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Melanin-based colouration as a signal of individual quality and its potential role in sexual selection in the Barn swallow (*Hirundo rustica*)

PhD Thesis

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Contents

1. Abstract

1.1 Abstract-English

Bird colours can be structural or can be due to a wide range of pigments incorporated into the feather keratin such as porphyrins, carotenoids and melanins. Melanins are not acquired through diet but are synthesized within dedicated organelles called melanosomes. Despite the strong genetic control of melanin synthesis, environmental variables and physiological condition can influence the expression of melanin ornaments, suggesting that the degree of melanism may honestly signal individual quality and have a role in sexual selection processes. However, studies on the association between melanin-based colour traits and life-history strategies have often provided inconsistent results. Thus, identifying any association between melanisation and fitness traits in populations under a sexual and natural selection regime is pivotal to any study of the evolution of melanism and, more generally, to research on sexual selection . The studies presented in this thesis aimed at investigating the role of melanin-based colouration in the Barn swallow (*Hirundo rustica*) as a signal of individual quality both in nestlings and adults and its role in sexual selection processes. Moreover, the results of a meta-analysis on the intensity of sexual selection on different phenotypic traits across the six Barn swallow subpopulations are presented. By correlative and experimental approaches, this thesis provides novel findings about the role of plumage colouration as a signal of individual quality. Indeed, I demonstrated the parental ability to differentially invest in male and female offspring according to a phenotypic melanin-based trait expressed during early development which will be involved in intersexual competition at sexual maturation; in addition, I provided unprecedented evidence that ventral plumage colouration, involved in parent-offspring communication, reflects telomere length and thus offspring quality. Moreover I demonstrated that, in adult Barn swallows, ventral plumage colouration is a signal of telomere length and predicts seasonal, but not lifetime, reproductive success. Ventral plumage colouration was also found to predict promiscuity in female Barn swallow, suggesting that it may affect male choice of social as well as of potential extra-pair mates. Finally, the present thesis provides a quantitative meta-analytic support for the evidences that different ornaments are subject to different selection regimes among geographically distinct Barn swallow populations, according to the hypothesis that sexual selection plays a major role in speciation processes.

1.2 Abstract-Italiano

Il colore del piumaggio degli uccelli può essere di tipo strutturale o essere dovuto ad un ampio spettro di pigmenti quali porfirine, carotenoidi e melanine, incorporati nella matrice di cheratina. Le melanine non sono acquisite attraverso la dieta ma sono sintetizzate in organelli cellulari detti melanosomi. Nonostante la sintesi delle melanine sia sotto controllo genetico, le condizioni ambientali e fisiologiche possono influenzare l'espressione dei caratteri ornamentali da esse determinati, suggerendo come la colorazione melanica sia un segnale onesto di qualità degli individui e possa avere un ruolo nei processi di selezione sessuale. Tuttavia, gli studi riguardanti l'associazione tra i caratteri melanici e le strategie legate alla *life-history* degli individui sono spesso contrastanti. Identificare l'associazione tra il colore melanico e i caratteri legati alla *fitness* in popolazioni sottoposte a selezione naturale e sessuale è quindi fondamentale al fine di analizzare l'evoluzione di caratteri legati alle melanine. I lavori inclusi nella presente tesi sono volti a saggiare il ruolo della colorazione melanica nella rondine (*Hirundo rustica*) come segnale di qualità individuale sia negli adulti che nei pulcini e a determinarne il ruolo nei processi di selezione sessuale. Inoltre, sono presentati i risultati di una meta-analisi riguardante l'intensità della selezione sessuale su diversi caratteri fenotipici nelle sei subspecie di rondine. Utilizzando un approccio sia correlativo che sperimentale, la presente tesi fornisce evidenze riguardo al ruolo della colorazione del piumaggio nella segnalazione onesta della qualità degli individui. In tale contesto, è stata dimostrata l'abilità dei genitori nell'investire in modo differente nella prole dei due sessi in relazione ad un carattere fenotipico espresso durante lo sviluppo potenzialmente implicato nella competizione tra sessi; inoltre, è stato dimostrato come la colorazione del piumaggio, utilizzata nella comunicazione tra genitori e prole, rispecchi la lunghezza dei telomeri e quindi la qualità dei pulcini. Inoltre, è stato dimostrato come, negli adulti di rondine, la colorazione del piumaggio sia un segnale delle dinamiche telomeriche e predica il successo riproduttivo realizzato nel corso di una singola stagione riproduttiva ma non quello realizzato nel corso dell'intera vita. La colorazione del piumaggio è stata inoltre dimostrata predire la promiscuità nelle femmine, suggerendone il ruolo svolto al fine di guidare la scelta dei maschi per la formazione della coppia sociale o per la scelta di una femmina extra-coppia. Infine, il presente lavoro fornisce un supporto quantitativo alla evidenza che differenti ornamenti sono soggetti a differenti regimi di selezione tra popolazioni geograficamente distinte, in accordo con l'ipotesi di un ruolo chiave della selezione sessuale nei processi di speciazione.

2. General introduction

2.1 Introduction

Bird plumage colouration has always been fascinating for scientist involved in studies of evolution of ornamental traits. Bird feather colours can be ascribed to two main categories: they can be structural or can be due to a wide range of pigments (Fox, 1976; Brush, 1978). Structural colours are produced by the light interacting physically with avian integument structures, while pigmentary colours are the result of pigments, such as porphyrins, carotenoids and melanins, incorporated into the matrix of keratin, affecting the absorption and the reflectance of incident light at different wavelengths.

Depending on the different refraction properties and the reciprocal arrangement of the nanostructures of the feather barbules as keratin, melanosomes and air vacuoles, three classes of structural colour can be produced (Prum, 1999):

1. white colourations, produced by unpigmented keratins and randomly distributed air vacuoles that incoherently scatter all visible light wavelengths (Fox, 1976);
2. Iridescent structural colourations, produced by structurally coloured feather barbules which scatter the light through arrays of melanin granules and/or air vacuoles suspended in the barbule keratin that create two-dimensional photonic sub-micron crystal-like or laminar structures (Zi et al., 2003; Li et al., 2005; Yoshioka and Kinoshita, 2002), where the light is split into rich component colours. Colours of iridescent feathers changes with the viewing or the illumination angle;
3. Non-iridescent blue, violet, green, and UV colourations, produced by feather structures such as the specialized, spongy, medullary layer of feather barbs. Colours are produced by three-dimensional spatial periodicity in feather barbs and changes in the observer angle do not lead to a change in colours (Proctor and Lynch, 1993).

Porphyrin pigments are ultimately derived from two modified amino acids -succinyl Coenzyme A and glycine- (Lascelles, 1964) and are known to be produced in at least 13 order of birds, among which the most commons are owls, goatsuckers and bustards. They are responsible of colours as pink, brown, red and green. However, studies regarding the biochemistry or the function of porphyrins in feathers are scant (McGraw, 2006).

Carotenoids are 40-carbon tetraterpenoid molecules consisting of a series of eight 5-carbon isoprene residues. The linear hydrocarbon skeleton of conjugated double bonds can exist alone or can be cyclized at one or both ends, and these end-rings are often substituted by different functional groups (McGraw and Hill, 2006). Carotenoids are present in

photosynthetic organisms such as plant, algae, fungi and bacteria, while animals are unable to synthetize them from the precursor (phytoene) and, thus, their only source of carotenoids is through diet. Birds obtain carotenoids by consuming algae, fungi and plant or, indirectly, by ingesting animal prey containing carotenoids also acquired with food. Despite birds cannot synthetize carotenoids, they can metabolize them into different forms and incorporate them into several tissues such as feathers, skin, scales, beaks, combs and wattles (Fox e Vevers, 1960; Fox, 1976; Brush, 1978). More than a carotenoid form can be present in a single integumentary tissue and the spectrum of colours produced is very broad, with red, orange, yellow and, less common, pink hues. Being highly conjugated molecules, carotenoids accept unpaired electron from reactive oxygen species produced during cellular metabolism and, thus, protect cells from oxidative damage (Burton and Ingold, 1984); moreover, they are involved in the enhancement of the immune system, since the immune response itself produces reactive oxygen species that disrupt the intercellular signals sent via lipid-rich, membrane-bound receptors (Chew, 1993). Animals may face a trade-off when allocating carotenoids acquired from the diet to health function and colouration purposes, and only the highest-quality individuals can incorporate more carotenoids into the integument for advertisement (Lozano, 1994); thus, carotenoid-based colours are signals of the foraging ability and nutritional state of individuals and, according to the handicap principle (Zahavi, 1975), are subject to mechanisms of sexual selection.

Melanins are biochemically classified as indole biochromes (Fox and Vevers, 1960) but, being unusually large and with strong cross-linking properties, their precise structure and characteristic have not been completely clarified yet (Ito, 2000). In animals there are two main melanin forms: eumelanin and pheomelanin. Eumelanin is the prevalent form in all animal taxa and confers black and brown hues, while pheomelanin gives red, yellow, chestnut and rufous colourations (Riley, 1997); the two pigments usually occur together in the feathers, and the colours are not shaped by the absolute amount of the two of them but, instead, by their relative concentration (Haase et al., 1992, 1995; Ito and Wakamatsu, 2011). Melanins are synthesized into dedicated organelles called melanosomes (Schraermeyer, 1996; Marks and Saebra, 2001), located in the melanocytes, which can be mainly found in the skin and the feather follicles. The melanocytes develop from melanoblasts, that are formed in the neural crest and subsequently migrate to the skin and feather follicles (Lucas and Stettenheim, 1972). Melanins are not acquired through the diet, but are synthetized endogenously starting from the amino acid tyrosine being oxidized to

dopaquinone by the enzyme tyrosinase (Wakamatsu and Ito, 2002) (Figure 1). The pathway to eu- or pheo-melanogenesis is at this point determined by the cysteine amino acid: a sufficient cysteine concentration in the melanosomes leads to the production of the precursors of pheomelanin cysteinyldopa, while its lack drives the pathway of production of eumelanin (del Mamol et al., 1996).

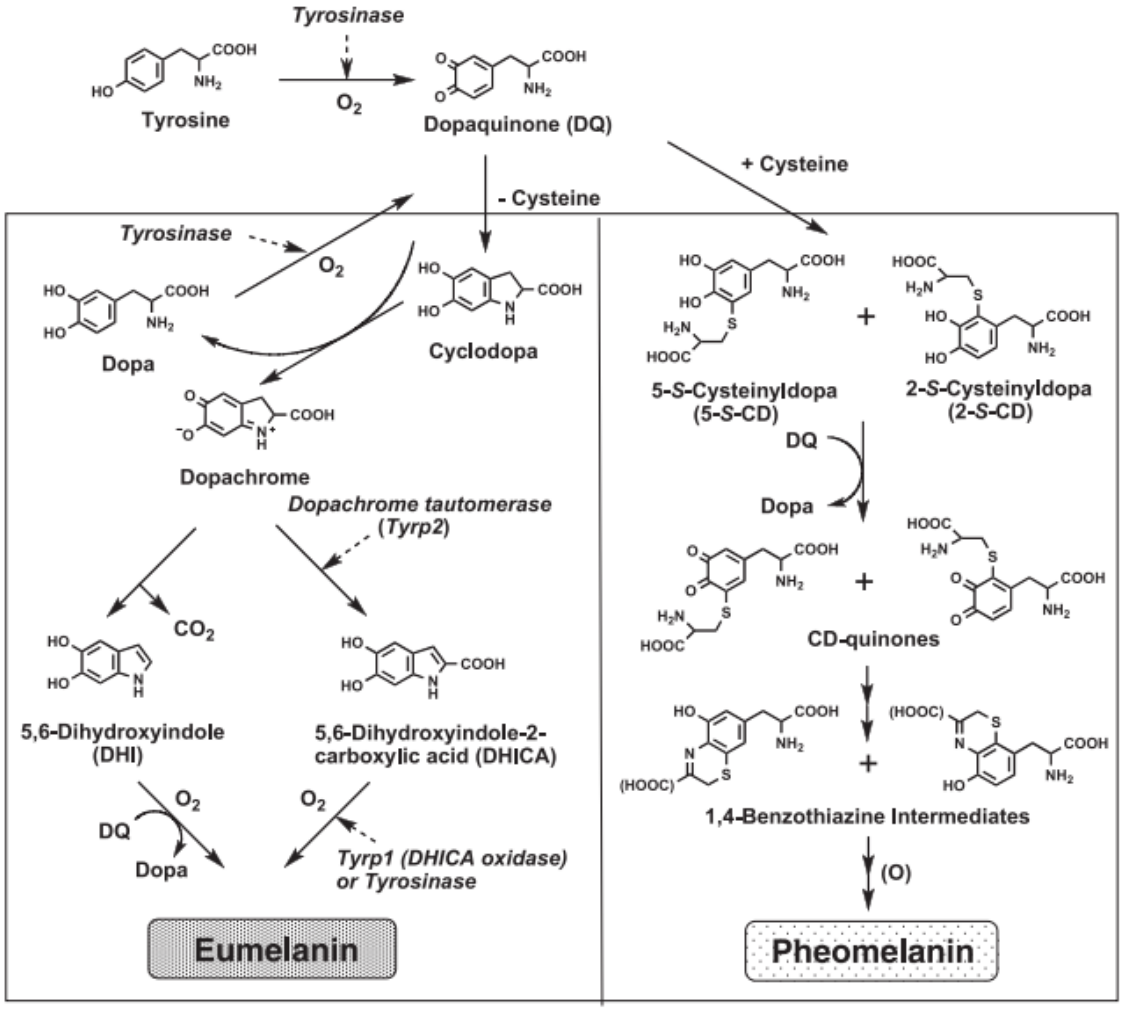

Figure 1. Biosynthetic pathways leading to eumelanin and pheomelanin production.
Ito and Wakamatsu, 2008

The relative amount of eumelanin to pheomelanin pigments is mainly regulated by a receptor belonging to the family of transmembrane G protein-coupled receptors (MC1-5Rs), the melanocortin-1 receptor (MC1R). In its active state, MC1R promotes the activity of eumelanin-related enzymes and proteins involved in melanosome biogenesis; on the contrary, when MC1R is in its inactive state there is an increase in pheomelanin pigment synthesis (Walker and Gunn, 2010). The switch between the eumelanin and pheomelanin production is mediated by the opposing effects of a pair of ligands: the agonist

melanocortins (α- β- γ-MSHs and the adrenocorticotropin hormone, ACTH) and the antagonist agouti signalling protein (ASIP) (Walker and Gunn, 2010). Melanocortins are cell-specific posttranslational products of the proopiomelanocortin (POMC) gene, highly conserved in their function and distribution across vertebrates (Schioth et al., 2005). They have neuroendocrine and paracrine functions through binding not only to MC1R but also to MC2–5Rs, which are responsible for several physiological and behavioural functions (Schioth et al., 2005). Thus, a covariation between melanin-based colouration and other phenotypic traits may exist. In many vertebrates, dark (relatively more eumelanic) and pale (relatively more pheomelanic) conspecifics differ in physiology and behaviour, an observation that stimulated researches aimed to understand the causes and consequences of the link between colouration and phenotypic traits (Ducrest et al., 2008; Galvan and Alonso-Alvarez, 2008; Jablonski and Chaplin, 2010). Knowledge of the pleiotropic effects of the melanocortin system appears useful to make predictions about how melanin-based colouration may covary with physiological and behavioural traits, and how colour plays a role in social interactions. According to Ducrest and co-worker (2008), compared to paler individuals, darker conspecifics may be predicted to:

1. be more aggressive: melanocortins promote aggressiveness by inducing the production of aggression self-stimulating pheromones (Morgan et al., 2004);
2. have higher exocrine gland function: via MC5R, melanocortins enhance the secretion and excretion of exocrine glands, such as the murine preputial, Harderian, lacrimal and sebaceous glands (Chen et al., 1997);
3. better maintain the energy balance between food intake and energy expenditure and have higher resting metabolic rate: in response to increased insulin and leptin inputs, melanocortins binding to neural MC3R and MC4R reduce food intake and coordinatively stimulate energy expenditure (Cone, 2006). Melanocortins also enhance energy expenditure through diet-induced thermogenesis. In addition, they regulate glucose homeostasis (Fan et al., 2005);
4. be less sensitive to stressful factors: the hypothalamic-pituitary-adrenal (HPA) axis is among the major regulators of the stress response. It consists of the hypothalamic corticotropin-releasing hormone, which stimulates the pituitaric ACTH and further activates the synthesis of glucocorticoids (cortisol and corticosterone) by binding to MC2R in adrenal glands (Charmandari et al., 2005);

5. have better anti-inflammatory, antipyretic and anti-oxidative responses: through binding to MC1R, MC3R and MC5R, melanocortins reduce acute, allergic and systemic inflammation and septic shock (Getting, 2006). In addition, α-MSH derived peptides have antipyretic activity through binding to MC4R (Roth et al., 2004). Finally, by binding to MC4R, melanocortins reduce apoptosis (Chai et al., 2006), oxidative stress and DNA damages induced by UV radiation in the skin (Bohm et al., 2005);
6. be sexually more active and have higher reproductive success: melanocortins enhance fertility, female sexual receptivity and male sexual motivation and performance (Shadiack et al., 2007). In addition, melanocortins have a positive effect on the production of sexual hormones (Eberle, 1988).

