are available in Online Resource (Tab. S3, Fig. S1).

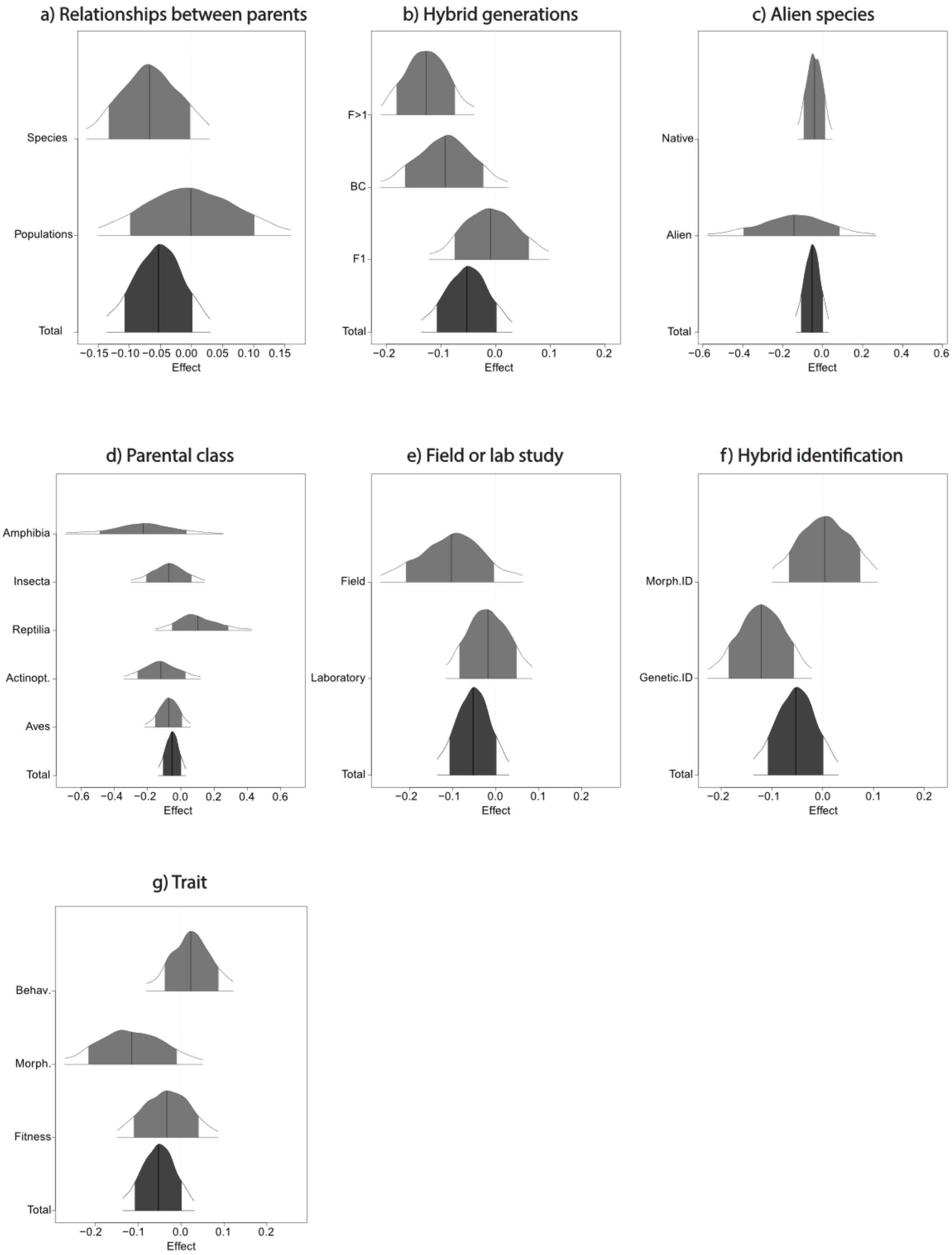

Fig. 2 Means of the effect sizes, as the difference in performance between hybrid and each parental population, in different subset of data. Density plots showing the means of the effect sizes for: **a** relationship between parents, **b** hybrid generations, **c** presence of an invasive population in parental cross, **d** parent's class, **e** laboratory vs field studies, **f** hybrid identification method, **g** trait categories used for the comparisons

Do divergence between parental lineage affects performance? Meta-regression

When considering all the effect sizes, there were no significant differences between intraspecific or interspecific hybrids using both comparison approaches (mid-parent approach: mean z = 0.03, 95% CI = -0.144/ 0.205; separate-parent approach: mean z = -0.054, 95% CI = -0.247/ 0.121, Tab. S4). However, when we only considered hybrids identified through genetical approaches (409 comparisons), hybrids from interspecific crosses showed a lower performance than intraspecific hybrids (separate-parent approach: mean z = -0.206, 95% CI = -0.395/-0.0195) (Tab. 1 a, b). Conversely, when we used the TIMETREE data to estimate interspecific divergence, we did not detect relationships between the amount of divergence and hybrid performance using both comparison approaches (mid-parent approach: mean z = -0.026, 95% CI = -0.103/-0.044, Tab. S4; separate-parent approach: mean z = 0.002, 95% CI = -0.009/-0.014). Results were consistent considering effect size of hybrids only genetically identified (separate-parent approach: mean z = 0.002, 95% CI = -0.014/0.018) (Tab. 1 c, d).

	Parameters of divergence	**Effect sizes considered**	**Mean z**	**95% CI**
a)	Intraspecific or interspecific	All	-0.054	-0.247/ 0.121
b)	Intraspecific or interspecific	Genetic identification	-0.206	-0.395/-0.0195
c)	TIMETREE data	All	0.002	-0.009/0.014
d)	TIMETREE data	Genetic identification	0.002	-0.014/0.018

Tab.1. Meta-regression models analysing whether divergence between parental lineages affected hybrid performance. **a**) discrimination between intraspecific or interspecific crosses using all the effect sizes, **b**) discrimination between intraspecific or interspecific crosses using effect sizes of hybrids only genetically identified, **c**) TIMETREE data (divergence in time) of parental lineages using all the effect sizes, **d**) TIMETREE data (divergence in time) of parental lineages using effect sizes of hybrids only genetically identified.

Overall assessment of factors potentially affecting hybrid performance

The meta-analysis including all the variables did not detect clear effects of any of the considered factors on hybrid performance comparing hybrid with both mid-parent value (Tab. S5) and each parent value separately (Tab. 2, Fig. 3a). Results were similar when we repeated the analysis only considering effect sizes obtained from studies that used genetic hybrid identification methods (Tab. 3, Fig. 3b).