Such multi-faceted associations with fitness traits suggest that melanin-based feather colours of many bird species may have evolved under the influence of inter- as well as intra-sexual selection for signals reflecting quality variation among individuals. However, since melanin synthesis and deposition are determined by a strong genetic control starting from basic dietary components that are not easily affected by environmental variation (Decker and McGinnis, 1947; Fox, 1976; Buckley, 1987; Veiga and Puerta, 1996, Hill, 2000), melanin-based plumage colouration has long been thought not to represent a reliable signal of individual quality (Hill and Brawner, 1998).

However, despite the strong genetic control of melanin synthesis, environmental variables and physiological condition such as food availability and changes in oxidative status can influence melanogenesis and, thus, melanin ornament expression, suggesting that the degree of melanism can honestly signal the quality of an individual, in accordance with the handicap principle of sexual selection proposed by Zahavi (1975). Studies regarding the dependency of melanin-based traits, both in terms of ornament size and colour, on condition have produced mixed results (Hill and McGraw, 2006, Meunier et al., 2011, Guindre-Parker and Love, 2014). For example, in a study conducted on the House sparrow, *Passer domesticus*, Veiga and Puerta (1996) demonstrated that, when fed ad libitum, both adults and juveniles develop badges of similar size, a condition that is not present in the field, where juveniles are excluded from the best feeding sites or lack the experience to obtain adequate food; however, in the same study, the blackness of the same patch was not affected by the nutritional status. In another study (Jawor and Breitwisch, 2004) on the Northern cardinals (*Cardinalis cardinalis*), the colour of the black face mask in males is

correlated with body condition early in the breeding season, reflecting the nutritional status during moult in the previous autumn, when the ornament was shaped.

The production of cytotoxic compounds during melanogenesis also suggests that melanin ornaments can serve as indicators of condition and of the ability to withstand physiological perturbances. Indeed, melanogenesis should be considered an oxidative process, as it basically consists in the oxidation of the amino acid tyrosine to dopaquinone, a biosynthetic process that generates reactive oxygen species (ROS) and other oxidative sub-products potentially toxic to melanocytes (Borovanský and Riley, 2011). Given the key role of oxidative stress (i.e., an imbalance between ROS production and antioxidant defences) as a determinant of life history strategies (Metcalfe and Alonso Alvarez, 2010), the type of melanin that is produced for pigmentation is expected to affect the evolution of life histories. ROS production is in fact higher in eumelanogenesis as compared to pheomelanogenesis, and the capacity to protect the organism against UV radiation is enhanced in eumelanogenesis. This may represent an evolutionary trade-off, whereby natural selection favours the evolution of different colour phenotypes according to the environmental conditions or the contingent physiological processes. For example, organisms exposed to high UV radiation may be selected to produce a larger amount of eumelanins, despite associated to oxidative costs that may hinder their ability to cope with further oxidative challenges. At the same time, being eumelanin based traits the most costly phenotypes to produce under high UV exposure, it would have a greater potential to evolve as honest signals of genotypic quality for intraspecific communication under these conditions (Galván and Alonso-Alvarez, 2009).

Melanin ornament expression may also be influenced by concentration of sex hormones, most importantly testosterone, during molt or during the breeding season. Aggressive and sexual behaviours in birds are generally enhanced by increased levels of testosterone (Wingfield et al., 1987), that has also been indicated as a mediator of intrasexual interactions (Hirschenhauser et al., 2003; Goymann et al., 2004; Garamszegi et al., 2005). In parallel, high testosterone levels not only favour intrasexual behaviour, but also determine the production of melanin-based ornamental traits, since melanocytes express cell surface receptors for testosterone. For example, in Mallards (*Anas platyrhynchos*), higher levels of testosterone preceding molt induce a more complete eclipse plumage (Haase and Schmedemann, 1992) and affect melanogenesis so that predominantly eumelanic feathers during the breeding period become mixed or predominantly

pheomelanic in nonbreeding periods (Haase et al., 1995). An opposite effect of testosterone has been demonstrated in European Starlings (*Sturnus vulgaris*), with higher levels inhibiting melanin deposition in the bill and favouring carotenoid deposition (Witschi and Miller, 1938). Those studies indicate that melanin ornaments are influenced by, and indicative of, levels of hormones that are important for breeding behaviour.

All these associations between melanin-based colouration and physiological traits raies the possibility that variation in melanin-based colourations advertises the genetic/phenotypic quality of an individual to same-sex competitors for mating opportunities or to choosy opposite-sex mates. A large number of studies has demonstrated the signalling role of melanin colouration in both intra- and inter-sexual selection. According to the game theory models (Smith and Harper, 1988) the honesty of an ornament, even if relatively inexpensive to produce, can be maintained if the ornament is constantly tested in male-male competition. In the Common yellowthroat (*Geothlypis trichas*), for example, males with larger melanin-based masks face higher frequency of male aggression over territories, food or mates (Tarof et al., 2005); moreover, males with enlarged melanin-based masks (a dishonest signal) were more likely to lose in agonistic interactions, supporting the prediction that cheaters would lose in a competitive interaction. Therefore, status signals such as melanin-based colouration plumage patches can be used by birds as honest indicators of a rival's age, rank, or fighting ability (Fugle et al., 1984; Jarvi and Bakken, 1984; Senar, 2006). Several studies adopted a manipulative approach to demonstrate the role played by melanin-based colouration in sexual selection. For example, Hoi and Griggio (2008) showed that, in the Bearded tit (*Panurus biarmicus*), females that spent more time with males that had an elongated rather than a shortened black, melanin-based beard. The preference for darker mates has been found also in other studies where the intensity of the melanin-based colouration (Safran et al., 2005) or the number of black feather spots (Roulin and Altwegg, 2007) were manipulated. A different experimental approach, whereby differently coloured males were presented to females in mate-choice trials, confirmed the role of melanin-based colouration as a mate-choice criterion (Cooke et al., 1972; Houtman and Falls, 1994; Pryke, 2010), although in these experiments it was not possible to distinguish among the effect that different phenotypic traits, such as body size or behaviour, may have on mate choice. The same result was obtained in the correlative study performed on the Eurasian Penduline tits *Remiz pendulinus* (Kingma et al., 2008),

where males with larger melanin-based masks were demonstrated to pair more quickly and have more mates during the breeding season as compared to males with smaller masks.

The above studies support the hypothesis that melanin-based colouration is a signal of individual quality that may also have a role in mate choice processes. However, the studies regarding the association between melanin-based colour traits and life-history strategies often provide inconsistent results. Thus, identifying any association between melanisation and fitness traits in populations under a sexual and natural selection regime is pivotal to any study of the evolution of melanism.

2.2 General aims

The present thesis is aimed at investigating the role of melanin-based colouration in the Barn swallow (*Hirundo rustica*) as a signal of individual quality both in nestlings and adults (chapters 1-3) and its role in sexual selection processes (chapter 4-5). Moreover, the results of a meta-analysis carried out to investigate the intensity of sexual selection on different phenotypic traits across the six Barn swallow subpopulations are presented (chapter 6).

- The aim of **chapter 1** was to investigate whether parents modulate their investment towards individual nestlings according to their ventral colour. Plumage colour of Barn swallow nestlings was experimentally manipulated and parental feeding effort monitored. In birds, nestlings may honestly display their quality by means of multiple acoustic and visual signals. Because of the potential reproductive advantage for males, but not females, in showing darker ventral feathers, parents were expected to preferentially allocate more resources towards male offspring displaying experimentally darkened plumage. In particular, when variation in plumage colour traits is related to reproductive success in one sex, parents should favour the offspring of the sex that will benefit the most by displaying such traits. However, to date, this hypothesis has received little attention.
- In **chapter 2** I investigated whether the preferential parental allocation of resources towards darker nestlings may be explained by the hypothesis that melanin-based plumage colouration represents a proxy of nestling telomere dynamics. Telomeres are nucleoprotein complexes located at the end of chromosomes that maintain chromosome integrity and have a major role in mediating individual response to endogenous and extrinsic factors. I thus manipulated brood size to test whether social stress influences nestling telomere dynamics and to investigate the covariation between telomere length and ventral plumage colouration. Parents were expected to preferentially allocate resources toward offspring showing traits that reliably reveal their resistance to stress in terms of telomere dynamics. This hypothesis is relevant to a core issue in the evolution of parent-offspring communication systems but has never been tested in any organism to date.
- Being telomere dynamics a key factor underlying variation in individual quality, they may also be relevant to sexual selection processes. The aims of **chapter 3** were threefold: to investigate the covariation between seasonal reproductive success and telomere length, expecting a higher reproductive success in individuals with larger telomere length; to investigate whether, according to the pleiotropic effect of

melanocortins, individual ventral colour reflects telomere length; to investigate whether seasonal reproductive success could be predicted by ventral plumage colouration: Despite in other Barn swallow populations darker males are known to produce a larger number of offspring per breeding season, in the European Barn swallow no study has ever been published on this relationship.

- In **chapter 4** I identified parentage of all offspring produced over three years at three colonies and measured male lifetime reproductive success to estimate selection on lifespan and on a number of secondary sexual traits such as the length and asymmetry of the outermost tail feathers, the area of white spots on the tail and the ventral plumage colouration. I expected that individuals with longer and more symmetric tails, larger white spots on tail and darker ventral plumage colouration had larger lifetime number of offspring, both in broods where they were the social parents and in broods other than their social broods. In addition, I expected lifetime reproductive success to increase with lifespan because the number of breeding events strongly increases with duration of life.
- Promiscuity can be adaptive not only for males but also for females. In **chapter 5** I scrutinized the phenotypic traits of females that may covary with their promiscuity, including feather morphological and colouration traits. I had no explicit predictions on the direction of any such relationship and we therefore interpreted any statistically significant relationship *a posteriori*. Moreover, we investigated whether female proneness to engage in extra-bond fertilization may be affected by the attractiveness of the social mate or, according to the mate compatibility hypothesis, the composition of the breeding pair also accounted for variation in female promiscuity.
- In **chapter 6** we carried out a meta-analysis of the intensity of sexual selection on the largest database to date for a single species, the Barn swallow, expressed as the strength (effect size) of the relationships between different plumage ornaments (tail length and asymmetry, size of white spots on tail, ventral and throat plumage colour and throat patch size) which have been shown to vary greatly among individuals within each sex and have previously been suggested to be relevant in intra- and inter-sexual interactions and several fitness proxies related to arrival date from spring migration, survival, reproduction, parental care and offspring quality. Moreover, we investigated whether sexual selection on different sexually dimorphic traits varied among subspecies; a difference in sexual selection among geographical populations would be consistent with a role of sexual selection in promoting phenotypic divergence and speciation.

2.3 Study species: the Barn swallow

The Barn swallow, (*Hirundo rustica*) (Figure 2), is one of 83 species of passerine birds belonging to the *Hirundinidae*, the family comprising martins and swallows (Turner, 2006). The small, highly aerodynamic body has a length of 17-19 cm, a wing span of 30-35 cm and a weight of about 20 g (Møller, 1994; Turner, 2006). The ventral part of the body has a whitish to rufous colouration, throat and forehead are brownish and the dorsal feathers are of an iridescent dark blue colouration. In a preliminary study conducted by Saino and co-workers (2013) on the same population focus of the present thesis, the melanin content of Barn swallow feathers from throat and ventral regions was quantified, and the covariation between feather colouration and absolute or relative concentration of either melanin form and their sex- and age- variation were investigated. The concentrations of eu- and pheomelanin, larger in throat than in ventral feathers, were poorly correlated both within and among plumage regions; moreover, both the absolute and relative concentration of the two melanin forms did not differed according to age. In both males and females, ventral feather colouration was associated with the absolute concentration of the two melanin forms, while the relative amount of either melanin form in the ventral feathers significantly predicted colouration only among males. In throat feathers, on the other hand, colour was predicted only by pheomelanin concentration in both sexes and pheo- to eu-melanin ratio in females.

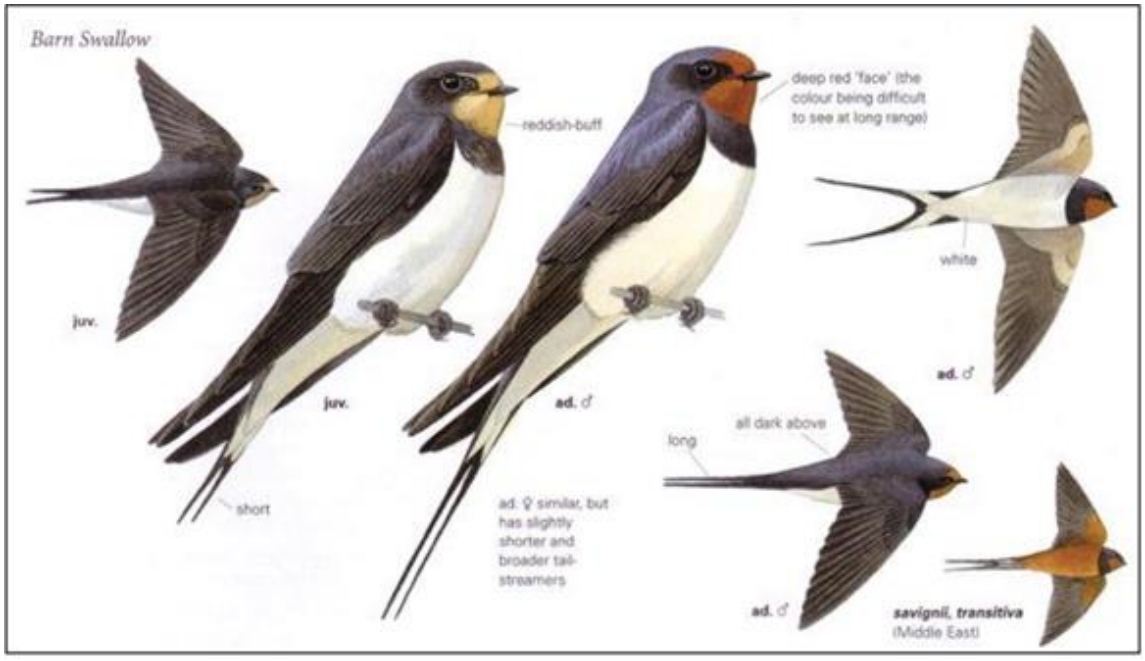

Figure 2. The Barn swallow. Collins Bird Guide

Sexual dimorphism in Barn swallow is small, and the main trait that allows to distinguish between the two sexes is the length of the outermost tail feather, longer in males (mean innermost tail feathers: 45mm, mean outermost tail feathers: 104 mm) than in females (mean innermost tail feathers: 45mm, mean outermost tail feathers: 90 mm) (Møller et al,. 1995). The sex of chicks and juveniles is morphologically indistinguishable before the first complete plumage moult, that takes place during the first wintering period in Africa; the colouration is paler than in the adult form and the tail feathers are considerably shorter. Nestling sex can thus be determined only by molecular techniques, for example by analysing the CHD gene (chromohelicase-DNA-binding gene), located on sex chromosome, that comes in two different forms: CHD-Z, with higher intron size, and CHD-W, with lower intron size. In birds, females are the heterogametic sex (W and Z sex chromosomes) and males are the homogametic sex (ZZ sex chromosomes): thus, a PCR amplification and the subsequent electrophoresis allows to distinguish between females, characterized by two amplicon bands, and males, characterized by a single amplicon band (Griffith et al., 1998, Saino et al., 2008).

The optimal habitat of this species is strictly interconnected to its trophic habits: the Barn swallow is an insectivorous bird that feeds almost exclusively on flying insects that can be found in association with livestock farming. During the last decades, European Barn swallow populations declined, probably due to factors in both wintering and breeding areas, including the progressive abandonment of traditional cattle sheds associated with presence of hayfields and pastures, higher insect availability and warmer indoor temperatures, in favour of less suitable modern and intensive sheds (Møller, 1994, Ambrosini et al., 2002, Turner, 2006). The six subspecies of Barn swallow have a widespread breeding distribution that encompasses the entire Holarctic region (Dor et al., 2010). The nominate *H. r. rustica* breeds in Europe, except the polar region, North Africa and Western Asia, while *H. r. erythrogaster* breeds across most of North America and Argentina. *H. r. gutturalis* and *H. r. tytleri* are the two Asian subspecies breeding from South to East Asia and in Northwest Asia, respectively. The four aforementioned subspecies migrate to tropical regions in Autumn for the wintering period and arrive in the breeding quartiers by the end of March-May. The two remaining subspecies are non-migratory and have a narrower distribution: *H. r. savignii* is found only in Egypt, and *H. r. transitiva* in the Middle East.

Importantly, Barn swallows of both sexes have extremely high breeding philopatry (Møller, 1994). Hence, birds can be followed throughout their life, and individuals that do not return to the colony where they bred the previous year can confidently be assumed to have died. Moreover, natal dispersal is very high (Balbontin et al., 2009), with the vast majority of yearling recruits immigrating from a colony different from their original one. Males usually arrive at the breeding ground earlier than females and establish a territory where they attract females by displaying their ornamental outermost tail feathers and by singing. Males and females build the nest where the female lays from 1 to 7 eggs, that incubates for approximately 14 days; hatch asynchrony is small, although not negligible. Parental cares are performed by both parents. Altricial nestlings fledge at an age of 18-20 days and parents eventually laid a second and even a third clutch. Nestling mortality is very low, sex-independent and mainly due to stochastic events. Barn swallows are socially monogamous, and pairs usually remain together in the same breeding season and in consecutive years. However, the proportion of offspring that are sired by a male different from the social father is high, although temporally and spatially variable, as is the frequency of broods where at least one offspring is sired by an extra pair male (Møller and Tegelstrom, 1997; Saino et al., 1997; Kojima et al., 2009).

2.4 Colour measurements

Birds depend on vision to gain information about the environment around them, more than any other animal *taxa*. Researchers involved in studies on birds necessarily need to take into account the way in which their visual systems work. Human colour vision, compared to that of birds, is not particularly well developed, relying on only three types of retinal cone photoreceptors that allow a three-dimensional ("trichromatic") mapping of colours, by adsorbing long, short and medium wave portions of the light spectrum. In contrast many vertebrates, including birds, have retained visual systems presumably similar to that found in the fish-like ancestor of the subphylum, that involves four types of retinal cones, which support tetrachromatic colour vision (Bowmaker and Hunt, 1999). The consequence of tetrachromacy is that birds see the world differently from humans. Colour vision does not provide a spectroscopic measure of the spectral reflectance of objects in the environment; instead, the neurons of the visual system map spectral radiance into a perceptual colour space that depends on the spectral sensitivities of the retinal receptors and on the neural processing in the central nervous system. Thus, colours, as perceived or assessed by an animal, do not provide an unambiguous measure of spectral radiance but are a species specific abstraction (Endler, 1990). For this reason, it is not possible to extrapolate from human colour experience to that of birds. Besides tetrachromacy, there are two other physiological differences that limit our appreciation of a bird's view of the world. First, most of the retinal cones of birds contain oil droplets that, by virtue of their high carotenoid content, act as spectral filters, narrowing the spectral sensitivities (Goldsmith, 1990). Second, since bird lens and cornea are UV transparent, birds are sensitive to ultraviolet wavelengths, whereas humans are not. Thus, the differences between human and avian vision imply that human vision is inappropriate for studying bird visual behaviour.

Very different methods have been historically adopted to analyse bird colours, among which the most used have been:

1. colour-ranking, based on some arbitrary, predetermined scale that may take into account colourfulness or vividness of colour; similarly, a rank series may be created based on different colour morph and, in the field, the individuals are compared to this catalogue;
2. colour swatch matching, accomplished by the visual matching of a reference colour swatch with the bird colour patch under standardized light condition;

3. photography. Photographs can be analysed using different software, although the accuracy of pictures is related to ambient light and quality of the camera. Moreover, pictures must be standardized including colour swatches in each photograph;

4. digital colour meters, designed to calculate tristimulus variables from measured reflectance spectra. This method, as well as the three listed above, does not provide information about the UV portion of the light spectrum that birds can see and thus does not allow to exhaustively describe colour as perceived by birds;

5. reflectance spectrometry. The most common method for measuring bird colour today is to determine a complete reflectance spectrum in the bird visible range using a spectrometer and then to calculate some indices of hue, saturation and brightness from those spectral data. In the present thesis, reflectance of one, randomly chosen ventral feather was recorded by means of an Avantes DH-2000 spectrometer equipped with a deuterium-tungsten halogen light source in a dark chamber and over a black background. Reflectance of the samples was always referred to white and black standards. The illuminated field covered an area of 2.5 mm^2 centred in the white to brownish region placed approximately at 2.5 mm from the distal end of the feather. Reflectance spectra were subsequently processed to quantify colouration according to the tetrachromatic colour space model using TetraColorSpace program (Version 1a) (Stoddards and Prume, 2008) run in MATLAB 7 (MathWorks, Natick, MA). This approach incorporates information on plumage reflectance spectra with those regarding bird cone sensitivity functions, allowing to estimate the relative stimulation of the retinal cones and thus to better model the colour as perceived by birds. The idealized stimulus QI of each retinal cone type by the reflectance of a colour patch can be estimated as: $QI = \int_{300}^{700} R(\lambda)Cr(\lambda)d(\lambda)$, where R(λ) is the colour reflectance spectrum and Cr(λ) is the spectral sensitivity function of cone type r. I assumed UVS cone type retina and adopted spectral sensitivity of the Blue tit (*Cyanistes caeruleus*), the species more phylogenetically close to the Barn swallow for which spectral sensitivity information is implemented in TetraColorSpace program. Idealized stimulation of the four cones were normalized to a sum of 1, so that each colour patch can be described in the tetrahedral colour space by a vector of {uv, s, m, l} values representing the relative stimulations of the ultraviolet-, short-, medium-, and long-wavelength-sensitive cones, respectively. Each colour vector in the tetrahedral colour space is then transformed to Cartesian coordinates that are subsequently converted into the spherical coordinates theta (θ), phi (φ) and rA (Figure 3). θ and φ represent the red-green-blue and the ultraviolet

components of hue, respectively, while rA is a measure of colour saturation. In the range of colours of Barn swallow throat and ventral feathers, increasing θ values indicate paler colouration. No verbal description can be provided for variation in φ because this mainly reflects ultraviolet colour components which cannot be sensed by the human eye. Because the colour space is a tetrahedron and not a sphere, different hues vary in their maximum potential chroma (rmax). Thus, in the analyses we used the 'achieved chroma', computed as rA = r/rmax. Repeatability of the three colour variables as estimated by measuring twice the same feather was high, as well as among-feathers repeatability of the three colour variables estimated by measuring two different feathers from the same region (Saino et al. 2013). Moreover, as demonstrated in Romano et al. (2015), the reflectance measurement of one ventral feather shows high consistency with the same measurement performed on three overlapping feathers and on the bird's body.

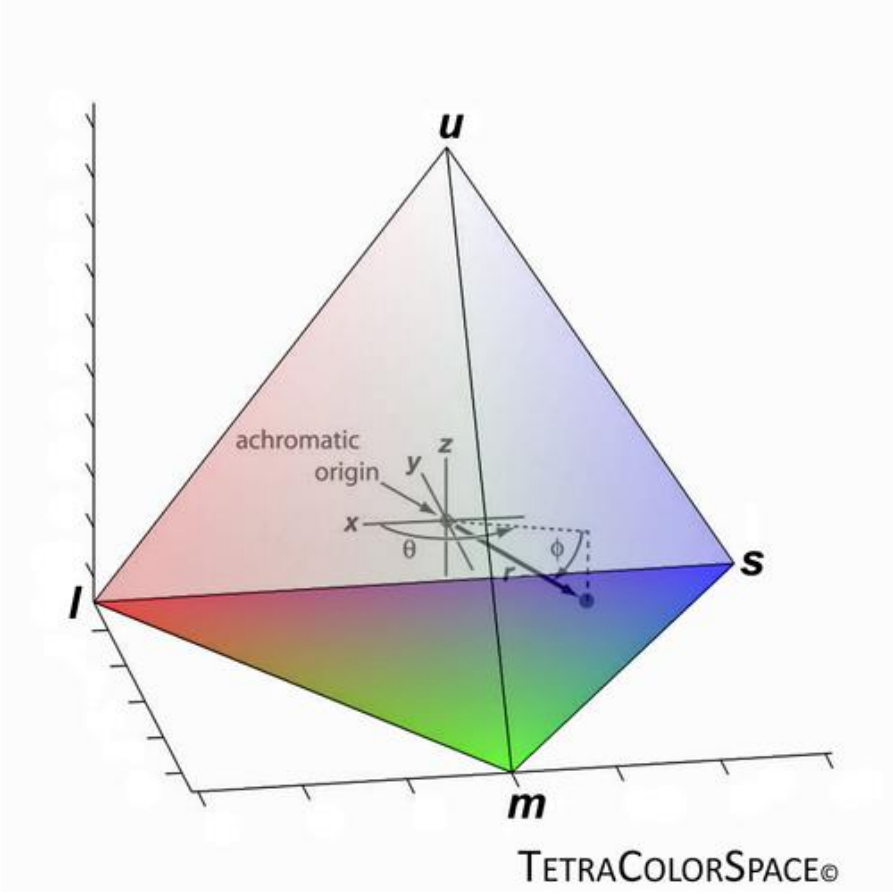

Figure 3. Tetrahedral colour space. Stoddard and Prum, 2008.

3. Outline of the study

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In chapter 1 and chapter 2 of the present thesis I investigated whether ventral plumage colouration could be a reliable signal of nestling individual quality and whether this trait could affect parental allocation strategies. In chapter 3, the covariation between ventral plumage colouration and individual quality have been investigated in adult birds.

Since biological parents are equally related to all their offspring, a similar parental allocation of limiting resources among the progeny should be expected (Trivers, 1974; Royle et al., 2012). However, when the quality of individual offspring differs, parents are expected to tune their parental investment accordingly (Mock and Parker, 1998; Lessells, 2002; Royle et al., 2012). Offspring quality should be related, for example, to their sex: indeed, male and female offspring may differ in several traits such as susceptibility to parasites or rearing conditions (Tschirren et al., 2003; Bize et al., 2005; Siefferman et al., 2007), absorption of food and energy expenditure (Martins, 2004) or competitive ability (Boncoraglio et al., 2008). Moreover, since parental lifetime fitness is profoundly influenced by the resource allocation strategy adopted, natural selection has favoured the evolution of offspring phenotypic traits, such as plumage colouration, that honestly convey reliable information about their quality (Kilner and Johnstone, 1997; Royle et al., 2012) allowing parents to adaptively modulate their investment and parental care (Saino et al., 2000; de Ayala et al., 2007; Aviles et al., 2011). However, not only the quality, but also the number of offspring that an individual can afford to produce at any breeding attempt can influence the evolution of breeding strategies (Roff, 1992). Despite in studies of reproductive trade-off between number and quality of nestlings offspring fitness has been estimated by focusing on specific classes of traits, such as development and somatic growth (Saino et al., 1997; Soler et al., 2003), the identification of general physiological mechanisms behind this trade-off remains to be elucidated. Recent studies have pointed at a major role of telomeres in mediating individual response to a host of endogenous and extrinsic factors such as environmental and social stress, including the number of competing siblings (Haussmann et al., 2012; Herborn et al., 2014). Telomeres are nucleoprotein complexes located at the ends of eukaryotic chromosomes that shorten at each cellular division (Palm and de Lange, 2008). Since telomere shortening below a certain threshold depresses organismal performance (Blackburn, 1991), and shortening rate depends on environmental conditions, telomeres are good candidates as mediators of reproductive trade-offs (Monaghan, 2010). Therefore, parents are expected to bias their

resource allocation toward offspring showing reliable proxies of large telomere length and/or low shortening rate. Moreover, since individual quality is strongly influenced by telomere dynamics we can hypothesize that plumage colouration, being a signal of telomere length, may also be relevant, in adults, in sexual selection processes. According to sexual selection theory, individuals of the sex investing more in reproduction should prefer high-quality individuals as mates because this will enhance their fitness. However, the components of variation in individual quality that are the target of mate choice are difficult to identify.

In **chapter 1** we manipulated siblings to obtain dyads of the same sex and similar body mass where one nestling was experimentally darkened, while the sham-coloured sibling served as control; we then compared both their mass variation and the proportion of feeding received during feeding trials of 90 minutes. The contour feather colouration is known to be heritable (Saino et al., 2013) and, in different populations, variation in reproductive success according to plumage colour is larger in males than in females (Romano et al., 2016); moreover, colouration at the nestling stage predicts colouration in adulthood (Hubbard et al., 2015). Because of the potential reproductive advantage for males showing darker ventral feathers, parents were expected to provide more food to their experimentally darkened male offspring, while this parental favouritism was not expected among females. The obtained results confirmed our hypothesis: experimentally darkened males, but not females, obtained more food and gained more mass than the control siblings. Thus, parents should enhance they future fitness by allocating more resources to darker sons, since they are expected to have shorter pre-laying period, larger fidelity by social mate and more success in sperm competition, while brownish females only produce slightly larger clutches than paler ones Moreover, a non-mutually exclusive interpretation regards the hypothesis that parents invest more in darkened nestlings because their darkness reliably signals better body condition or quality compared to paler siblings, as shown in other species.

In **chapter 2**, I tested the hypothesis that the trade-off between offspring number and quality is mediated by the negative consequences that adverse rearing conditions have on telomere dynamics; moreover, the covariation between telomere length and ventral plumage colouration was investigated. A recent study has demonstrated that telomeres undergo significant shortening during the Barn swallow nestling stage but smaller shortening later in life, implying that nestling stage is crucial to telomere dynamics

(Parolini et al., 2015). I thus altered nestling social environment by manipulating brood size and measured telomere length in blood cells 12 days after hatching, when colouration of contour feathers has just become visible and cannot influence parental allocation of resources. The documented effects of brood size manipulation on specific fitness proxies and competitive interactions among siblings, and sensitivity of telomere dynamics to rearing conditions led us to expect that nestlings in enlarged broods had reduced telomere length at growth completion as compared to nestlings reared in reduced broods. We found that telomere length was larger in nestlings from reduced as compared to enlarged broods, suggesting that telomere dynamics may be one of the mechanisms that mediate the trade-off between number and quality of offspring. Moreover, we provided unprecedented evidence that signals involved in parent-offspring communication, such as ventral plumage colouration, reflect telomere length and thus offspring reproductive value.

In **chapter 3** we suggested, for the first time in any wild organism, that variation in telomere length may be a major component of variation in adult individual quality, and may therefore covary with the expression of sexually dimorphic traits potentially under directional inter-sexual preference, such as melanin-based ventral colouration. The aims of chapter 3 were thus threefold. 1. to test if telomere length covaries with seasonal reproductive success; 2. to test if ventral plumage colouration reflects telomere length; 3. to analyse the relationship between colouration and seasonal reproductive success. This study was only correlative and involved information on reproductive performance of 65 breeding pairs. The relationship between seasonal breeding success, measured in terms of number of nestling fledged, and telomere length was in the expected direction, according to the hypothesis that large telomere length enhances individual performance. Since assortative mating for telomere length exist in the same Barn swallow population studied here (Khoriauli et al., submitted), an explanation of the relationship between telomere length ad seasonal reproductive success is that high-quality males with long telomeres mate with relatively more fecund females which also have long telomeres, and/or that pairs with relatively long telomeres perform better at parental duties. In chapter 3 we also found a covariation between telomere length and ventral plumage colouration, that may serve to explain the assortative mating found for telomere length, as individuals of both sexes can use colouration to adaptively choose high-quality mates. Moreover, in chapter 3 we showed for the first time in any European Barn swallow population that individual variation in adult ventral plumage colouration predicts annual reproductive success.

However, in the same study population darker males suffer a viability disadvantage compared to paler males (Saino et al., 2013), suggesting that fecundity and viability selection may concur in maintaining extensive, genetically based polymorphism in colouration.

The association between phenotypic traits, including ventral colouration, and sexual selection is also the focus of chapter 4 and chapter 5.