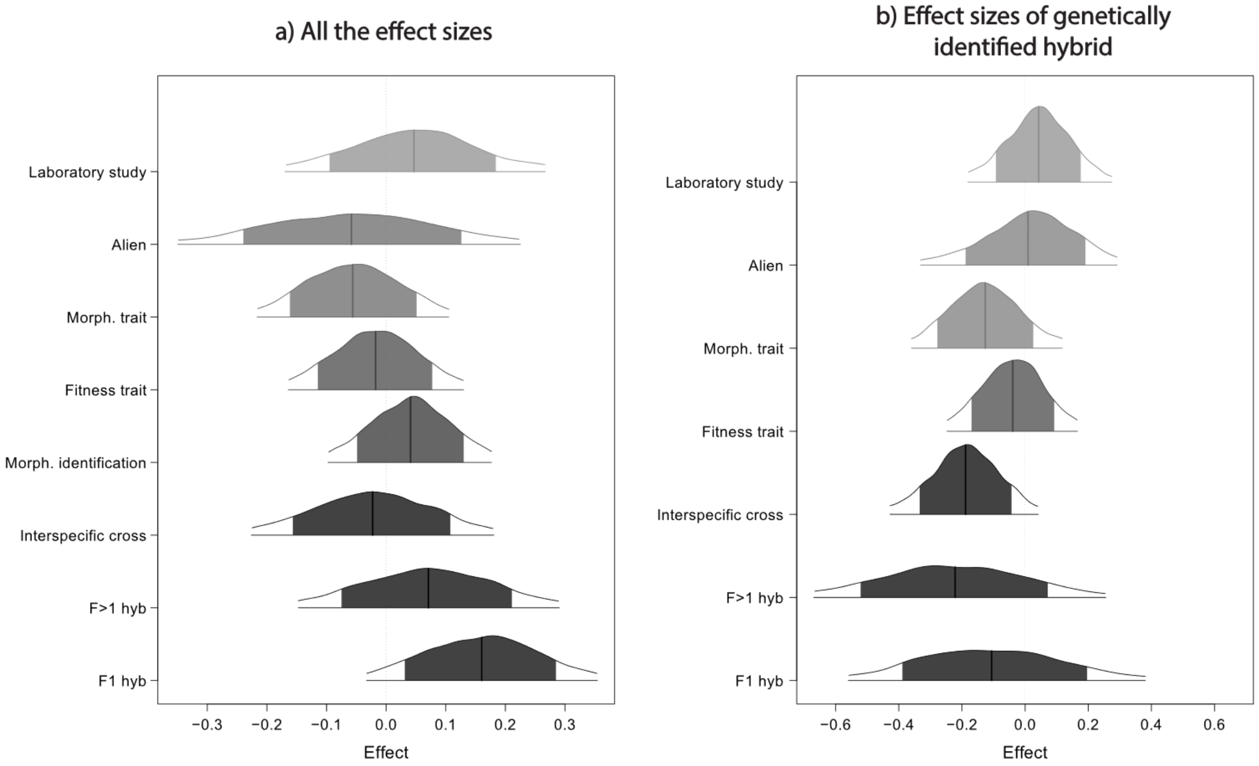

Fig. 3. Overall separate-parent approach meta-regression of factors potentially affecting hybrid performance considering: **a** all the effect sizes, **b** the effect sizes of only genetically identified hybrids

	mean z	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
Intercept	-0.192	-0.543	0.142	0.273
F1 hybrid	0.160	-0.037	0.345	0.110
F>1	0.072	-0.149	0.278	0.502
Interspecific cross	-0.021	-0.229	0.181	0.820
Morph. identification	0.042	-0.089	0.179	0.564
Fitness trait	-0.016	-0.161	0.126	0.831
Morph. Trait	-0.055	-0.227	0.101	0.507
Alien	-0.063	-0.338	0.217	0.681
Laboratory study	0.047	-0.160	0.263	0.656

Tab.2. Meta-regression model analysing the factors that potentially affected hybrid performance.

	mean z	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
Intercept	0.182	-0.327	0.714	0.463
F1 hybrid	-0. 087	-0.518	0.384	0.674
F>1	-0.201	-0.642	0.257	0.378
Interspecific cross	-0.187	-0.440	0.032	0.102
Fitness trait	-0.045	-0.236	0.171	0.654
Morph. Trait	-0.123	-0.353	0.111	0.296
Alien	-0.004	-0.360	0.308	0.962
Laboratory study	0.040	-0.186	0.268	0.715

Tab. 3. Meta-regression model analysing the factors that potentially affected hybrid performance, using effect sizes for genetically identified hybrid only.

Discussion

Despite long interest on hybrids, it remains difficult to identify a general trend for hybrid performance. By synthesizing 982 performance comparisons between hybrid and their parents, our meta-analysis provided insights on the role of several biological and methodological processes that could affect the outcome performance assessments. A large number of studies observed significant differences in performance between hybrids and parental lineages, and the variation in performance clearly was in different directions, with a comparable number of studies showing higher or lower performance, compared to parental lineages.

Two main approaches have been used to compare the fitness between hybrids and parental lineages, each of which can help identifying different facets of fitness variation during the hybridization process. Some studies have compared hybrid traits with the mid-parent value to investigate additive or nonadditive genetic effects of hybridization. However, this approach was only used by the minority of studies, focusing on the match or mismatch between hybrid performance and the intermediate features of parents (Atsumi et al. 2021, Thompson et al. 2021). The separate-parent approach was more common, because it can easily allow testing different patterns of hybrid performance, has less assumptions on the performance of hybrids, and does not require having accurate performance of both parental species. The two approaches yielded comparable conclusions, even though the mid-parent approach showed lower mean effect sizes compared to separate-parent approach (Fig. 4). Furthermore, the mid-parent approach allowed to include a lower number of studies than the separate-parent approach, and this reduced the statistical power of meta-analyses.

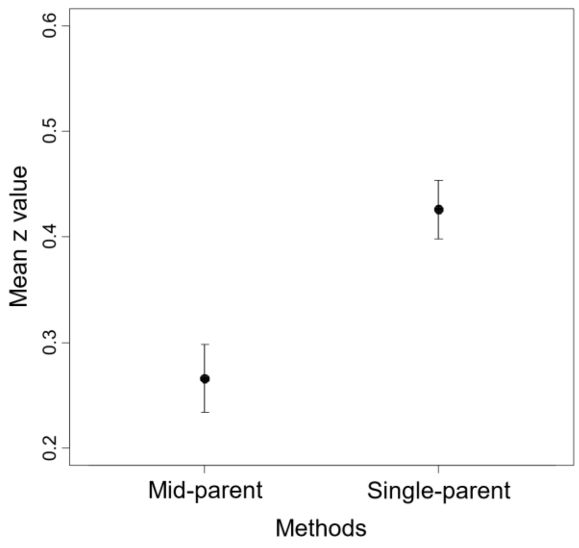

Fig. 4. Mean *z* value of hybrid compared to parental populations using mid-parent and separate-parent approaches; mid-parent: hybrid compared with mid-parent value of performance, separate-parent: hybrid compared with each parental lineage separately

Generally, the average performance of hybrids was slightly lower than the one of their parents, but the differences in performance were extremely small, with strong heterogeneity across studies and approaches. Such heterogeneity is probably related to the very diverse processes that occur in different species, and can range from mortality and stillbirths, low viability, fertility, and survival (Stelkens et al., 2015, Fukui et al., 2018), to hybrid vigour, and adaptive advantages (Abbott et al. 2013, Meier et al. 2019). Such differences are probably linked to intrinsic differences across study systems, for instance to very different genetic architectures of animal species. Interactions between genotype and environment can also play an important role, thus the same system can show different outcomes depending on conditions experienced by individuals (Grant &

Grant 1996, Arnold & Martin 2010). Furthermore, we found limited effect of the considered moderators on performance, and we only found some support for an effect of divergence between parental lineages, with hybrids between different lineages of the same species performing better than hybrid between different species.

Among the biological effects considered, we observed some support that the genetic distance between parents could be a driver of hybrid performance. Indeed, hybrids between distinct species showed lower performance than hybrids between lineages attributed to the same species (Tab 1 a, b). This result might be related to the combined effects of heterosis and hybrid breakdown. Heterosis is often observed in hybrids between genetically close parents, and in some cases determine better fitness of hybrids (Dagilis et al. 2019, Atsumi et al. 2021). Our result aligns with a recent meta-analysis showing that genetically similar parents tend to produce hybrids with larger body size and reduced the phenotypic variability, while genetic distance between parental lineages increased this variability. Indeed, heterosis promotes developmental stability in hybrid between genetically close parental lineages (Atsumi et al. 2021). On the other hand, hybrids between different species often show hybrid breakdown. Hybrids between species can be inviable or sterile because the accumulation of genes that are regularly functional in pure-species, but produce negative epistatic interactions in hybrids. These postzygotic incompatibilities increase rapidly with the divergence between species (Dobzhansky 1937). For instance, in *Drosophila* the amount of genes involved in hybrid breakdown increases with the divergence between two pairs of parental species (Matute et al. 2010). The genetic distance hypothesis would also predict that, for interspecific crossings, crossings involving distantly related species have lower fitness than the ones involving closely related species. However, we did not find evidence of relationships between relatedness (measured on the basis of TIMETREE) (Kumar et al. 2017) and hybrid performance (Tab. 1 c, d). This is partially in contrast with what we observed for the comparison intraspecific vs. interspecific crossings, and can be related to different causes. First, the TIMETREE data had a limited sample size, because genetic distances are not available for all the considered lineages. Furthermore, TIMETREE provides divergence time (in years) for a pair of taxa, but the time of divergence is not necessarily relevant as the same temporal divergence lead to a different genomic outcome depending, for example, on generation time and factors closely dependent on intrinsic characteristics of species (e.g. insects have much faster generations than vertebrates). Unfortunately, information on generation time is too scanty to be tested in this study.

It is known that different biological processes can affect hybrid performance and have been described quite well relative to a specific cross in the literature (e.g: Campbell & Meinke 2010, Casas et al., 2012). However, our meta-analysis did not identify a clear effect on these biological processes on hybrid performance, as only the divergence between parents affect hybrid performance. We found limited differences among hybrid generations, although it is known that F1 can be characterized by heterozygote advantage (Fitzpatrick & Bradley Shaffer 2007). Nevertheless, advanced hybrid generations (F>1) tended to have poorer fitness than e.g. F1 (Fig. 2b). These hybrids mostly represent a mix of different advanced generations (e.g. F2/F10), and under these conditions hybrids could suffer from hybrid breakdown, consequently this generation category showed lower performance than parents (Dobzhansky 1970, Burton 1990). In fact, the main hybrid breakdown is expected after F1 hybrid generation, when heterosis decreases and genetic incompatibilities increase (Dobzhansky 1947). For instance, hybrid breakdown occurs in cichlid fish in F2 generation which shows particularly reduced fitness compare to parental species and F1 hybrids (Stelkens et al., 2015).

Finally, hybrids involving non-native lineages showed a performance similar to the ones only involving native lineages. Hybridization is often described as a major process determining the success of invasive alien species and it could lead to the loss of native populations through genetic pollution (Allendorf et al. 2001, Mooney and Cleland 2001, Falaschi et al. 2020a). For example, Italian Crested Newts, *Triturus carnifex*, were introduced in Western Switzerland, within the range of the native Great Crested Newts, *T. cristatus*. This introduction caused a massive introgression in Great Crested Newts and, sometimes, the total replacement of pure native species (Dufresnes et al. 2016). Hence, hybrids involving non-native lineages could have high performance in some ecological contexts (Ryan et al. 2009). Nevertheless, alien invasive species often show extremely high performance, for example at traits that allow to cope with novel environments or climate changes (Blackburn et al., 2009, Shik & Dussutour 2020, Da Silva et al., 2021). Hence, in several cases the performance of native × introduced hybrid can be lower than the performance of the invasive parental species, even though higher or similar than the native parental line. Unfortunately, this expectation cannot be tested here, as most of native × introduced hybrid studies compared hybrid performance only with the native parental lineages. Finally, we did not detect differences in performance between native × introduced hybrid and native × native hybrid, inasmuch the strong data heterogeneity did not allow the delineation of a general trend.

When we analyzed the processes related to study design and methodology that could lead to different hybrid performance outcomes, we observed some difference between wild and laboratory hybrids. Compared to laboratory, in the wild hybrids showed a higher incidence of lower-performance comparisons. In the last decades, some debate existence on the consistence between the results obtained by field and laboratory studies. Laboratory studies have better control of experimental conditions (e.g. physiological and motivational variables), and limit the interactions with other species or individuals that can affect the outcomes of the experiment (Campbell et al., 2009). However, assays in captivity do not necessarily reflect the conditions in natural habitats, and laboratory environment could induce unpredictable effects and stressful conditions (Joron & Brakefield 2003, Ficetola & De Bernardi 2005, Niemelä & Dingemanse 2014). Conversely, field studies avoid the removal of animals from their natural context and artificial responses of individuals to unnatural stimulations (Fisher et al., 2015, Osborn & Briffa 2017). Nevertheless, field studies can be affected by uncontrollable environmental variation (Campbell et al., 2009), and can have limited replication levels because they are sometimes expensive in terms of money and time, or because of the complexity to tagging, tacking, and monitoring wild animals (Campbell et al. 2009, Fisher et al. 2015). Furthermore, measuring individual performance in the wild is challenging and, without genetic data, it is difficult to ascertain the introgression status of individuals. Some analyses revealed poor agreement between field and laboratory researches (e.g. Bezemer & Mills 2003, Joron & Brakefield 2003), while others suggested that laboratory studies provide a good representation of patterns occurring in the wild (Mathis et al., 2003, Hillebrand & Gurevitch 2014). However, we did not detect clear differences between these study typologies, supporting the idea that well-planned laboratory studies can provide results consistent with what is observed in the wild (e.g.: Herborn et al., 2010). In the context of hybrid performance studies, both lab and field studies have their own advantages, and the selection of the most appropriate approach can be dictated by species-specific technical constraints (e.g. feasibility of studying animals in the lab vs. in the lab), as well as by study aims.

The same hybrid can have lower, upper or similar performance compared to parental lineages based on the trait category considered for comparisons (e.g. breeding success, morphology, behavior…) (e.g. Campbell & Meinke 2010, Casas et al., 2012, Gélin et al., 2019). For instance, Bryden et al., (2004) examined 12 performance traits in Chinook salmon comparing hybrid and parental lineages. Introgressed salmons showed better performance at growth-related traits, but also a poor resistance to pathogens. Although such differences can be easily determined for specific crosses, the meta-analytic approach failed to find for which trait category hybrids are more or less performing compared to parental lineages, probably because of the huge variety of investigated traits across studies or species.

We found differences between studies using genetic vs. non-genetic approaches for the identification of parental lineages and their hybrids. Hybrids and parental lineages showed similar performance if only morphology was used to identify hybrids, while the lower performance of hybrids was evident in studies using genetic identification. In several cases, it is extremely difficult to identify pure or hybrid lineages in absence of genetic data, thus these differences can be related to misidentification that can occur using morphological approaches (Dowling et al. 2015). Phenotypic traits are not always a reliable diagnostic method to recognize different lineages as they can vary strongly depending on the life histories (Vanhaecke et al. 2012). For instance, widely proposed morphological approach do not allow the perfect discrimination between the marsh frog (*Pelophylax ridibundus*) and the hybrid edible frogs (*Pelophylax* kl. *esculentus*), inasmuch several morphological characters greatly overlap between them (Pagano & Joly 1999). Our results highlight the importance of genetic analyses for the correct identification of hybrids and avoid classification errors. Only after we limited our analyses to genetically-identified hybrids, we detected a negative relationship between genetic distance of parental lineages and the performance of hybrids (Table 1b). Thus, hybrid identification through genetic methods provides higher power to any kind of analysis. The growing availability and decreasing cost of genetic markers now enables fast identification of hybrids even in complex situation (Della Croce et al., 2016). Genetic analysis can also detect different rates of introgression in individuals, even when low introgression occurs. In fact, the amount of introgression can elicit different performance of the hybrids, and the extent or the direction of introgression can lead to different hybridization outcomes (Aboim et al. 2010, Payseur 2010). For instance, in some systems the performance of hybrids can decrease at increasing proportion of introgression (Muhlfeld et al. 2009).