Variance in male reproductive success can arise in the context of sexual selection processes (Andersson, 1994), whereby males differ in the number and/or the quality of the females that they can monopolize and whose preference is often non-randomly distributed with respect to the expression of male ornamental traits (Jennions and Petrie, 1997; Møller and Ninni, 1998; Wong and Candolin, 2005); moreover, it can be affected by the success of males in securing the paternity of the offspring generated by their social mate (within-pair offspring) and in siring offspring by fertilizing females different from their social mate (extra-pair offspring) (Lebigre et al., 2012). Despite numerous studies have quantified sexual selection and reproductive success with respect to heritable traits in single breeding season, only few studies have investigated the role of sexual selection on multiple sexual ornaments by estimating reproductive success realized by males of iteroparous species during their entire life cycle, combining information regarding the duration of life, that affects the lifetime number of reproductive events, and the number of viable offspring produced per breeding event. Indeed, estimate lifetime reproductive success in species with intense sperm competition is difficult, as it requires a large effort of marking and monitoring of all the individuals in a population during their entire life; moreover, 'edge effects', whereby the study sample reproductively interacts with the individuals breeding just outside the study area, can lead to inaccurate lifetime reproductive success estimates due to missed paternity events by the focal males (Webster et al., 1995, 2001; Sheldon and Ellegren, 1999). In addition, collecting exhausting data on all within- and extra-pair offspring sired by individual males may be hardly feasible. However, promiscuity should be adaptive not only for males but also for females. According to the hypotheses formulated to explain the evolution of female promiscuity, females may acquire either direct (Birkhead and Møller, 1992; Sheldon, 1994; Nakamura, 1998) or indirect benefits for their progeny, if the extra-bond male carries 'compatible' genes with those of the choosy female or if is of superior genetic quality as compared to the social mate (Møller and Ninni, 1998; Jennions and Petrie, 2000). However, since many studies have failed in

identifying any net advantage of promiscuity to females, it has been speculated that female promiscuity can also arise as a consequence of the positive genetic correlation between male and female sexual behaviour (Forstmeier et al., 2014). Such correlation may operate both at the within-sex level, with females more responsive to courtship by their social mate being also more responsive to courtship by extra-bond males, and at the between-sexes level, with genes that promote adaptive sexual promiscuity in male offspring also having positive pleiotropic effects on promiscuity in daughters. These hypotheses rest on the implicit assumption that females can 'intrinsically' differ in promiscuity, and such individual variation is the target of selection driven by the benefits and costs of extra-bond fertilizations.

In **chapter 4** I identified parentage of all the 829 offspring produced at three colonies over three years (2013-2015) and measured lifetime reproductive success of males (including extra-pair paternities) to estimate selection differentials and partial selection differentials (i.e. selection gradients controlling for the effect of selection on correlated traits) on lifespan, on different secondary sexual traits (such as ventral colouration, length and asymmetry of outermost tail feathers and white spots on tail) which have been shown to have a role in natural and sexual selection in one or more of the geographical populations/subspecies of this species and "ordinary" (i.e. non-sexually-selected) traits, such as wing size, that is known to affect flight performance, and tarsus length, that is a proxy of body size. I found selection on lifespan mediated both by within- and extra-pair fertilization success and selection on tail length mediated by within- but not extra-pair fertilization success. In addition, I found selection on tail white spots via extra-pair fertilization success after controlling for selection on other traits. Conversely, plumage melanin-based colouration seems not to be currently under directional selection mediated by reproductive success. Hence, natural and sexual selection mediated by lifespan reproductive success operates on lifespan, tail length and size of the tail white spots in Barn swallows.

In **chapter 5** I exploratively investigated whether female promiscuity, i.e. the proneness of females to engage in extra-bond fertilizations, can be predicted by a number of different phenotypic traits, including size-related traits (bill length, keel length, wing length, length of the innermost tail feather), ventral colouration and the expression of sexually dimorphic traits currently under directional sexual selection in males (outermost tail feather length), while controlling for age effects. Moreover, I investigated whether female promiscuity is

an attribute of the social breeding pair, in accordance with the genetic compatibility hypothesis, or is affected by the attractiveness of the social mate, measured in terms of tail length that, in chapter 4, was demonstrated to represent the only morphological trait of males to predict paternity in our study population. I thus relied on extensive genetic parentage assessment of offspring to test for variation in intrinsic promiscuity of 91 females and their 89 social mates that were monitored during 201 multiple breeding events over three years. The results obtained in the present studies showed that paternity (the proportion of offspring in a brood sired by the social father) depended not only on the tail length of the social male but also on morphological and colour traits of the mother. This finding is in accordance with the hypothesis that female promiscuity can represent an individual intrinsic characteristic, and female traits reflecting promiscuity exist. Since, in the Barn swallow, assortative mate choice have been showed to exist, a male should rely on these traits to choose as social mate a faithful female because this will increase his within-pair reproductive success.

In the last chapter of this thesis, **chapter 6**, we conducted a meta-analysis on the intensity of sexual selection in the Barn swallow, expressed as the strength of the relationships between plumage ornaments that have been suggested to be relevant in intra- and inter-sexual interactions in the six Barn swallow subspecies (tail length, tail asymmetry, white spots on tail, ventral plumage colour, throat plumage colour and throat patch size) and several fitness proxies concerning reproduction, parental care, offspring quality, arrival date from spring migration and survival. Meta-analysis is defined as the quantitative summary of research domains, and it refers to a specific set of statistical quantitative methods that are designed to compare and synthesize the results of multiple studies (Arnqvist and Wooster, 1995). We thus tested whether the intensity of sexual selection varied between sexes, according to age and during the course of the breeding season. In addition, we investigated whether the intensity of sexual selection varies among different plumage ornaments and among subspecies; we also tested the effect of the interaction between plumage ornament and subspecies to investigate whether the intensity of sexual selection on different plumage ornaments varied among subspecies, since a difference in the patterns of sexual selection among geographical populations would confirm the role of sexual selection in promoting phenotypic divergence and speciation. The strength of the relationship between plumage ornaments and fitness proxies has been estimated in terms of effect size. The intensity of sexual selection was found to be stronger in males than in

females, while no difference existed between individuals of different age. The existence of a selection force acting on female ornamental traits should be explained by both a directional mate selection for conspicuous female ornament or by a positive genetic correlation in plumage traits between sexes. The intensity of sexual selection was found to change across the different breeding stages, spanning from arrival date from spring migration to parental care of the first brood, being higher at the beginning of breeding season and progressively decreasing starting from egg fertilization. This result is in accordance with the observation that competition for a mate and for paternity occurs during the pre-zygotic, most promiscuous part of the breeding cycle. While the intensity of sexual selection did not varied across subspecies and plumage ornaments, sexual selection on different plumage ornaments varied among the six subspecies and, in particular, between the phylogenetically less related subspecies. This result is consistent with the hypothesis that sexual selection plays a major role in speciation processes, since sexual selection of different traits may result in divergence among geographical populations and hence in a differentiation among taxa (Kirkpatrick and Ravigné, 2002).

4. Conclusion

4. Conclusion

The present thesis was aimed at deepening the knowledge about the covariation between melanin based colouration and different life-history traits by investigating the role of ventral plumage colour in the assessment of individual quality and, consequently, to investigate a possible role of ventral plumage colouration as mediator of sexual selection using the European Barn swallow (*Hirundo rustica rustica*) as a model species. To this aims, I used both correlational and experimental approaches and, moreover, I used a meta-analytic approach to review the entire literature regarding sexual selection on different morphological traits, including ventral plumage colour, in the focal species.

First, this thesis provides novel findings about the role of plumage colouration as a signal of individual quality. Indeed, contrary to carotenoid-based colouration, that is known to be sensitive to environmental factors such as food availability, body condition and parasites, melanin based colouration is under strong genetic control and, because of this reason, it was hypothesized to not reveal individual quality. However, in the last years, a large number of studies demonstrated that, contrary to the past believes, the expression of melanin-based pigmentation covaries with several physiological and behavioural traits, including immune or hormonal functions, response to stressors and aggressiveness. The second main finding of this thesis is that, being a proxy of individual quality, melanin-based colouration can be exploited in intersexual interactions, and individual fitness may thus be influenced by the expression of melanin-based sexually selected traits.

In **chapter 1** we experimentally demonstrated that nestling plumage colouration drives the allocation of parental cares, in term of food provisioning, provided by both parents. To the best of our knowledge, the only previous study that investigated a parental favouritism according to nestling plumage colour, performed on the Eastern Bluebird (*Sialia sialis*), showed a preferential post fledging defence behaviour toward the most ornamented sons (Barrios-Miller and Siefferman, 2013) and a preferential food allocation toward brighter males (Ligon and Hill, 2010). Our novel results are therefore consistent and complementary to those obtained in the Eastern Bluebird, providing further evidence on the parental ability to differentially invest in male and female offspring according to a trait expressed during early development, which will be involved in intersexual competition for access to mates at sexual maturation.

In **chapter 2** I further investigated the association between melanin-based colouration and individual quality by testing the covariation between ventral plumage colouration and telomere length. Darker colouration was found to reliably reflect telomere length of individual nestlings and, as a consequence, broods with relatively dark colouration can be perceived by parents as consisting of nestlings with on average longer telomeres, and thus better quality, than broods with relatively pale nestlings. Unfortunately, to the best of our knowledge, no mechanistic links have been proposed so far between melanogenesis and telomere dynamics, despite an interplay might be suggested by the observation that altered telomerase activity affects the expression of tyrosinase, an enzyme involved in early melanogenesis pathways (Bagheri et al., 2006). In addition, a covariation between colouration and telomere length may be established thanks to the pleiotropic effects of the melanocortines, which may also influence telomere dynamics.

The findings of chapter 2 are therefore compatible with the idea, expressed in chapter 1, that the observed parental favoritism for darker nestlings reflects adaptive parental strategy of preferential allocation of care to the offspring with larger expected reproductive value.

The expected association between plumage colouration, telomere length and reproductive success was tested in **chapter 3**, where it has been correlatively investigated in adult birds. Seasonal reproductive success has been shown to be predicted by ventral plumage colouration, that ultimately signals telomere length. The approach of this study was, unfortunately, only correlative, because an experimental manipulation of telomere length would be hardy feasible in the wild. Thus, future studies should be designed to modulate, for example, the activity of the telomerase, either to promote the elongation of telomeres or to reduce their attrition (de Jesus et al., 2011).

Despite, in chapter 3, an association between plumage ventral colour and seasonal reproductive success has been disclosed, this association was not found in **chapter 4**, where I analysed the intensity of sexual selection on lifespan and sexually selected traits during an individual lifetime by considering its success both in within- and between-pair fertilization. Indeed, in this chapter, lifetime reproductive success was demonstrated to be strongly influenced by the number of breeding season experienced by an individual, by the length of the outermost tail length and by the area of white spots on tail. However, no association between lifetime reproductive success and ventral plumage colouration was found. Interestingly, in the same Barn swallow population studied in the present thesis, darker males suffer a viability disadvantage as compared to paler conspecifics (Saino et al.,

2013), suggesting that conflicting fecundity and viability selection may differently influence the seasonal and lifetime reproductive success of an individual. Moreover, the results of chapter 4 are consistent with the meta-analytic results of chapter 6, indicating that different plumage ornaments are differently selected in distinct Barn swallow subspecies.

In studies of extra-pair paternity and sexual selection, the role of female traits is generally under-studied. However, to gain a holistic understanding of extra-pair mating behaviour, it is imperative to gain a better understanding of variation in female intrinsic promiscuity. The results of **chapter 5** suggest that females differ in promiscuity independently of the identity of the social mate and of the positive effect of sexual ornamentation of the social male mate on paternity. Hence, phenotypic female traits that reliably reflect female promiscuity, such as feather morphological and colouration traits, exist, and may therefore affect male choice of social as well as of potential extra-pair mates. In addition, the results of chapter 5 lend support to the hypothesis that female realized promiscuity depends on compatibility with the social male mate, since extra-pair fertilizations have been suggested to depend on the specific composition of the breeding pair.

Finally, in **chapter 6** we conducted a meta-analysis of sexual selection in the Barn swallow, a classic model system for studies of sexual selection thanks to its extremely high breeding philopatry, the synantrophy, relative low longevity, ease of capture and accessibility to the nest and the large number of extra-pair offspring. Thanks to these characteristics, we have been able to gain information from the literature regarding all the six subspecies found across the Holartic region. Intensity of sexual selection on morphological traits has been found to be stronger in males than in females and more intense in the pre-zygotic, most promiscuous part of the breeding cycle, when competition to acquire a mate is intense. More important, this work provide a quantitative meta-analytical support for the evidences that different ornaments are subject to different selection regimes among geographically distinct populations, and particularly between the phylogenetically less related subspecies. This is consistent with the hypothesis that sexual selection plays a major role in speciation processes, since it may result in divergence among geographical populations and hence differentiation among taxa.

5. References

5. References

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6. Chapter 1

Nestling sex and plumage colour predict food allocation by barn swallow parents.

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Original Article

Nestling sex and plumage color predict food allocation by barn swallow parents

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Despite parents are equally related to all of their progeny, they may differentially invest in offspring that provide the highest fitness return. Sons and daughters can differ in reproductive value, especially in species where fitness is predicted by the expression of sexually selected traits. In many birds, offspring plumage coloration functions as a honest signal of individual quality, thus allowing parents to differentially invest in offspring of either sex accordingly. Here, we tested whether parents allocate different amounts of food depending on plumage color of their male and female offspring. As a model, we used the barn swallow (*Hirundo rustica*), a species where large among- and within-brood variation in ventral plumage color exists and male reproductive success varies according to ventral plumage coloration. We recorded the proportion of feedings obtained and body mass variation by dyads of same-sex and similar-sized nestlings subjected to either experimental darkening of their ventral plumage color or to a sham treatment. Plumage darkening enhanced food provisioning and body mass gain of males but not of females. Because darker ventral coloration is associated with larger reproductive success in male barn swallows, these results suggest that parents tune their effort toward more valuable male offspring that are likely to provide the greatest fitness returns. Our study thus suggests that parents are selected to differentially invest in offspring of either sex according to a trait expressed in early life, which is relevant to intrasexual competition for access to mates at sexual maturity.

Key words: **food allocation, parental investment, parent–offspring conflict, plumage color, sexual selection, sibling competition.**

INTRODUCTION

In multiparous species with altricial offspring, parents have to decide how to partition limiting resources among the progeny in order to maximize their fitness. Because biological parents are equally related to all of their offspring, an even parental investment in individual members of the progeny should theoretically be expected (Hamilton 1964; Trivers 1974; Royle et al. 2012). However, whenever individual offspring differ in quality and viability, parents will benefit from providing greater care to the more valuable descendants (Haig 1990; Mock and Forbes 1995; Stenning 1996; Mock and Parker 1997, 1998; Lessells 2002; Glassey and Forbes 2002; Royle et al. 2012).

One crucial source of variation in offspring reproductive value is sex: Owing to fundamental genetic differences, male and female offspring may differ in several characters, such as developmental trajectories (Seller and Perkins-Cole 1987; Cook and Monaghan 2004), susceptibility to parasites or rearing conditions (Nager et al. 2000; Tschirren et al. 2003; Bize et al. 2005; Siefferman et al. 2008; Romano et al. 2011), absorption of food or energy expenditure (Martins 2004; Vedder et al. 2005), begging for food or competitive ability (Fargallo et al. 2003; Boncoraglio et al. 2008), and hence fitness return per unit parental investment. Parents are therefore expected to tune their parental investment accordingly (Trivers and Willard 1973; Charnov 1982; Lessells 2002; Uller 2006). In addition, sex-related variation in reproductive value is expected to be larger in species where male breeding success depends on the expression of sexually dimorphic traits under female preference (Komdeur 2012). This is the case because of the unequal breeding opportunities for males and females of different phenotypic qualities, leading to a larger variance in reproductive success of males (Trivers and Willard 1973). Indeed, high-quality males are expected to produce more offspring than females of the same quality, whereas the opposite holds true for poor-quality individuals.

Because decisions by parents about resource allocation in a brood according to offspring reproductive value and sex can have profound consequences on their lifetime fitness, natural selection should have favored the evolution of offspring phenotypic traits that convey reliable information about their quality (Kilner and Johnstone 1997; Wright and Leonard 2002; Royle et al. 2012), allowing parents to adaptively modulate their investment (e.g., Kilner 1997; Saino et al. 2000; Fargallo et al. 2003; de Ayala et al. 2007; Dugas 2009; Avilés et al. 2011). In birds, nestlings

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may honestly display their quality, in terms of current need of care or condition, by means of multiple visual and acoustic signals (Johnstone 1995, 1996; Kilner and Johnstone 1997; Wright and Leonard 2002), and parents may also exploit various other types of reliable cues (e.g., body mass) potentially revealing the reproductive value of their progeny (see Kilner and Johnstone 1997; Wright and Leonard 2002; Royle et al. 2012). Although it is rare for immature birds to have conspicuous feather coloration (but see Krebs and Putland 2004), increasing evidence exists that plumage chromatic traits also serve as signals of offspring quality to parents (Galván et al. 2008; Tanner and Richner 2008). Indeed, many studies have demonstrated that nestling plumage coloration can be a condition-dependent trait, which may, for example, mirror parasites infection, nutritional state, or rearing conditions (Hõrak et al. 2000; Tschirren et al. 2003; Fitze et al. 2003; Jacot and Kempenaers 2007; Siefferman and Hill 2007; Siefferman et al. 2008; Parejo and Silva 2009). In addition, it has been shown that the expression of ornamental plumage traits in juveniles may also influence the level of parental care (Lyon et al. 1994; Griggio et al. 2009; Ligon and Hill 2010; Barrios-Miller and Siefferman 2013; but see Tschirren et al. 2005).