In conclusion, hybrid performance can be extremely variable. Hybrids often show significantly different performance compared to their parental lineages, still the very strong heterogeneity across studies makes it difficult to determine a general pattern of performance variation. Here, we have shown how both biological (genetic divergence) and methodological (hybrid identification method) factors may influence the detected hybrid performance. Hence, heterosis and hybrid breakdown could play a key role in the evolutionary dynamics of animal hybrids, and genetic approaches are fundamental to improve our understanding of these complex systems. Despite the huge amount of work on hybrid systems in the last decades, we are far from exhaustive knowledge of the factors determining the variation of hybrid performance. Nevertheless, the growing methodological (e.g. genomic analyses) and conceptual developments are opening new study avenues that can improve our understanding of hybridization as major component of the evolutionary processes.

Acknowledgement

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Supporting Information

Supporting Information is available at: https://link.springer.com/article/10.1007/s11692-022-09585-x

Supplementary material

Appendix S1: 60 journal articles were considered for the meta-analysis by using separate-parent comparison approach, the 33 underlined references were retained also for the meta-analysis by using mid-parent value approach.

Alatalo R V., Eriksson D, Gustafsson L, Lundberg A (1990) Hybridization between Pied and Collared Flycatchers—sexual selection and speciation theory. Journal of Evolutionary Biology 3: 375–389. https://doi.org/10.1046/j.1420-9101.1990.3050375.x

Audet CL, Wilson CC, Pitcher TE (2017) Effects of intraspecific hybridisation between two hatchery-reared strains of Atlantic salmon, *Salmo salar*, on juvenile survival and fitness-related traits. Fisheries Management and Ecology 24: 1–9. https://doi.org/10.1111/fme.12195

Baker MC, Boylan JT (1999) Singing behavior, mating associations and reproductive success in a population of hybridizing Lazuli and Indigo Buntings. The Condor 101: 493–504. https://doi.org/10.2307/1370179

Barreto FS, Burton RS (2013) Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. Proceedings of the Royal Society B: Biological Sciences 280. https://doi.org/10.1098/rspb.2013.1521

Burton RS (1990) Hybrid breakdown in developmental time in the copepod *Tigriopus californicus*. Evolution 44: 1814–1822. https://doi.org/10.1111/j.1558-5646.1990.tb05252.x

Campbell LA, Meinke LJ (2010) Fitness of *Diabrotica barberi*, *Diabrotica longicornis*, and their hybrids (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 103: 925–935. https://doi.org/10.1603/AN10031

Casas F, Mougeot F, Sánchez-Barbudo I, Dávila JA, Viñuela J (2012) Fitness consequences of anthropogenic hybridization in wild red-legged partridge *(Alectoris rufa*, Phasianidae) populations. Biological Invasions 14: 295–305. https://doi.org/10.1007/s10530-011-0062-3

Chan WY, Peplow LM, Menéndez P, Hoffmann AA, van Oppen MJH (2018) Interspecific hybridization may provide novel opportunities for coral reef restoration. Frontiers in Marine Science 5: 1–15. https://doi.org/10.3389/fmars.2018.00160

Cullum AJ (1997) Comparisons of physiological performance in sexual and asexual whiptail lizards (Genus *Cnemidophorus*): Implications for the role of heterozygosity. American Naturalist 150: 24–47. https://doi.org/10.1086/286055

Culumber ZW, Shepard DB, Coleman SW, Rosenthal GG, Tobler M (2012) Physiological adaptation along environmental gradients and replicated hybrid zone structure in swordtails (Teleostei: *Xiphophorus*). Journal of Evolutionary Biology 25: 1800–1814. https://doi.org/10.1111/j.1420-9101.2012.02562.x

Debes P V., Fraser DJ, McBride MC, Hutchings JA (2013) Multigenerational hybridisation and its consequences for maternal effects in Atlantic salmon. Heredity 111: 238–247. https://doi.org/10.1038/hdy.2013.43

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Tab S1: Estimates of the meta regressions excluding articles in which hybrids were compared with only one parent: **a)** meta-regression analysing whether divergence between parental lineage affects hybrid performance (genetic distance = relationships between parents); **b)** meta-regression model analysing the factors that potentially affected hybrid performance.

a)	**Effect sizes considered**	**mean z**	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
Intraspecific or interspecific	All	-0.037	-0.211	0.165	0.695
Intraspecific or interspecific	Genetic identification	-0.186	-0.411	-0.006	0.05

b)	**mean z**	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
Intercept	-0.206	-0.549	0.125	0.221
F1 hybrid	0.159	-0.027	0.258	0.583
F>1	0.059	-0.174	0.258	0.583
Interspecific cross	0.028	-0.179	0.236	0.794
Morph. identification	0.017	-0.122	0.159	0.792
Fitness trait	-0.032	-0.187	0.123	0.696
Morph. Trait	-0.098	-0.268	0.069	0.246
Alien	-0.122	-0.422	0.209	0.441
Laboratory study	0.104	-0.119	0.328	0.371

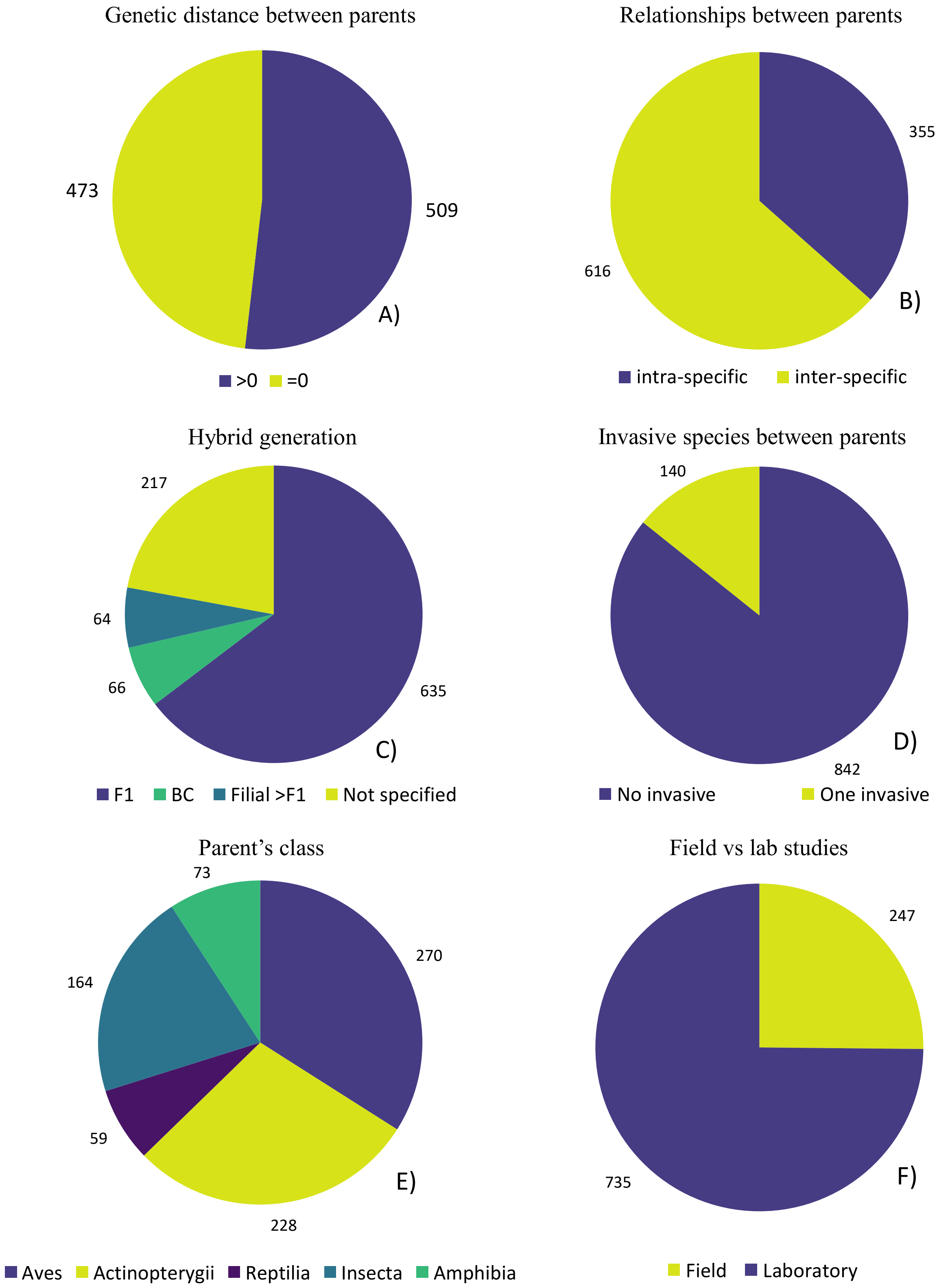
Tab. S2: Estimates from Bayesian mixed-effects meta-regression analyses of the mean performance of hybrid relative to their mid-parent performance value in different subsets of data (95% CI: credible interval, N effect sizes = number of comparisons).

Subsets	**Category**	**Mean**	**95% CI**	***P* MCMC**	**N effect sizes**
All data		0.027	-0.05|0.113	0.508	357
Genetics distance		0.071	-0.026|0.179	0.172	189
Relationships between parents	intra-specific	0.002	-0.18|0.189	0.956	138
	inter-specific	0.034	-0.06|0.12	0.45	219
Hybrid generation	F1	0.013	-0.09|0.114	0.768	227
	BC	0.032	-0.091|0.15	0.539	25
	F>1	-0.004	-0.14|0.128	0.993	105
Alien parental	no invasive population	0.023	-0.073|0.111	0.631	299
	one invasive population	0.07	-0.1|0.219	0.315	58
Parent's class	Aves	0.03	-0.115|0.173	0.631	98
	Actinopterygii	0.09	-0.146|0.325	0.433	99
	Insecta	0.029	-0.164|0.223	0.77	59
Laboratory vs field studies	Field	0.038	-0.158|0.234	0.63	90
	Laboratory	0.014	-0.08|0.121	0.759	267
Hybrid identification	Morphological	0.008	-0.127|0.103	0.972	190
	Genetic	0.065	-0.053|0.186	0.258	167
Hybrid identification 2	Controlled crosses	0.021	-0.116|0.167	0.752	148
	Morphological	-0.068	-0.21|0.087	0.273	42
Trait for the comparisons	Fitness	-0.014	-0.1|0.059	0.705	200
	Morphological	0.084	-0.071|0.226	0.246	124
	Behavioral	0.028	-0.77|0.152	0.599	33

Tab. S3: Estimates from Bayesian mixed-effects meta-regression analyses of the mean performance of hybrid relative to their parents in different subsets of data (95% CI: credible interval, N effect sizes = number of comparisons).

Subsets	**Category**	**Mean**	**95% CI**	***P* MCMC**	**N effect sizes**
All data		-0.052	-0.134|0.033	0.234	982
Genetics distance		-0.051	-0.129|0.032	0.226	509
Relationships between parents	intra-specific	-0.002	-0.165|0.154	0.958	355
	inter-specific	-0.068	-0.174|0.027	0.177	616
Hybrid generation	F1	-0.012	-0.123|0.087	0.806	635
	BC	-0.094	-0.222|0.009	0.094	66
	F>1	-0.147	-0.254|-0.044	0.012	217
Alien parental	no invasive population	-0.04	-0.126|0.046	0.353	842
	one invasive population	-0.149	-0.604|0.253	0.417	140
Parent's class	Aves	-0.068	0.207|0.071	0.308	270
	Actinopterygii	-0.121	-0.352|0.108	0.27	228
	Reptilia	0.125	-0.229|0.473	0.411	59
	Insecta	-0.087	-0.301|0.130	0.375	164
	Amphibia	-0.225	-0.648|0.239	0.234	73
Laboratory vs field studies	Field	-0.109	-0.268|0.05	0.161	247
	Laboratory	-0.019	-0.118|0.089	0.696	735
Hybrid identification	Morphological	0.003	-0.111|0.116	0.948	573
	Genetic	-0.116	-0.219|-0.015	0.03	409
Hybrid identification 2	Controlled crosses	-0.001	-0.123|0.133	0.999	460
	Morphological	0.027	-0.197|0.261	0.726	113
Trait for the comparisons	Fitness	-0.035	-0.146|0.088	0.532	581
	Morphological	-0.116	-0.271|0.049	0.157	296
	Behavioral	0.023	-0.073|0.112	0.619	105

Fig. S1: Pie charts representing the frequencies of the subset categories used for the meta-regression analysis. The numbers indicate the frequency of the categories in hybrid-parental comparisons. **A)** Genetic distance between parents; **B)** Relationships between parents; **C)** Hybrid generations, **D)** Invasive species between parents; **E)** Parent's class; **F)** Field vs laboratory studies **G)** Author identification method for hybrid; **H)** Trait considered to the comparisons.