Because plumage coloration honestly signals individual quality to competitors and potential mates in sociosexual contexts (see Roulin 2004; Hill and McGraw 2006; McGraw 2008 for reviews), in species where nestlings plumage color shows large interindividual variability, parents may differentially invest in male and female offspring according to their feather coloration. In particular, when variation in heritable plumage color traits is related to reproductive success in one sex, parents should favor the offspring of the sex that will benefit the most by displaying such traits (i.e., generally males). To date this hypothesis has received little attention: To our knowledge, only 2 studies on the same species, the eastern bluebird (*Sialia sialis*), provided evidence of sex-related parental care according to offspring plumage color traits that are associated with fitness traits in adults (Ligon and Hill 2010; Barrios-Miller and Siefferman 2013).

Here, we first provide a description of natural among-brood and between-sex variation of barn swallow (*Hirundo rustica*) nestling white to brownish coloration of ventral body feathers. Like in several other Palearctic birds, barn swallow juveniles fledge with a plumage that is a paler representation of that of the adults (Moreno and Soler 2011; Hubbard et al. 2015), suggesting that adult-like plumage in immature individuals could be mainly due to selection pressures on adults (Hawkins et al. 2012). We thus investigated whether parents provide a different amount of food to their offspring according to experimental alteration of nestling plumage. We tested this hypothesis by recording parental food allocation (e.g., proportion of feedings and body mass variation) to dyads of same-sex nestlings subjected to a different manipulation of their ventral contour feathers coloration: One nestling was experimentally darkened within the range of natural variation, while a sham-colored sibling served as control.

In adult barn swallows, ventral plumage shows moderate sexual dichromatism, males having darker pheomelanin- and eumelanin-based ventral coloration than females, but also large within-sex variability (Saino, Romano, Rubolini, Ambrosini, et al. 2013; Saino, Romano, Rubolini, Teplitsky, et al. 2013). The contour feather coloration is heritable (Saino, Romano, Rubolini, Ambrosini, et al. 2013; Hubbard et al. 2015; Vortman et al. 2015), and in different populations, paler individuals of either sex pay sexual selection costs compared with darker ones (Safran and McGraw 2004; Safran et al. 2005; Vortman et al. 2011, 2013), with variation in reproductive success according to plumage color being larger in males than in females (Safran et al. 2005; Vortman et al. 2011). Moreover, within individuals, coloration at the nestling stage predicts coloration in adulthood (Hubbard et al. 2015). Because of the potential reproductive advantage for males showing brownish ventral feathers, parents were expected to favor their male offspring displaying experimentally darkened plumage by providing them with more food compared with the control sibling. This parental favoritism was not expected among females.

MATERIALS AND METHODS

Study species

The barn swallow is a small (ca. 20 g) semicolonial migratory passerine bird, breeding synanthropically in rural buildings. Females lay 1–3 clutches of 1–7 eggs (modal size: 5 eggs) per breeding season and incubate them for circa 14 days (Møller 1994). Nestlings are fed by both parents and display sex-related begging features, thus allowing parents to recognize offspring of either sex (Saino et al. 2003; Saino, de Ayala, et al. 2008). Nestlings fledge when they are circa 20 days old, and parents usually provide food to a single nestling per visit to the nest (Romano et al. 2011, 2012).

General field procedures and sex determination

The present study was carried out between April and July 2014 in 4 barn swallow colonies (= farms) located in the countryside southeast of Milan (Northern Italy). All nests were visited every second day to record breeding events. Considering the day of hatching of the first egg(s) in a nest as the day 0, at day 7 all nestlings in broods with 3 or more nestlings were banded with an aluminum ring. Nestlings were sexed molecularly (Griffiths et al. 1998; Saino, Martinelli, et al. 2008) using a small blood sample (ca. 40 µL) collected by puncturing their brachial vein. We thus identified the sex of all nestlings before the day of the manipulation of plumage color and the assessment of its potential effect on parental food allocation.

Plumage color manipulation

At 15 days of age, we manipulated the ventral plumage color of half of the male and half of the female nestlings (hereafter, "darkened" nestlings) within each brood by using a nontoxic marker (Letraset Promarker, satin, item code Y129). A similar procedure was used to manipulate color of adult barn swallows in previous studies (Safran et al. 2005, 2008; Vitousek et al. 2013). At this age, the ventral part of the body is almost entirely covered by contour feathers and nestlings are already soliciting parental care by protruding part of their body from the edge of the nest cup (i.e., showing to parents not only the gape as younger nestlings do but also the breast plumage; Romano A, personal observation) to achieve an advantage in scrambling for food. A small number (3–5) of feathers were plucked both before and after the experimental treatment for spectrometric color analyses (see below). To control for the effect of marker application, a sham-control treatment was performed on the remaining nestlings of the same broods (hereafter "sham-colored" nestlings) by painting their ventral plumage with a transparent marker (Letraset Promarker, clear blender, item code BL; see also Safran et al. 2005).

Nestlings were randomly assigned to the darkened or control treatment. Whenever the number of nestlings of either sex was

odd, the odd nestling was assigned randomly to either treatment. Overall, we darkened the ventral coloration of 58 nestlings (29 males and 29 females) from 28 broods, whereas 63 siblings served as controls (32 males and 31 females). However, not all nestlings were included in feeding trials (see below).

Feeding trials

To test for a difference in the food allocation between darkened and sham-colored nestlings, we compared both body mass variation and the proportion of feedings received during feeding trials, including pairs of same-sex and opposite-treatment siblings (i.e., darkened male vs. sham-colored male; darkened female vs. sham-colored female; hereafter "dyads"). We decided to focus on dyads of nestlings, rather than on the entire brood, in order to adhere to theoretical models of parental food allocation, which were developed for pairs of offspring (e.g., Godfray 1995; Johnstone 2004), and to simplify the behavioral observations. Because in the barn swallow the sex of nestlings and that of their siblings are known to affect parental food allocation (Bonisoli-Alquati et al. 2011), we tested only same-sex dyads in order to experimentally account for this confounding effect. In addition, because in the barn swallow the allocation of food varies in relation to nestling size (Bonisoli-Alquati et al. 2011), dyads were composed by selecting the 2 nestlings showing the least difference in body mass within the brood.

Feeding trials were performed 10–15 min after the color manipulation in order to allow the plumage to dry after the application of colored and sham marker, and started at 8.00–9.00 AM. Feeding trials were carried out as following: First, we measured body mass of the 2 focal nestlings with an electronic scale (accuracy 0.1 g) and their tarsus with a digital caliper (accuracy of 0.01), randomly marked them on their forehead with 2 white spots (aligned longitudinally or transversally) to make them individually recognizable, and left them in their nest for 90 min while removing all their siblings. Concomitantly, the nestlings not included in the feeding trial were kept in a safe place at ambient temperature. All parental feeding visits were videotaped with a Sony DCR-SR72E camera placed in front of the nest so as to identify which nestling received each food item provided by parents. At the end of the feeding trial, the focal nestlings were weighed again to record body mass variation, indicating individual food intake and also accounting for the balance between cost and benefit of scrambling and for the defecation rate (see also Boncoraglio et al. 2008; Romano et al. 2011, 2012).

When 2 other nestlings of the same-sex and opposite treatment existed in the same brood, the above-described procedures were repeated to obtain information for an additional dyad. However, no nestling was used in more than 1 feeding trial. Although nestlings in different dyads from the same brood had different levels of hunger (i.e., the second dyad was always tested after a period of food deprivation during the feeding trial of the first dyad), we emphasize that nestlings within each dyad were always tested under the same hunger level. Indeed, despite the fact that the feeding rate was greater in second tested dyads compared with first tested ones (Poisson mixed model including dyad order as fixed effect and nest identity as random effect: $F_{1,14} = 20.77$; $P = 0.0004$), dyad order did not affect the quality of results (see Supplementary Materials).

The number of feedings provided by parents to each nestling was counted on video recordings by using VLC Media Player 2.2.1 (Free Software Foundation, Inc., Boston, MA). The proportion of feedings received was computed as the ratio between the number of feedings obtained by each nestling and the total number of feedings delivered by parents to the nest. The proportion of feedings obtained by each nestling was used to assess whether food allocation differed between experimental and control nestlings. However, because feeding rates do not account for variation in quality and size of individual food item, body mass variation during trial was also used to account for the whole food allocation in different nestlings by parents.

In the barn swallow, parental food allocation to individual nestlings is positively related to the intensity of their begging behavior (Saino et al. 2000; Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2012). Thus, we also compared the intensity of postural begging between darkened and sham-colored nestlings. To this aim, the intensity of postural begging (hereafter "begging intensity") during each feeding visit by parents was scored on a 4-level scale ranging from 0 (nestling did neither open the mouth nor move toward the attending parent) to 3 (nestling begged intensely by shaking head and fully stretching neck toward the attending parent) (see Romano et al. 2012 for details).

The whole protocol was accomplished for 36 dyads (18 male and 18 female dyads). For practical reasons (e.g., impossibility of placing the camera in front of the nest), for other 10 dyads (6 male and 4 female dyads), only the data on body mass variation during the feeding trial, not food provisioning data, were available. Before the experimental manipulation of plumage color, no significant difference in body mass and tarsus length was observed between darkened and sham-colored nestlings, both considering all dyads (paired $t_{45} = -0.30$; $P = 0.77$) and when analyses were separated for males (paired $t_{23} = -1.30$; $P = 0.21$) and females (paired $t_{21} = 0.73$; $P = 0.48$).

At the end of the experiment, all nestlings were returned to their nest. We did not observe any nest desertion by parents nor mortality of nestlings during the study.

Control broods

To test for the possibility that a larger investment in the darkened nestlings was rather caused by a disadvantage paid by their sham-colored siblings because of the sham treatment on plumage (i.e., sham-colored nestlings may have been made less valuable for parents by the experimental manipulation), feeding trials were also established on a sample of 11 control broods, including sham-colored and un-manipulated nestlings. Un-manipulated nestlings were handled for the same amount of time as their sham-colored siblings, while on their plumage, we did not apply any colored or sham marker. For practical reasons, only data concerning the body mass variation during the feeding trial were available for these broods. On the whole, feeding trials were carried out for 10 male and 8 female dyads in control nests. No difference in body mass and tarsus length was observed between sham-colored and un-manipulated nestlings before the feeding trials (in both sexes: paired $t < 1.19$; $P > 0.26$).

Color measures

Reflectance spectra of a single ventral feather per nestling were measured in a dark chamber, using an Avantes DH-2000 spectrometer. Every measure was taken twice. Importantly, as previously demonstrated (see Romano et al. 2015), color measurements taken on a single feather, on 3 feathers or directly on ventral plumage of the individuals from which the feathers were plucked provide very consistent estimates (correlation coefficients between the color variable values obtained by the 3 methods were $r > 0.88$ for θ and $r > 0.91$ for both φ and rA). Color quantification was performed

relatively to white and black standards by using the tetrachromatic color space model with the TetraColorSpace program version 1a (Stoddard and Prum 2008). This program also includes information about bird cone sensitivity and therefore provides biologically realistic color metrics (Antonov et al. 2011; Saino, Romano, Rubolini, Ambrosini, et al. 2013). Here, we assumed a ultraviolet-sensitive cone-type retina and used spectral sensitivity of the blue tit (*Cyanistes caeruleus*), the most closely phylogenetically related species to the barn swallow for which information on cone spectral sensitivity is available in TetraColorSpace. For each measurement, the program provided 3 color components: θ, φ, and *rA* (see Stoddard and Prum 2008; Antonov et al. 2011). φ and θ, respectively, reflect the ultraviolet and the red–green–blue components of hue, whereas *rA* is a proxy of color saturation. For barn swallow ventral feathers, decreasing θ indicates darker, brownish coloration and a higher concentration of both eumelanin and pheomelanin (see Saino, Romano, Rubolini, Ambrosini, et al. 2013). Further details on color measures are provided in Supplementary Materials.

The experimental manipulation enhanced ventral plumage coloration within 1 or 2 standard deviation (SD) of the mean value of un-manipulated nestlings depending on the color variable considered (see Supplementary Materials). The sham treatment slightly altered *rA* of nestling plumage coloration. However, we stress that the alteration of the plumage color of the sham-colored nestlings was in the same direction with respect to that observed in darkened nestlings, indicating that our results were conservative (see also Results for analysis of "control broods"). Importantly, after the experimental manipulation (i.e., during the feeding trial), darkened nestlings were significantly darker than their sham-colored siblings (see Supplementary Materials).

Statistical analyses

Natural among-brood variation in ventral plumage coloration was investigated by estimating the contribution of brood identity to the variance of the 3 color variables (θ, φ, and *rA*). This was done by means of variance components analyses, including nest identity as a random factor, while accounting also for the effect of nestling sex included as a fixed factor. The same analyses were also performed on male and female nestlings separately. Broods containing a single nestling of either sex were removed from both analyses. In addition, sex-related variation of color variables was analyzed in linear mixed models (LMMs) including the sex of individual nestling as a fixed effect and brood identity as a random effect. These analyses were performed using ventral feathers plucked before the application of the color marker or the sham marker.

All the analyses on parental food allocation were performed for males and females separately because dyads were composed by same-sex nestlings, thus preventing us to test the effect of the statistical interaction between treatment and sex on the whole dataset (see also Romano et al. 2011).

We investigated whether the proportion of feedings received by the darkened nestling in each dyad deviated from 0.5 (i.e., the value expected in case of 2 same-sex and similar-size nestlings in the absence of any parental favoritism) by means of intercept-only binomial mixed model where the dependent variable was the proportion of feedings obtained by the darker nestlings over the total number of feeding events (i.e., events/trials syntax), and brood identity was included as a random intercept effect. This analysis is mathematically equivalent to a mixed model of the absolute number of feeding received by each nestlings in each dyad assuming a Poisson error distribution, including experimental manipulation (sham colored vs. darkened) as a 2-level fixed effect, with brood and dyad identity as random intercept effects (details not shown for brevity).

The difference in body mass between the end and the start of the trial (hereafter "body mass variation") was analyzed by means of LMMs with experimental manipulation (sham colored vs. darkened) as a 2-level fixed effect, with brood and dyad identity as random intercept effects. The analysis of body mass variation in the sample of control dyads was carried out by LMMs with the same random structure as above and with treatment (un-manipulated vs. sham colored) as a fixed effect.

Variation in begging intensity between control and darkened nestlings was analyzed in multinomial mixed models for ordered outcomes by using the cumulative logit link function (Schabenberger 2006; see also Romano et al. 2012) with experimental manipulation as a fixed effect, and brood and dyad identity as random intercept effects.

All analyses were carried out using SAS 9.1.3 (SAS Institute 2006). The analyses of variability of color variables among nests were carried out with the VARCOMP procedure, whereas mixed models analyses using the MIXED and GLIMMIX procedures. In LMMs, degrees of freedom were computed with the Kenward–Roger approximation, and in multinomial generalized linear mixed models, the degrees of freedom were conservatively set equal to the number of dyads included in each model.

RESULTS

Natural variation in nestling plumage color

All color variables significantly varied among broods (analysis of variance tests: θ: $F_{27,91} = 2.10$, $P = 0.005$; φ: $F_{27,91} = 2.33$, $P = 0.002$; *rA*: $F_{27,91} = 3.24$, $P < 0.001$), indicating that ventral plumage color was more similar between nestlings reared in the same nest than between nestlings of different broods. However, the variance explained by the nest of rearing accounted for less than half of the total observed variance (variance components analyses: θ: 0.20; φ: 0.24; *rA*: 0.34), as previously demonstrated in nestlings of a different subspecies of the barn swallow (*Hirundo rustica erythrogaster*; Hubbard et al. 2015). This result was confirmed when the analyses were carried out on males (variance components analyses: θ: 0.26; φ: 0.25; *rA*: 0.52) or females (variance components analyses: θ: 0.33; φ: 0.33; *rA*: 0.29) separately. These findings thus imply that large differences existed in ventral coloration among same-sex siblings. A large within-brood variability in color is a fundamental prerequisite to enable parents to differentially allocate parental care among individual progeny members based on their ventral plumage coloration. The within-brood variation was not explained by nestling sex, as no statistically significant difference in any color variable was observed between males and females (mixed models with brood identity as a random factor; effect of sex: θ: $F_{1,90} = 1.11$, $P = 0.29$; φ: $F_{1,90} = 0.28$, $P = 0.60$; *rA*: $F_{1,90} = 0.07$, $P = 0.79$; see Supplementary Figure S1).

Parental food allocation according to experimental manipulation of nestling ventral coloration

The mean number of feedings delivered by parents during feeding trials was 20.69 ± 12.35 SD, ranging from 4 to 64 feeding events per trial. Analyses on male dyads showed that darkened nestlings obtained a larger proportion (and number) of feedings than

sham-colored siblings, whereas this was not the case among female dyads (Table 1; Figure 1). Indeed, in 14 out of 18 male dyads, darkened males obtained more food items than the sham-colored siblings, indicating a nonrandom food allocation by parents (binomial test: $P = 0.031$). On the other hand, darkened females obtained more food than sham-colored siblings in 9 out of 18 cases (binomial test: $P = 1.00$), as expected in case of random food allocation. As a consequence, color darkening had also a positive effect on male, but not female, body mass gain (Table 1).

Begging intensity did not differ between darkened and sham-colored nestlings of both sexes (males: $F_{1,18} = 1.13$; $P = 0.30$; females: $F_{1,18} = 0.11$; $P = 0.74$). Therefore, begging intensity did not confound our results. Finally, no significant difference in body mass variation during the feeding trials was observed between sham-colored and un-manipulated nestlings in the control broods in dyads of either sex (males: $F_{1,9} = 1.22$; $P = 0.23$; females: $F_{1,7} = 0.18$; $P = 0.69$), indicating that the sham treatment did not affect food allocation to the nestlings.

DISCUSSION

We showed that barn swallow parents modulated their investment in male, but not female, offspring according to plumage coloration. In fact, experimentally darkened males obtained more food and gained more mass compared with their sham-colored siblings. As expected, this pattern of differential food allocation between offspring displaying different plumage colors was not observed in females. The mean standardized effect size $\mathcal{Z}$ associated with the relationships between experimental manipulation and measures of parental investment (i.e., considering the proportion of feedings obtained and the body mass variation) was 0.50 and 0.21 in male and female dyads, respectively, indicating that the effect of treatment in males was more than twice as large as in females.

Between- and within-sex variation in plumage melanin-based coloration is common in birds (Hill and McGraw 2006). Melanogenesis, and thus the expression of melanin-based pigmentation, is also known to covary with several physiological and behavioral traits, including immune or hormonal functions, response to stressors, as well as social aggressiveness and sexual behavior (Roulin 2004; Ducrest et al. 2008; Roulin et al. 2008; Galván and Alonso-Alvarez 2009). Variation in melanin-based colorations can therefore reliably indicate individual genetic/phenotypic quality, which can be used in intrasexual and intersexual interactions, as showed by a large variability in breeding performance according to melanization, with darker individuals being generally more sexually active and obtaining larger benefits in terms of reproduction (Roulin 2004; Meunier et al. 2011). This is also the case for the species we studied here, in which darker individuals, especially males, accrue breeding advantages over paler ones (Safran and McGraw 2004; Safran et al. 2005; Vortman et al. 2011, 2013). Therefore, by allocating more resources to darker sons, which will be recruited as darker adult breeders in the next breeding season (Hubbard et al. 2015), parents were likely favoring the more valuable offspring, which should provide them the largest fitness return. This is consistent with our expectation because there is greater variance in reproductive success associated with brownish coloration in males than in females. While brownish females only produce slightly larger clutches than paler ones (Safran and McGraw 2004), darker males have shorter prelaying period, enjoy larger fidelity by social mate, prevail in sperm competition, and extrapair copulations, thus resulting in a larger production of biological offspring in each breeding season (Safran and McGraw 2004; Safran et al. 2005; Vortman et al. 2011, 2013). Larger parental investment in darkened males may thus function to enhance parental fitness.

Our results are also compatible with the non-mutually exclusive interpretation that parents invested more in darkened nestlings because their darkness reliably signals better body condition or quality compared with siblings, as shown in other species (Tschirren et al. 2003; Fitze et al. 2003; Parejo and Silva 2009). However, a brood size manipulation experiment carried out on 12 pairs of broods that were either increased or decreased in size showed that an experimental increase in brood size (see Bonisoli-Alquati et al. 2008 for details of the experimental protocol) negatively affected body mass (LMM with brood of origin and of rearing, and dyad as a random effects; effect of treatment: $F_{1,64} = 4.68$; $P = 0.034$), but did not alter nestling ventral coloration (LMMs as above; effect of treatment on θ, φ, and rA: all $P \geq 0.24$; Costanzo A et al., unpublished data). The lack of effect of brood size manipulation on plumage coloration does not support the hypothesis that coloration is a condition-dependent trait. However, other sources of nongenetic variation, such as maternal and paternal effects, external environment (e.g., rearing temperature and air pollution), and social environment (e.g., sex ratio of siblings), may contribute to variation in plumage coloration of nestlings. In fact, in a recent study on the same species, Hubbard et al. (2015) showed that only half of the variance in nestling plumage color is explained by the combination of genes and brood of rearing, suggesting that other sources of environmental influences affect plumage coloration.

In addition, plumage color may mirror other aspects of the nestling quality. In the same population where this study was performed, we showed that darker nestlings of both sexes harbor longer telomeres (Costanzo A et al., unpublished data). Individuals with longer telomeres and/or smaller rates of their shortening are generally more viable (Barrett et al. 2013; Boonekamp et al. 2014) and have better reproductive and physiological performance (Le Vaillant et al. 2015). It is therefore possible that barn swallow

Table 1

Mixed models of the effects of experimental manipulation (darkened or sham colored) on proportion of feedings received by darkened nestlings and on body mass variation by dyads of same-sex nestlings

	Males				Females			
	Coefficient (SE)	$F/\mathcal{Z}$	df	P	Coefficient (SE)	$F/\mathcal{Z}$	df	P
Proportion of feedings	0.243 (0.106)	2.30[a]	—	0.021	0.142 (0.103)	1.38[a]	—	0.17
Body mass variation	0.175 (0.082)	4.54	1,23	0.044	0.045 (0.084)	0.29	1,21	0.59

Analyses were performed for nestlings of either sex separately. $\mathcal{Z}$ values were computed by dividing the intercept estimate by the associated standard error in intercept-only logistic regression models. See Methods for details and sample sizes. df, degrees of freedom; SE, standard error.
[a] $\mathcal{Z}$ value.

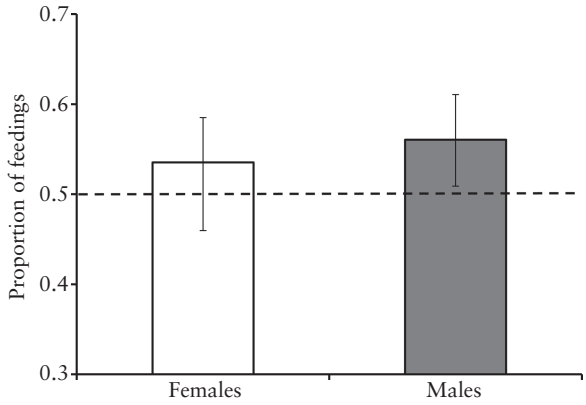

Figure 1
Proportion of feedings received during feeding trials by darkened nestlings in 18 male and 18 female dyads. Values are estimates from mixed models shown in Table 1, together with the 95% confidence intervals reported on the original scale. The broken line indicates the expected proportion of feedings received in case of 2 same-sex and similar-size nestlings in the absence of any parental favoritism. The *y* axis is bound between 0.3 and 0.7 for ease of representation.

parents invested more resources in darker nestlings because they were honestly advertising their quality in terms of telomere length. Although darkened females also obtained more (though statistically nonsignificantly so; Figure 1) food than their control female siblings, the effect of darkening on parental investment was markedly larger among male offspring, possibly because they benefit more than females from carrying this sexually selected trait at sexual maturity. We emphasize however that, irrespective of the mechanism that generated preferential food allocation toward darker sons, our results are consistent with the idea that barn swallow parents adaptively modulate their parental investment based on offspring coloration.

Proximately, although larger investment in darkened nestlings should not increase their fledging success, because the mortality in the last few days of rearing is almost negligible (Møller 1994), an increased food intake could favor their survival in the very crucial postfledging period, when mortality is very high (Grüebler et al. 2014).

Favoritism toward darkened males emerged after controlling for the effect of sex of their direct competitors (i.e., always males), and irrespective of difference in body size and begging behavior, which are known to affect competitive interactions among siblings and food allocation in the barn swallow (Saino et al. 2000; Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2011). Thus, our results should reliably reflect parental decisions on food allocation according to offspring reproductive value, as clearly indicated by the observation that in a very large proportion of male dyads the darkened nestlings obtained more food than their sham-colored siblings. In addition, the differential investment toward darkened males or females was observed despite a lack of sex-related difference in plumage coloration in this sample of immature barn swallows (but see Costanzo A et al., unpublished data), as in most of bird species (Moreno and Soler 2011; Hawkins et al. 2012). This is not surprising because the parental ability to distinguish offspring of either sex, and therefore to differently invest in them, is mediated by other traits, such as vocalizations and gape coloration, which are known to differ between male and female nestlings (Saino et al. 2003; Saino, de Ayala, et al. 2008). The lack of sex-dependent coloration in nestlings used in the present study is partly consistent with the evidence from a recent study on the North-American subspecies (*H. r. erythrogaster*), where a slight difference between male and female nestlings was observed in plumage saturation (*rA*), but not in the other color variables considered here (Hubbard et al. 2015).

In our study population, darker males are less viable than paler ones once they reach sexual maturity (i.e., between the first and the second breeding season; Saino, Romano, Rubolini, Teplitsky, et al. 2013). However, viability according to ventral plumage color has not been investigated in other populations, and it is still unknown if survival in the first year of age varies according to coloration, thus preventing to generalize to all age classes the pattern observed between the first and the second breeding seasons (Saino, Romano, Rubolini, Teplitsky, et al. 2013). Indeed, there is no a priori reason to discard the possibility that an opposite pattern of color-related mortality may occur between fledging and sexual maturation, approximately 1 year later. In addition, the barn swallow is a short-lived species with a life expectancy at sexual maturation of 1–2 years; indeed, most adults only enjoy a single breeding season in their life (Møller 1994; Saino et al. 2012). Furthermore, although the variance in the number of clutches, of eggs, and of fledglings is very small (Møller 1994; Saino N, unpublished data), sperm competition is intense, leading to a high level of extrapair paternity (in our population, the biological sire of up to 30% of nestlings is not the social father; Saino et al. 1997). This suggests that the cost paid by darker adult males in terms of survival may be overcompensated by the larger success in sperm competition, thus justifying an investment toward brownish male nestlings.

The above speculation rests on the assumption of a role of plumage color in affecting male lifetime breeding success in our focal population, which is matter of current investigation. However, in a recent study (Romano et al. 2015), we demonstrated that male-like females with darker ventral plumage produce male-biased broods. Because plumage color is a heritable trait (Saino, Romano, Rubolini, Ambrosini, et al. 2013; Hubbard et al. 2015; Vortman et al. 2015), this finding suggested an overproduction of "sexy sons" implying a possible role of ventral ornamentation in increasing male attractiveness. Taken together, the present findings and those by Romano et al. (2015) therefore indicate the possibility that the white to brownish plumage color in males may be under sexual selection also in European barn swallows.

Male and female parents may differently contribute to bias food allocation according to the phenotype of their offspring (Tschirren et al. 2005; Barrios-Miller and Siefferman 2013). Unfortunately, in the present study, we had no information about the feeding behavior of individual parents, and we therefore have no clue as to whether differential food allocation toward darker sons was driven more by mothers or fathers, or both parents. Further investigations by identifying the sex of attending parents during feeding trials would disclose this interesting issue.

Previous studies of other bird species demonstrated that plumage color can convey reliable information about nestling quality, allowing parents to adjust food allocation accordingly (see above and Introduction for details). This is also the case for the expression of colored plumage patches that can confer direct fitness benefits in intrasexual competition at sexual maturity, as suggested by studies on great tits (*Parus major*; Galván et al. 2008; Tanner and Richner 2008), eastern bluebirds (Ligon and Hill 2010; Barrios-Miller and Siefferman 2013), and rock sparrows (*Petronia petronia*; Griggio et al. 2009). However, to the best of our knowledge, only a single study investigated whether parental favoritism according to nestling plumage color varied between sons and daughters: Male, but not

female, eastern bluebird parents showed a preferential postfledging defense behavior toward the most ornamented sons (Barrios-Miller and Siefferman 2013). In addition, preferential food allocation toward brighter male nestlings has been observed in the same species, but differential food provisioning according to offspring coloration was not tested in females (Ligon and Hill 2010).

Our novel results are therefore consistent and complementary with those obtained in the eastern bluebird. They in fact provide further evidence on the parental ability to differentially invest in male and female offspring according to a trait expressed during early development, which will be involved in intrasexual competition for access to mates at sexual maturation by adding new knowledge about the mechanisms of parental favoritism of more valuable offspring.

A.R. conceived of the study, designed the study, collected data in the field, performed data analyses, drafted the manuscript; G.B. collected data in the field; M.Ca. carried out the molecular lab work; M.Co. collected data in the field; A.C. carried out the color measures; D.R. conceived of the study, participated in data analysis, helped draft the manuscript; N.S. conceived of the study, designed the study, helped draft the manuscript. All authors gave final approval for publication.

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SUPPORTING INFORMATION

Materials and methods

Colour measures

Reflectance spectra of one ventral feather per nestling were measured in a dark chamber, using an Avantes DH-2000 spectrometer equipped with deuterium-tungsten halogen light source (see Saino et al. 2013a for details). Every measure was taken twice. Importantly, as previously demonstrated (see Romano et al. 2015) colour measurements taken on a single feather, on three feathers of the same individual or directly on ventral plumage of individuals provide very consistent estimates (correlation coefficients between the colour variable values obtained by the three methods were $r > 0.88$ for θ and $r > 0.91$ for both φ and rA). Colour quantification was performed relatively to white and black standards by using the tetrachromatic colour space model with the TetraColorSpace program version 1a (Stoddard and Prum 2008). This program includes information about bird cone sensitivity and therefore provides biologically realistic colour metrics (see also Antonov et al. 2010; Saino et al. 2013a). Here, we assumed a UVS cone type retina and used spectral sensitivity of the blue tit (*Cyanistes caeruleus*), the most closely phylogenetically related species to the barn swallow for which information on cone spectral sensitivity is available in TetraColorSpace.

Idealized stimulation of the four cone types of passerines were normalized to a sum of 1, so that tetrahedral coloration was described by a vector of {uv, s, m, l} values, which represents stimulation of the cones sensitive to ultraviolet, short, medium and long wavelengths, respectively. Tetrahedral colour space vectors were then transformed into the spherical coordinates θ, φ, and r (see Antonov et al. 2010; Stoddard and Prum 2008). φ and θ respectively reflect the ultraviolet and the red-green-blue components of hue, while r is a proxy of colour saturation. For barn swallow ventral feathers, decreasing θ indicates darker, brownish coloration and a higher concentration of both eu- and pheo-melanin (see Saino et al. 2013a). Because the tetrahedral colour space is not a sphere and different hues vary in maximum potential saturation (r_{max}), we therefore computed a 'achieved saturation' as the ratio between the obtained and the maximum potential value of saturation ($rA = r/r_{max}$).

Effects of experimental manipulation on plumage colour

We tested whether the experimental manipulation increased the ventral plumage colour in mixed models including sex, experimental manipulation (sham-coloured vs. darkened), and sequence (before or after manipulation) as fixed effects, and brood and nestling identity as random intercept effects. The experimental manipulation by experimental phase interaction term was also included in the model. The

same model was applied to all colour variables (θ, φ, and rA). Colour measurements of three individuals were removed from the analyses because outliers.

The results of experimental manipulation on plumage colour of the two experimental groups are shown in Table S1. No differences in plumage colouration between male and female nestlings were observed for all colour variables. Plumage colouration significantly varied both according to experimental manipulation and sequence (Table S1). Their interaction was statistically significant for θ and rA, but not for φ. Fig. S1 shows the spectral curves for ventral plumage colouration of male and female nestlings before and after experimental manipulation for control and treatment groups.