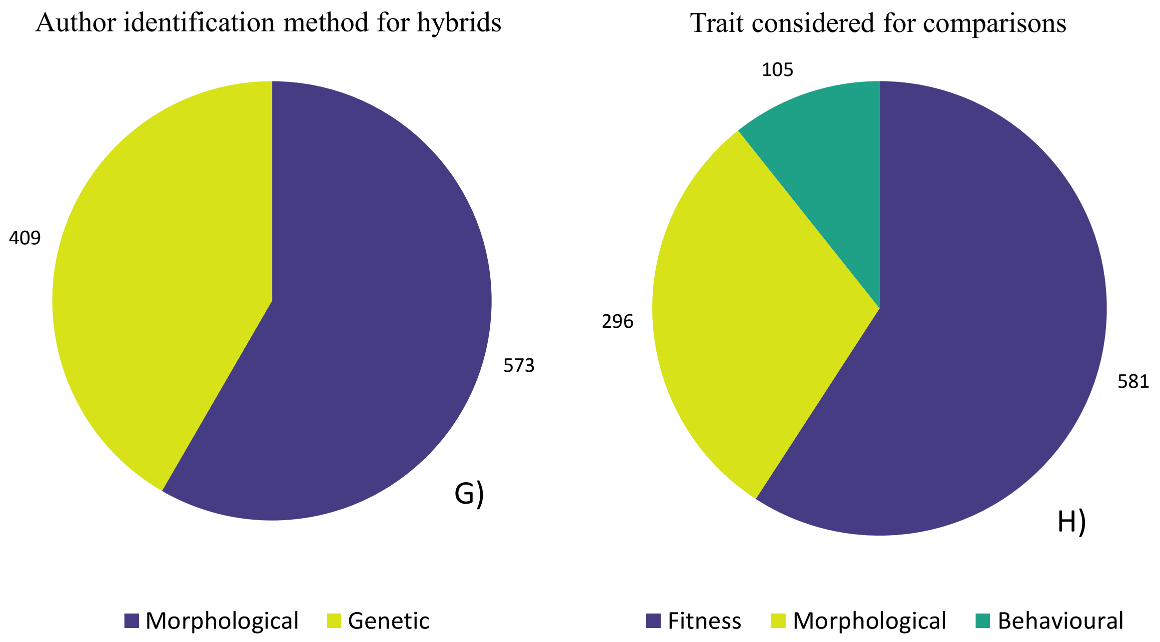
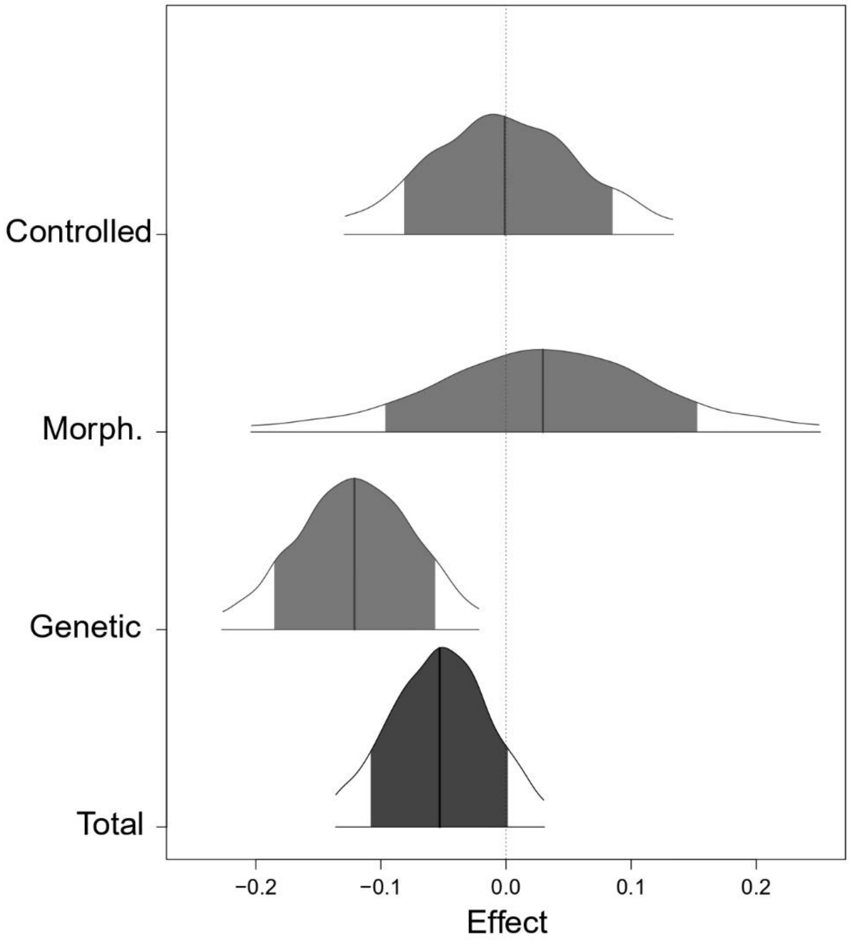


Fig. S2: Density plot of the mean of hybrids performance relative to their parents considering the author method for hybrid identification, subdividing morphological identification method in: morphological and controlled crosses in laboratory.



Tab. S4: Meta-regression models, using mid-parent approach, analysing whether divergence between parental lineages affected hybrid performance. **a)** discrimination between intraspecific or interspecific crosses, **b)** TIMETREE data (divergence in time) of parental lineages.

Parameters of divergence	**mean z**	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
a) Intraspecific or interspecific	0.03	-0.144	0.205	0.745
b) TIMETREE data	-0.026	-0.103	0.044	0.466

Tab. S5: Meta-regression model analysing the factors that potentially affected hybrid performance comparing hybrid with mid-parent performance value.

	mean z	**lower 95% CI**	**upper 95% CI**	P_{MCMC}
Intercept	0.226	-0.146	0.587	0.22
F1 hybrid	-0.107	-0.303	0.075	0.264
F>1	-0.151	-0.351	0.071	0.155
Interspecific cross	-0.008	-0.249	0.198	0.931
Morph. identification	-0.103	-0.301	0.088	0.288
Fitness trait	-0.037	-0.194	0.125	0.672
Morph. Trait	-0.001	-0.161	0.165	0.999
Alien	0.044	-0.254	0.3254	0.774
Laboratory study	-0.007	-0.242	0.239	0.962

CHAPTER 5 | DISCUSSION

5.1 | Phenotypic response to global change

Even though studying the consequences of global changes is complex, it is possible to delineate phenotypic changes in animal populations in response to global change. Some species have the ability to adapt in the face of environmental change according to the amount of genetic variability of individuals within populations. In this thesis, I investigated phenotypic variability of animal populations in response to three key pressures of global change: invasive species, resource availability and hybridization.

5.1.1 | Anti-predator strategies against alien species

Alien species are one of the main factors affecting global biodiversity (Blackburn et al. 2019). These species have changed the species composition, structure and function of ecosystem. For these reasons, biological invasions are an outstanding factor of global environmental change (Kassas and Batanouny 2022). Amphibians are particularly sensitive to the introduction of alien invasive predators that can drive native populations to loss of fitness, decline or extinction (Ficetola et al. 2011c, Bucciarelli et al. 2014, Nunes et al. 2019). Even though, several amphibians can modify their phenotype implementing anti-predator strategies against alien species (e.g. Warkentin 2005, Melotto et al. 2020). Anti-predator strategies can be the result of phenotypic plasticity or local adaptation. These mechanisms can allow native preys to cope with new selective pressures and drive evolutionary changes that can improve the withstanding against alien predators (Moore et al. 2004, Nunes et al. 2014b). In this thesis, I observed the expression of anti-predator strategies, concerning the modulation of ontogeny, in response to an invasive predator in a threatened frog. These strategies could be adaptive, hence, the modulation of phenotype in response to an invasive predator may improve the persistence of native populations (Chapter 2).

In Chapter 2, I evaluated whether individuals can modulate their phenotypic traits in response to predation pressure by combining field observations and a common rearing experiment. This chapter focused on the anti-predator strategies expressed by Italian agile frog (*Rana latastei*) populations under the heavy predation pressure of an alien species, the red swamp crayfish (*Procambarus clarkii*). Field observations revealed that this threatened frog showed strong variation in parental investment among populations, but this variation was not related to the presence of the red swamp crayfish in the pond of origin. This suggested that mothers did not modulate parental investment in relation to the presence of this alien predator. However, embryos developed faster when clutches and tadpoles were exposed to the cues of the invasive crayfish in a common rearing experiment. Moreover, embryos from crayfish-invaded sites reached Gosner's development stage 25 faster than those from non-invaded sites. If these anti-predator strategies are effective, they could lead to adaptation. This study represents an example of how a possible adaptive and plastic variation of the phenotype could improve the survival of native populations against predator pressure of an invasive species. Although is demonstrated that biological invasions are a major threat of amphibian biodiversity, studies on ecological responses of amphibians to invasive species and their evolutionary outcomes are still rare in the literature. This study evaluates the amphibian capacity to withstand invasive species and can provide crucial information to implement appropriate conservation plans (Mooney and Cleland 2001). In general, this study underlines the importance of analysing phenotypic variation to understand evolutionary responses of populations to a drastic environmental change.

5.1.2 | Resource availability

Knock-on effects of global changes cause modification of resource availability of many species and their ability to translate resources into fitness, resulting in a greater number of animals affected by environmental stresses (Vitousek 2009, Mason et al. 2014, Gibson et al. 2018, Young et al. 2019, Auer et al. 2020). Resource availability is among the most important environmental features for animal performance determining survival, growth, reproduction and other animal traits (Couret et al. 2014, Limongi et al. 2015, Shu et al. 2022). Animals can respond to changes in resource availability via phenotypic plasticity and evolutionary adaptation of several traits such as metabolic rate, morphology and behaviour (Hawlena et al. 2011, Auer et al. 2020, Gunn et al. 2022).

Variation in phenotypic traits is strictly related to individual condition that, in turn, is a product of resource availability and the individual’s efficiency at translating the available resources into fitness (Bonduriansky and Rowe 2005). Consequently, animal phenotype often shows strong plasticity across gradients of resource availability. Global changes can alter resource availability such as primary productivity and temperature. In consequence, individual condition might drastically decrease (Reading 2007, Brodersen et al. 2011, McLean et al. 2020, Brusch et al. 2022). The ongoing changes in global temperature, rainfall patterns and land use can modify primary productivity which have the potential to vastly alter food webs and may affect animal reproduction and survival (Wu et al. 2011, Mastrantonis et al. 2019, Serrouya et al. 2021). Determining how phenotype is shaped by changes in resource availability is crucial for predicting the impact of global changes on populations. In this thesis, I evaluated the role of body condition, primary productivity and temperature in the variation of sexually selected traits, which are costly for individuals, in lizard populations. The phenotypes of males and females responded differently to environmental gradients and males’ differences across environment were both condition- and context- dependent. This variation is the result of phenotypic plasticity and/or local adaptation (Chapter 3).

Chapter 3 focused on phenotypic variation in response to environmental heterogeneity at both the within- and among-populations scales. I studied two sexually selected morphometric traits in island populations of the Italian wall lizard (*Podarcis siculus*) to investigate whether ecomorphological variation differs between sexes across environmental gradients. I showed that the body size and head shape of the Italian wall lizards strongly vary among islands of the Aeolian archipelago, which might reflect plastic and/or local adaptive responses to resource availability. Three potential predictors of differences in head shape and body size between sexes were tested. These predictors were individual body condition, ecosystem productivity and temperature, used as proxy for resource availability. Both body condition and ecosystem productivity appeared to be the main drivers of body size sexual dimorphism (SD) variation, and body condition alone for head shape SD, whereas temperature played a minor role. These results were congruent at within- and among-populations scales and demonstrate that sexual dimorphism is both condition- and context-dependent. This study highlights that multi-scale approaches are useful to understand the role of environment in the expression of phenotypic traits. This study increases the knowledge on how phenotypic plasticity or adaptation can shape the expression of sexually selected traits involved in mate choice and, thus, in animal fitness in response to heterogeneous environments. Studying the expression of phenotype across heterogeneous environment may allow to understand the adaptability of a species in a context of global change that leads to the alteration of resource availability in time and space.

5.1.3 | Hybridization

Populations and species can respond to the contemporary global changes through shifts in their geographic distributions (e.g. Forcada and Trathan 2009, Taylor et al. 2015). This range shift increases the probability of sympatry between divergent populations or species and, consequently, the potential for hybridization (Garroway et al. 2010, Taylor et al. 2015). Hybridization can quickly alter the evolutionary trajectory of species and can play a crucial role in speciation, extinction, and adaptive radiations (Sakai et al. 2001, Seehausen 2004, Mallet 2005, Capblancq et al. 2015, Kagawa and Takimoto 2018). Understanding hybrid performance may allow to predict evolutionary trajectory of parental species and the outcomes of hybridization. In this thesis, I studied differences in performance between hybrids and their parents in different ecological tasks, analyzing different methodological approaches. Hybrid performance was generally similar to the performance of their parents, but lower when the genetic distance between the parents is high (Chapter 4).

In Chapter 4, I performed a meta-analysis to assess the effect of hybridization on hybrid performance. I included 60 studies on performance traits or their underlying phenotypic traits, resulting in 982 hybrid-parents comparisons. Moreover, I assessed whether biological and methodological processes could drive to the observed differences in performance between hybrids and their parents. Hybridization led to extremely variable performance outcomes. Hybrid performance was often significantly higher or lower compared to the mean performance of their parents. Nevertheless, on average hybridization resulted in similar performance between hybrids and their respective parental lineages. Performance was generally lower for hybridization events between genetically distant parental lineages. Indeed, hybrids between distinct species showed lower performance than hybrids between lineages of the same species. This finding might be the result of the combined effects of heterosis and hybrid breakdown. Heterosis occurs often in hybrid between genetically close parents and can increase hybrid fitness (Dagilis et al. 2019, Atsumi et al. 2021). On the other hand, accrued genetic difference between parents can affect hybrid fitness because the accumulation of genes that are regularly functional in pure-species, but produce negative epistatic interactions in hybrids. These postzygotic incompatibilities increase rapidly with the divergence between species (Dobzhansky 1937, Matute et al. 2010). These findings align with a recent meta-analysis that hypnotized heterosis promotes developmental stability in hybrid between genetically close parental lineages resulting in a reduced phenotypic variability of hybrid. Conversely, phenotypic variability is larger in hybrid with developmental instability determined by genetic difference between parents (Atsumi et al. 2021). The results also revealed that study settings can bias our understanding of factors determining hybrid performance, especially the use of imprecise approaches for hybrid identification (e.g. morphology-based). Hybrid identification through genetic data enables more certain identification of individuals and provides information on levels of introgression that could be related to different hybrid performance. This meta-analysis provides insights on the role of biological and methodological processes that influence the outcome of performance assessments and clarifies the effects of hybridization on phenotypic variation.

5.2 | Methods implemented to analyse performance

As showed in this thesis, several methods can be employed for studying different facets of animal phenotypic variability. A common rearing experiment allowed to detect the phenotype changes induced by an alien predator pressure in a native species, excluding other factors that could have affected the findings of experiment (Chapter 2). The common rearing experiments are often effective in discerning between phenotypic plasticity and adaptation. In Chapter 2, it was possible to delineate the role of phenotypic plasticity and adaptation. I observed a plastic ontogenetic shift in tadpoles reared in the presence of the invasive predator, independently of the site of origin, as these tadpoles exhibited a faster development. Furthermore, I detected differences between crayfish-colonised and crayfish-free populations in development time as tadpoles from invaded sites, regardless of being exposed to the crayfish or not, developed faster. This finding supports the idea that adaptation may occur through the rapid fixation of genotypes showing adaptive plasticity (Levis et al. 2018). Field studies are less effective in distinguishing between phenotypic plasticity and adaptation than rearing experiments. Through a field study (Chapter 3), it was not possible to determine whether the phenotypic variation among individuals was triggered by phenotypic plasticity or adaptation.