Table S1. Mixed models of the effects of offspring sex, experimental manipulation (sham-coloured vs. darkened), sequence (before or after manipulation) and their interaction on θ, φ, and rA. Brood and nestling identity are included as random effects in the models.

	Coefficient (SE)	F	DF	P
θ				
Sex	-0.016 (0.012)	1.72	92	0.19
Experimental manipulation	-0.081 (0.016)	13.04	92	0.0005
Sequence	-0.093 (0.013)	27.76	92	<0.0001
Experimental manipulation × sequence	0.074 (0.021)	12.44	92	0.0007
φ				
Sex	-0.013 (0.022)	0.34	92	0.36
Experimental manipulation	-0.088 (0.031)	3.96	92	0.11
Sequence	-0.142 (0.027)	18.42	92	<0.0001
Experimental manipulation × sequence	0.103 (0.042)	2.36	92	0.016
rA				
Sex	0.009 (0.009)	1.27	92	0.26
Experimental manipulation	0.070 (0.012)	20.95	92	<0.0001
Sequence	0.089 (0.011)	51.18	92	<0.0001
Experimental manipulation × sequence	-0.060 (0.017)	12.85	92	0.0002

We thus explored the differences between darkened and sham-coloured nestlings before and after the experimental manipulation by means of post-hoc tests (Figure S2). Before the marker application no difference in any colour variable was observed between nestlings included in either sham-coloured or darkened group (θ: t_{92} = -0.40, P = 0.69; φ: t_{92} = 0.51, P = 0.61; rA: t_{92} = 0.84, P =

0.40). In addition, the experimental manipulation enhanced the colour of darkened nestlings, as indicated by the significant difference between all colour variables before and after experimental manipulation (θ: t_{92} = -6.92, P < 0.0001; φ: t_{92} = -5.30, P <0.0001; *rA*: t_{92} = 8.44, P < 0.0001). Moreover, the sham-coloured manipulation also altered *rA* of nestlings (*rA*: t_{92} = 2.31, P = 0.023). However, we stress that the alteration of the plumage colour of the sham-coloured nestlings was in the same direction with respect to that observed in darkened nestlings, indicating that our results were therefore conservative. Importantly, the sham-colour treatment did not affect the θ and φ of ventral plumage colour of sham-coloured nestlings (θ: t_{92} = 1.13, P = 0.26; φ: t_{92} = -1.19, P = 0.24). This means that the red-green-blue component of hue, which consists in the spectral location representing the position of a spectrum in the colour wheel, did not change.

Importantly, after the markers application (i.e. at the moment of feeding trial), all colour variables of darkened nestlings were significantly different from those of their sham-coloured siblings (θ: t_{92} = -5.04, P < 0.0001; φ: t_{92} = -2.87, P = 0.005; *rA*: t_{92} = 5.79, P < 0.0001), indicating that the experimental manipulation significantly darkened ventral plumage colour (Figure S2).

Finally, the mean values of θ and φ of experimentally darkened nestlings were shifted by approximately 1 SD of the mean values of the natural colour of the nestling plumage (mean θ ± SD of all nestlings before experimental manipulation: 0.207 ± 0.076, range 0.015 - 0.438, N = 119; mean θ ± SD of darkened nestlings after experimental manipulation: 0.120 ± 0.076, range 0.013 - 0.341, N = 56; mean φ ± SD of all nestlings before experimental manipulation: -0.580 ± 0.177, range -0.777 - -0.055, N = 119; mean φ ± SD of darkened nestlings after experimental manipulation: -0.706 ± 0.069, range : -0.802 - -0.376, N = 56). The mean values of *rA* of experimentally darkened nestlings were shifted by approximately 2 SD from the mean value of the natural colour of the nestling plumage (mean *rA* ± SD of all nestlings before experimental manipulation: 0.106 ± 0.056, range 0.041 - 0.280, N = 119; mean *rA* ± SD of darkened nestlings after experimental manipulation: 0.191 ± 0.068, range 0.057 - 0.306, N = 56).

Figure S1. Average reflectance curves (± SD) for ventral plumage colouration of male (solid line) and female (dashed line) bar swallow nestlings both before (a: nestlings of sham-coloured and darkened groups pooled) and after experimental manipulation of plumage colour (b; sham-coloured nestlings; c: darkened nestlings). Sample size is 44 female (16 sham-coloured and 28 darkened) and 50 male (22 sham-coloured and 28 darkened) nestlings.

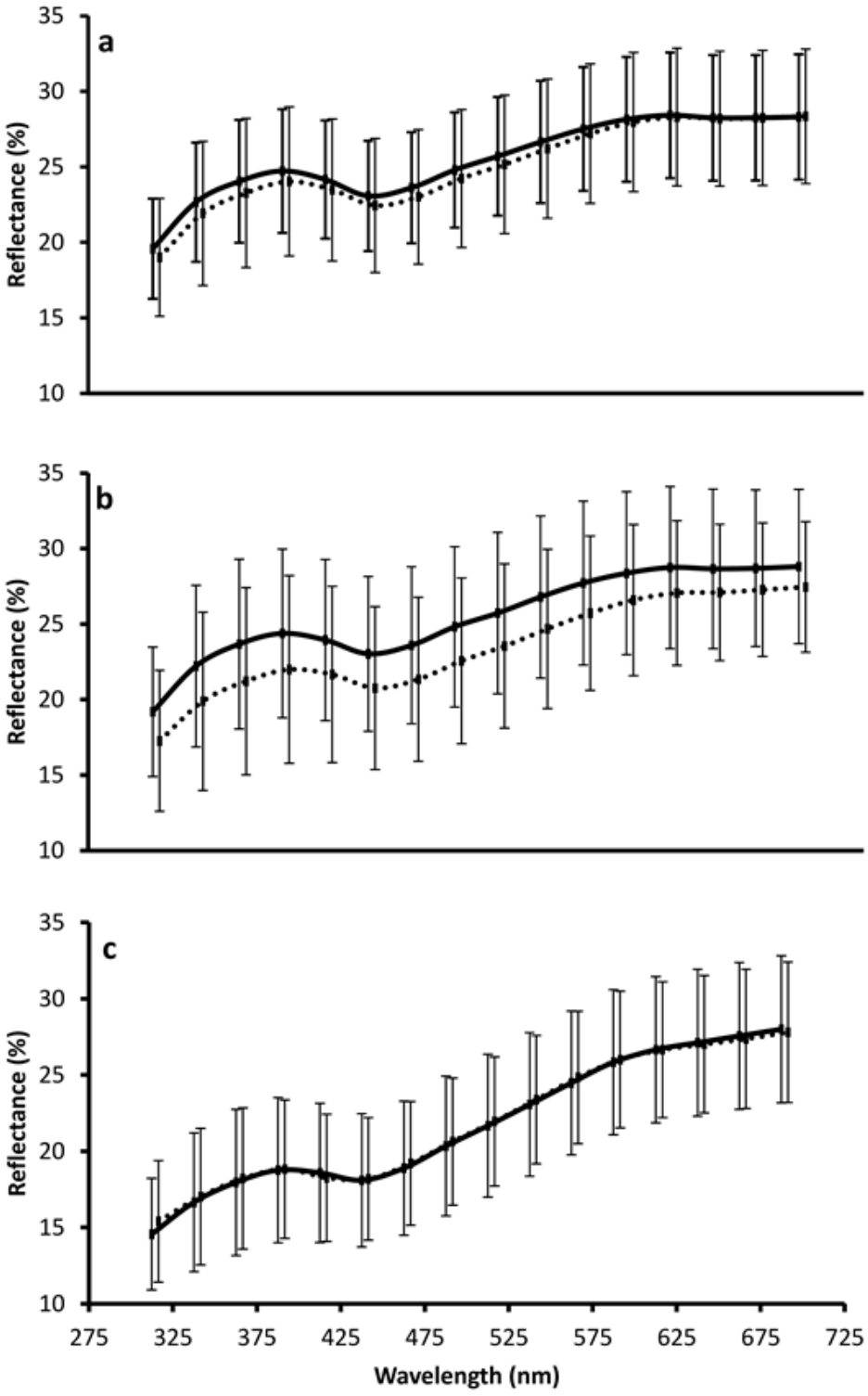
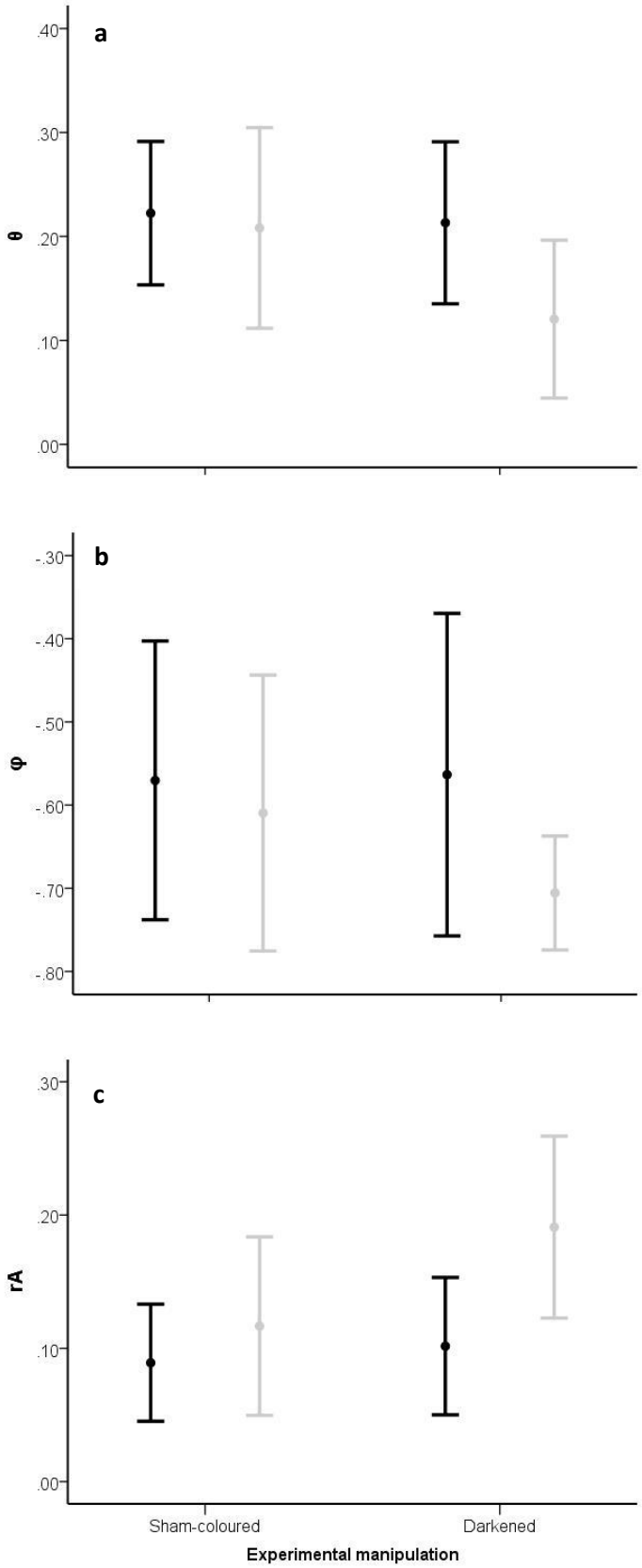


Figure S2. Mean (± SD) values of θ (a), φ (b) and rA (c) of sham-coloured (N = 38) and darkened (N = 56) nestlings before (dark bars) and after (light bars) the experimental manipulation of ventral plumage colour.



Results

Parental food allocation according to nestlings plumage colour while accounting for the 'dyad order'
For several broods (body mass variation: N = 21; number of feedings: N = 15) we performed feeding trials for two dyads differing in hunger level. This was the case because the second dyad was always tested after a period of food deprivation during the feeding trial of the first one. In the barn swallow, food deprivation is well known to increase hunger and begging intensity, and consequently parental feeding rate (see Saino et al. 2000; Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2011; 2012). This is also the case for the present study as feeding rate to the second-tested dyads was larger than that of the first-tested ones (Poisson mixed model including dyad order as a fixed effect and brood identity as random effect: $F_{1,14} = 20.77$; $P = 0.0004$). To account for the different feeding rate between first- and second-tested dyads for each brood, the analysis on body mass variation was therefore repeated while also including a two-level fixed effect indicating the *dyad order*. We did not repeat the analysis on the proportion of feeding received by darkened nestlings including the dyad order because this dependent variable was not affected by dyad order ($F_{1,13} = 1.86$; $P = 0.20$). This was expected because the proportion of feedings received by each nestling is not dependent on parental feeding rate.

As shown in Table S2, the dyad order significantly predicted body mass variation of nestlings, with nestlings belonging to the second-tested dyad gaining more body mass than siblings tested in the first one. However, and importantly, the effect of the experimental manipulation was qualitatively similar to that presented in Table 1. Indeed, experimental manipulation significantly predicted the body mass variation in male, but not in female, dyads.

Table S2. Mixed models of the effects of experimental manipulation (darkened or sham-coloured) and dyad order on body mass variation by dyads of same-sex nestlings. The analyses were performed for nestlings of either sex separately. See the main text for details and sample sizes.

	Coefficient (SE)	F	DF	P
Males				
Experimental manipulation	0.175 (0.082)	4.54	1,23	**0.044**
Dyad order	0.434 (0.108)	16.27	1,20.1	<**0.001**
Females				
Experimental manipulation	0.045 (0.084)	0.29	1,21	0.59
Dyad order	0.388 (0.190)	4.19	1,6.76	0.08

7. Chapter 2

Brood size, telomere length, and parent-offspring color signaling in barn swallows.

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Original Article

Brood size, telomere length, and parent-offspring color signaling in barn swallows

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Trade-offs select for optimal allocation of resources among competing functions. Parents are selected to maximize production of viable offspring by balancing between progeny number and "quality." Telomeres are nucleoproteins, at the ends of eukaryotic chromosomes, that shorten when cells divide. Because shortening below a certain threshold depresses organismal functioning and rate of shortening depends on environmental conditions, telomeres are good candidates as mediators of trade-offs. We altered brood size of barn swallow *Hirundo rustica* and found that brood enlargement caused a reduction in relative telomere length (RTL). Reliable signals of offspring quality should evolve that mediate adaptive parental care allocation. Because nestlings with darker coloration receive more care, we analyzed the covariation between RTL and coloration and found that RTL increased with plumage darkness, both within and between broods. Hence, we provide unprecedented evidence that signals relevant to parent-offspring communication reflect telomere length and thus offspring reproductive value.

Key words: **brood size, *Hirundo rustica*, parent-offspring communication, plumage color, telomere.**

INTRODUCTION

The number and quality of the offspring that individuals can afford to produce at any breeding attempt are limited by reciprocally constraining relationships. Physiological trade-offs between current progeny number and viability is a major force driving the evolution of breeding strategies (Roff, 1992). Empirical tests of reproductive trade-offs have typically relied on the experimental manipulation of parental effort that parents devote to individual progeny members and the assessments of the effect on progeny fitness proxies (Santos and Nakagawa, 2012). For example, brood size manipulation experiments in birds have demonstrated that increased brood size leads to stronger competition among siblings, ultimately resulting in a deterioration of average offspring condition (e.g. reduction of average body mass and immune response) compared to broods where sib-sib competition is alleviated by reducing brood size (Saino et al., 1997). Optimal parental investment also depends on allocation strategies to individual offspring. This is the case because parents investing more in offspring with larger reproductive value will accrue a fitness advantage relative to parents adopting even or random allocation strategies. However, adaptive differential parental allocation according to offspring quality requires that reliable signals of offspring reproductive value have evolved (Royle et al., 2012). For example, traits that young birds use to solicit care provisioning by their parents have been suggested to honestly advertise parasite load (Tschirren et al., 2003), competence of the immune system (Moreno-Rueda, 2010), and body size or need of additional care (Kilner, 1997).

In studies of reproductive trade-offs, offspring fitness has been typically estimated by focusing on specific classes of traits, such as development and somatic growth and/or functioning of the immune system (e.g. Saino et al., 1997; Soler et al., 2003). Albeit important, these proxies of offspring fitness likely provide only a partial representation of overall phenotypic quality and therefore of offspring reproductive value. Hence, the identification of general physiological mechanisms behind offspring number-quality trade-offs has proven elusive.