The field study (Chapter 3) allowed to detect the main environmental factors driving two sexually dimorphic traits at two spatial scales. Implementing the analysis at two scales allowed to evaluate whether scaling issues affect the detection of the drivers of SD (Chapter 3). Multi-scales approach is useful to solve scaling issues comparing results of different scales. Multi-scales analyses are increasingly performed in ecological studies for a range of aims, including the study of habitat use (e.g. Cossa et al. 2022), the influence of landscape features on species and community (e.g. Ficetola et al. 2011a), species-richness analyses (e.g. Rahbek 2005), predator-prey dynamics (e.g. Schellhorn and Andow 2005), pattern of distribution of species (e.g. Ficetola et al. 2007) and geographic variation of phenotype (e.g. Petrusková et al. 2010). Multi-scales approach can be used for several purposes as comparing analyses among intra-population, inter-population, species and community levels (e.g. Schellhorn and Andow 2005, Petrusková et al. 2010, Ficetola et al. 2011a) or to compare patterns of biological processes (e.g. Ficetola et al. 2007, Cossa et al. 2022). However, analyses at different scales can have opposite results and each scale has its own limits. The outcomes of analyses at different spatial scales can be incongruent because the importance of ecological variables and processes often depend on the spatial scale at which the variables are measured (Hill and Hamer 2004). Finally, each spatial scale has its own limits; for example, in Chapter 3 island scale has less sample size compared to individual scale and, as a consequence, less statistical power. On the other hand, the analysis at island spatial scale is crucial to understand the overall pattern of condition- and context- dependent sexual dimorphism. For these reasons, the delineation and choice of scale can directly affect the outcomes of analysis and the comparability of similar studies (Hill and Hamer 2004, Rahbek 2005).

I used a meta-analysis to summarize the performance consequences of hybridization. This meta-analysis filled a gap in knowledge about the effect of hybridization on hybrid performance traits and evaluated the parameters that could determine differences in performance between hybrids and parental populations (Chapter 4). Previous meta-analyses provided important insight on hybrid phenotypic variability, however these were related only to F1 generation and investigated only genetic diversity between parents as driver of phenotypic variation (Atsumi et al. 2021, Thompson et al. 2021). In the meta-analysis, I also studied how methodological approaches can affect the assessment of hybrid performance. While field and laboratory studies were comparable and the performance outcomes were similar regardless of the trait considered for the comparisons, studies that include the genetic identification of hybrid were needed to avoid identification error.

These findings underline that well-planned laboratory studies can provide results consistent with what is observed in the wild (e.g.: Herborn et al. 2010). However, hybrid identification through morphological data is not always precise as identification with genetic methods that provide higher power to any kind of analysis. The growing availability and decreasing cost of genetic markers enables fast and more certain identification of hybrids (Della Croce et al. 2016). This study highlights the importance of appropriate hybrid identification for a realistic understanding of the evolutionary impacts of hybridization. Moreover, genetic data are necessary to distinguish among different levels of individual introgression that in some system could be related to different performance (Muhlfeld et al. 2009). On the other hand, meta-analyses inevitably bring together studies that differ in design, conduct and outcomes. Such heterogeneity among studies can be a limitation of using broad inclusion criteria, because it does not allow to explain all of the variability detected by the meta-analysis (Gurevitch et al. 2018). Ideally, multi-species studies with standardized approaches would be needed to reduce heterogeneity among studies and, consequently, improve meta-analysis conclusions.

5.3 | Future research directions

Further studies are needed to investigate: i) the drivers of maternal investment and the effectiveness of anti-predator strategies in Italian agile frog; ii) the relationship between genetic distances among populations and phenotypic differences in the Italian wall lizard; iii) possible competition between hybrid of closely related parents and their parents, and if the difference in performance between hybrid and their parents is related to a shift in the niche.

The modulation of frog parental investment (egg number and egg size) across frog populations (Chapter 2) is still unclear. I demonstrated that this modulation was unrelated to climate conditions and to the presence of the alien crayfish. However, I selected a homogeneous pool of populations to better evaluate the impact of the alien crayfish in this study, but it will be interesting to assess these patterns across a broader climatic gradient. Climatic conditions and other environmental features could induce variation in female size and conditions (e.g. Reim et al. 2006, Roitberg et al. 2013) that, in turn, are frequently related to clutch features (Prado and Haddad 2003, Tessa et al. 2009, Chen et al. 2012, Sinsch et al. 2015). Future investigations are needed to analyse whether maternal investment is condition- and context- dependent in this system increasing the number of populations, environmental conditions and geographic range of the study. This information will allow to understand whether mothers can modify the phenotype in response to the environment in order to improve the survival of the offspring. To broaden the conclusions of Chapter 2, the effectiveness of anti-predator strategies in Italian agile frog and their costs on the life history traits should be analysed. The demonstrated shift in ontogeny of frog embryos could be fundamental to increase survival and populations fitness. However, this is strictly related to the effectiveness of this trait variation and the costs of these anti-predator strategies can be high for the populations. Long-term studies can provide key insights on the presence of local adaptations in invaded populations to determine their evolutionary trajectories.

Regarding Chapter 3, it would be interesting to expand the study area and integrate genetic data in order to better assess the role of population history and environmental variation in shaping the spatial variation of phenotypic variation of Italian wall lizards. It will be important to analyse both neutral and adaptive genetic variation. Neutral genetic variation in unaffected by natural selection and unrelated to individual fitness. Nevertheless, neutral genetic variation allows to investigate processes such as gene flow, migration and dispersal. On the other hand, adaptive genetic variation, genetic variation under natural selection, is fundamental to study the evolutionary and adaptive potential of a populations or species (Holderegger et al. 2006, Merilä and Hendry 2014). In collaboration with other researchers, we are analysing RAD-seq data and we have already collected a large amount of data on phenotypic traits around the whole Italian peninsula. Phenotypic variation and environment can vary substantially across broad spatial scales. Attention to geographic variation in phenotypic traits, and its genetic basis, to environment may improve the knowledge of plasticity and/or adaptation and the history of this species studying mutations and genetic drift.

Finally, further investigations on hybrid performance (Chapter 4) should focus on hybrid performance of closely related parents to investigate the possible competitiveness between hybrid and parents. The performance of hybrid of closely related parents is similar to the performance of parents and they might be in competition for resources. This will clarify the evolutionary trajectory of hybrid and their parents. Moreover, future studies should address whether the different performance of hybrid is related to a niche shift compared to parental lineages. This data could explain if closely related individuals (hybrid and parents) can partition resources and coexist stably and clarify the ecological outcomes of hybridization. Such knowledge will allow to broaden scientific understanding on patterns of phenotypic variability and adaptation to environmental changes.

5.4 | Conclusions

The phenotype and its related performance can strongly vary according to the environment, and we expect strong phenotypic variation in response to global changes. Animals can modulate phenotypic expression in response to a predatory risk (Chapter 2), can increase the expression of some phenotypic traits in favourable environmental conditions (Chapter 3) and, finally, phenotypic variation was related to lower performance in hybrid genome with accumulation of genes that produce epistatic interactions in individuals (Chapter 4). The modulation of phenotype in response to different stress factors can be very variegated and elaborated. Future researches should focus on the combined effects of several environmental stressors on animal phenotype. The findings of this thesis represent a step toward a better knowledge on phenotypic variation and its related performance in different ecological tasks of animals, highlighting the role of the environment and hybridization. Studies on phenotypic variation could be central for comprehending whether and how animals can adapt to the contemporary global change.

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