Recent studies have pointed at a major role of telomeres in mediating individual response to a host of endogenous and extrinsic factors, suggesting that telomere dynamics may integrate the information of individual history of exposure to stressful conditions (Haussmann et al., 2012; Herborn et al., 2014; Asghar et al., 2015b). Telomeres are nucleoprotein complexes located at the ends of eukaryotic chromosomes. Vertebrate telomeric DNA is composed by the tandem repetition of the hexamer TTAGGG and is tightly associated to a multiprotein complex (shelterin), which

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ensures proper regulation and protection of chromosome ends (Palm and de Lange, 2008). Due to the inability of DNA polymerase to fully replicate linear DNA, in normal somatic cells, telomeres physiologically shorten at each cell division. When telomeres reach a threshold length, cells enter either replicative senescence or apoptosis (Blackburn, 1991). Consequently, telomere shortening can reduce the renewal capacity of tissues and might depress organismal functioning and performance (Blasco, 2005; Monaghan and Haussman, 2006). The length and shortening rate of telomeres depend on genetic background (Asghar et al., 2015a; Atema et al., 2015), but also on diverse factors including exposure to oxidative stress (Beaulieu et al., 2011), food availability and quality (Badás et al., 2015), exposure to elevated levels of hormonal mediators of the hypothalamic-pituitary-adrenal (HPA) physiological stress response (Haussmann et al., 2012; Herborn et al., 2014), parasitism (Asghar et al., 2015b) as well as various forms of environmental and social stress (Kotrschal et al., 2007; Mizutani et al., 2013; Hau et al., 2015; Meillère et al., 2015), such as the number of competing siblings (Boonekamp et al., 2014; Reichert et al., 2015).

Telomere length and/or rate of attrition, in turn, have been shown to positively predict viability (Haussmann et al., 2005; Pauliny et al., 2006; Bize et al. 2009; Barrett et al., 2013; Boonekamp et al., 2014), fecundity (Le Vaillant et al., 2015), and also a number of important morphological (Kim and Velando, 2015), physiological (Le Vaillant et al., 2015), and behavioral traits (Nettle et al., 2015), with longer telomeres and smaller rate of attrition being generally associated with better performance.

Consistency in the general patterns of telomere dynamics across vertebrates, the impact that telomere shortening has on individual performance, and susceptibility of the telomere shortening process to extrinsic as well as endogenous factors, lead to expect that telomere dynamics underpin evolutionary and physiological trade-offs that reciprocally constrain the expression of fitness traits (Monaghan, 2010). Along the same line of reasoning, parents are expected to have selected for offspring traits that reliably reveal the quality of individual progeny in terms of telomere length and/or rate of telomere shortening, because offspring with long telomeres may have larger expected reproductive value and/or higher chances of survival. The latter hypothesis is relevant to a core issue in the evolution of parent-offspring communication systems but, to the best of our knowledge, has never been tested in any organism to date.

Parents have been shown to rely on several, diverse traits to tune their strategies of allocation of care based on offspring traits that are involved in parent-offspring communication (Royle et al., 2012). Such parental decisions may serve to maximize parental fitness by favoring the offspring with larger expected reproductive value. For example, parental discrimination has been observed in favor of offspring displaying more brightly colored mouth tissues or darker melanin-based coloration (Kilner, 1997; Romano et al., 2016, and references therein). To the best of our knowledge, no mechanistic links have been proposed so far between melanogenesis and telomere dynamics. However, an interplay between melanogenesis and telomere dynamics might be suggested by the observation that telomerase activity can affect early melanin biosynthetic pathways (Bagheri et al., 2006). In addition, a covariation between coloration and telomere length may be established because genes that control melanogenesis have pleiotropic effects on physiological, immune, and behavioral traits (Ducrest et al., 2008; Emaresi et al., 2013), which may in turn influence telomere dynamics.

In this study of the barn swallow (*Hirundo rustica*), a small passerine bird, we first test the hypothesis that the trade-off between offspring number and quality is mediated by the negative consequences that adverse rearing conditions have on telomere dynamics. Previous experiments on the same barn swallow population have shown that an experimental increase in brood size causes reduced growth and immune response in nestlings (Saino et al., 1997) and also an increase in ectoparasite infestation (Saino et al., 2002). In addition, enlarged broods are a socially stressful environment, as scramble competition for food is harsher than in reduced broods (Saino et al., 2000). A recent study has demonstrated that telomeres undergo significant shortening during the barn swallow nestling stage and suggests that telomere shortening later in life may be small, implying that the nestling stage is crucial to telomere dynamics (Parolini et al., 2015). The documented effects of brood size manipulation on specific fitness proxies and competitive interactions among siblings, and sensitivity of telomere dynamics to rearing conditions, led us to expect that nestlings in enlarged broods had reduced telomere length at growth completion as compared to nestlings reared in reduced broods.

Attending an enlarged brood increases parental effort and reduces annual parental survival in the barn swallow (Saino et al., 1999). Because parental care entails a measurable cost in terms of survival, selection on parents that adaptively modulate parental effort according to the reproductive value of the offspring should have resulted in the evolution of reliable signals of offspring quality. Nestling barn swallows vary in melanin-based chestnut coloration of their ventral plumage both at the within- and the among-family levels. An experiment with a within-brood manipulation design showed that darkening of the ventral plumage shortly before fledging (day 16 after hatching) caused an increase in parental food provisioning, implying that parents favor offspring that show darker melanin-based coloration (Romano et al. 2016). Here, we therefore tested if melanin-based coloration can reliably reveal nestling quality in terms of telomere length. To this aim, we measured telomere length in blood cells at an age (12 days after hatching) when it cannot be influenced by differential parental allocation according to nestling coloration because the coloration of the developing contour feathers has just become visible. We predicted that telomere length is larger in darker nestlings, as these have been experimentally shown to attract more care compared to paler nestlings (Romano et al., 2016).

METHODS

The barn swallow is a socially monogamous semicolonial passerine bird with biparental care of the progeny (Turner, 2006). Females lay 1–3 clutches per breeding seasons. Incubation lasts ca. 14 days and eggs hatch with small asynchrony (i.e. all nestlings usually hatch within 24 h of hatching of the first egg). Altricial, nidicolous nestlings fledge ca. 20 days after hatching. Osteometric growth is completed by day 11, while peak body mass is attained around day 12–13 (Turner, 2006). Contour body feathers start to emerge by day 6–7. The overall pattern of nestling coloration, determined by pheomelanins and eumelanins, is similar to that of adults. In spring 2014, in a study area located near Milan (Norther Italy; center of the study area: 45°36′N, 8°37′E), we performed a brood size manipulation experiment. We reciprocally swapped an unbalanced number of individually marked, randomly chosen nestlings between pairs of broods ("dyads") where hatching occurred on the same or in consecutive days. Hatchlings were swapped between

nests according to an unbalanced, partial cross-fostering design at the age of 0–1 days, when all nestlings of both broods in the dyad had hatched. The number of nestlings that were transferred was such that the size of either, randomly chosen, brood was increased, whereas the other was reduced by one nestling (see Saino et al., 1997). At the end of this procedure, both broods in the dyad contained both biological and foster nestlings. The sample included 24 broods. The size of enlarged broods (mean: 5.33 ± 0.26 standard error [SE] nestlings) was significantly larger than that of reduced broods (mean: 3.42 ± 0.23 SE nestlings; paired *t*-test: $t_{11} = 6.67$, $P < 0.001$), and the difference between the size of matched broods did not significantly differ from 2 nestlings, as expected ($t_{11} = 0.29$, $P = 0.777$). When nestlings were 12 days old, we measured body mass and tarsus length as a proxy of skeletal body size. In addition, a blood sample was taken for telomere analysis and identification of nestling sex by molecular tools (Saino et al., 2008). When nestlings were 16 days old, we plucked 5–10 growing feathers from the ventral plumage region for color analysis. At the time of measurement and blood sampling, the 24 broods in the sample contained a total of 106 nestlings. Information on tarsus length, plumage coloration, and telomere length was available for 105 nestlings for each variable, whereas body mass could be recorded for 97 nestlings. Two ϕ values (see Color analysis) were excluded from the analyses because they deviated by more than 3.5 standard deviation (SD) from the mean value. In all univariate and bivariate analyses, the largest available sample was used.

Because assignment of broods to either treatment group was randomized, we have no reason to suspect that any effect of brood size manipulation on telomere length was the spurious consequence of a difference in mean telomere length at hatching due to, for example, genetic variation or early maternal effects mediated by egg quality on telomere length. In fact, this assumption could not be tested because sampling of even a small amount of blood from ca. 1.5 grams hatchlings is hardly feasible in the field.

Color analysis

Color of one ventral feather, reflecting the coloration of the entire ventral plumage (Romano et al., 2015), was quantified recording the reflectance spectra of its distal end (Saino et al., 2013). Reflectance data were subsequently processed according to the tetrahedral color space model. Feather color was thus described by the 2 spherical coordinates that represent color hue: ϑ, which accounts for the "visible" component and ϕ, which account for the ultraviolet component (Goldsmith, 1990; Stoddard and Prum, 2008).

Telomere analysis

Telomere length analysis was performed according to the method described by Parolini et al. (2015). Genomic DNA was extracted from 10 to 20 µl of red blood cells using 1 ml TNSE buffer (10 mM Tris HCl, 400 mM NaCl, 100 mM EDTA, and 0.6% SDS) and a standard phenol/chloroform method. DNA samples of nestlings from the same nest were extracted in the same batch. We measured the quantity and purity of the extracted genomic DNA using a Nanophotometer (IMPLEN). DNA degradation was checked by electrophoresis in 1% agarose gel. Telomere length was measured by the monochrome multiplex quantitative PCR method (MMQPCR; Cawthon, 2009) on a PikoReal 96 thermal cycler (Thermo Scientific): telomere length was measured as the ratio (T/S) of the amount of telomeric repeats (T) to the amount of a single copy gene (S), relative to a reference sample. By this method, telomere length is evaluated indirectly by measuring the relative number of telomeric repeats in a genome and it is indicated from now as relative telomere length (RTL). The sequences of telomeric primers for MMQPCR were (telg 5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′; telc 5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′), while the single copy sequence used as control was a fragment from the 12th exon of the swallow CTCF gene (CCCTC-binding factor zinc finger protein). The CTCF primers used were: forward (5′-CCCGCGGCGGGCGGCGCGGGCTGGGCGGCTCCCAATGGAGACCTCAC-3′) and reverse (5′-CGCCGCGGCCCGCCGCGCCCGTCCCGCCCATCACCGGTCCATCATGC-3′); these primers are composed of a swallow genomic sequence and a GC-clamp at the 5′ end (underlined) to increase the melting temperature of the PCR product. Since the melting temperature of telomeric and CTCF PCR products are different, both primer pairs could be used in the same reaction. PCR reactions were prepared using 30 ng of genomic DNA as template, 1× DyNAmo ColorFlash SYBR Green qPCR Master Mix (Thermo Scientific), telomeric, and CTCF primers at a final concentration of 1,000 nM and 500 nM each, respectively. Three-fold serial dilutions of a barn swallow reference sample (from 5.5 to 150 ng) were included in each plate to produce a standard curve to measure reaction efficiency and quantify the amount of telomeric repeats and single copy gene in each sample. We used the same reference sample per plate. All reactions were run in triplicate and 6 plates containing 25 samples each were performed. Samples from the same dyad were randomly included into the same plate. Cycling parameters for the PCR reactions were as follows: Stage 1: 15 min at 95 °C; Stage 2: 2 cycles of 15 s at 94 °C, 15 s at 49 °C; and Stage 3: 35 cycles of 15 s at 94 °C, 10 s at 62 °C, 15 s at 74 °C with signal acquisition, 10 s at 84 °C, 15 s at 88 °C with signal acquisition. The PikoReal Software (Thermo Scientific) was used to calculate the amount of telomeric repeats (T) for each sample by interpolation of the quantification Cycle (Cq) into the linear function $y = ax + b$ of the standard curve of the telomeric primers. Similarly, the software calculates the amount of the single copy gene (S) for each sample. Mean values for T and S for each sample were used to calculate the T/S ratios. The mean reaction efficiency (±SD) for telomere and CTCF amplifications was 87 ± 5% and 86 ± 4%, respectively. The intraplate and interplate repeatability of RTL measures, expressed as intraclass correlation coefficient, was 0.79 and 0.81, respectively. The intraplate and interplate coefficient of variation (±SD) of RTL measures was 12 ± 8% and 10 ± 3%, respectively. Because of consistency of telomere length during the nestling period within individual nestlings (Parolini et al., 2015), we could assume that nestlings with relatively long telomeres at the age of blood sampling (12 days), also had relatively long telomeres at the age when ventral coloration was measured (16 days).

Statistical analyses

The effect of brood size manipulation and sex (2-levels fixed-effect factors) on nestling phenotype was analyzed in univariate linear mixed models (LMMs) while including dyad of broods, brood of rearing, and brood of origin as random effects. The correlation between traits was analyzed in bivariate LMMs with a repeated-measures design, where brood of rearing was included as a grouping factor, according to the procedure outlined in Dingemanse and Dochtermann (Dingemanse and Dochtermann, 2013), while adopting restricted maximum likelihood estimation of parameters. The within- and between-brood correlations between pairs of traits

were computed using the variance and covariance estimates according to equations 7c and 7d in (Dingemanse and Dochtermann, 2013). The significance of the within- and, respectively, between-broods correlation coefficients was estimated by likelihood-ratio tests (maximum likelihood estimation) comparing the full model with the model constraining the R or, respectively, the G matrix to a covariance of 0 (Dingemanse and Dochtermann, 2013). Bivariate LMMs were also run while considering nest of origin, rather than nest of rearing, as a grouping factor. Because the bivariate correlations obtained while including either grouping factor were generally qualitatively consistent (see Results), only the results based on the model including nest of rearing are presented in details. To represent the bivariate relationship between RTL and the morphological or the coloration variables at the between- or, respectively, the within-brood level, we computed the within-brood means or, respectively, the within-brood residuals of both variables relative to the brood mean. To better visualize the bivariate relationship we performed a type II (major-axis) regression analysis. Univariate and bivariate LMMs were run in SAS 9.2. Major-axis regression analysis was performed by the "lmodel2" package in R (version 3.2.3).

RESULTS

In LMMs, nestling body mass, tarsus length, RTL, and plumage color variables were not significantly predicted by the interaction effect between brood size treatment and nestling sex ($P > 0.23$ for all traits). Simplified models only including the main effects of sex and brood size treatment showed that RTL and body mass had significantly smaller mean phenotypic values in enlarged as compared to reduced broods, whereas the effect of brood size manipulation on the other traits was nonsignificant (Table 1; Figure 1). Male nestlings had significantly darker coloration of the ventral plumage as compared to females, consistent with adult sexual dichromatism, while the other traits did not significantly vary according to sex (Table 1; Figure 1). Parameter estimates for random effects are presented in Supplementary Table S1.

We tested for covariation between nestling RTL and the other phenotypic traits both at the between- and at the within-brood level in bivariate LMMs with a within-brood of rearing repeated-measures design (see Statistical analyses). Body mass and tarsus length did not significantly covary with RTL both at the between- and at the within-brood level (Table 2). However, the correlation coefficients for body mass and tarsus length at the between-broods level were large and negative (Table 2), suggesting that the lack of statistical significance could be due to the relatively small number of broods included in the analyses (sensu Nakagawa and Cuthill, 2007). The ϑ component of ventral plumage coloration negatively covaried with RTL both at the between- and at the within-brood level (Table 2; Figure 2), implying that nestlings with darker ventral coloration had larger RTL. In addition, the ϕ color component negatively covaried with RTL at the within-brood levels, whereas the significance of the correlation at the between-brood levels could not be estimated because the reduced model failed to converge (Table 2).

Bivariate LMMs where phenotypic traits where modeled while adopting a within-brood of origin repeated-measures design confirmed the results of models with brood of rearing as a grouping factor, with the only exception that the negative association between RTL and ϑ at the between-broods level turned to nonsignificant ($P = 0.100$; Table 2).

DISCUSSION

We manipulated the social environment of barn swallow nestlings by altering the size of the brood where they were reared and found that the length of their telomeres at somatic growth completion was smaller in enlarged as compared to reduced broods. In addition, independently of sex and brood size manipulation, nestlings with darker melanin-based coloration had longer telomeres, providing evidence that telomere length is reliably reflected by an offspring trait relevant to communication with parents.

Life-history theory posits that parents are selected to strike the optimal balance between their own survival and the number and quality of their offspring (Roff, 1992). In barn swallows, these major parental fitness components have been experimentally shown to be linked by reciprocally constraining relationships: brood enlargement depresses parental survival (Saino et al., 1999) and has adverse effects on the offspring (Saino et al., 1997). Per capita food intake is reduced in enlarged broods (Saino et al., 1997; Saino et al., 2000). The negative effect of brood enlargement on somatic growth and immune response likely mediated by poor nutritional conditions has been repeatedly documented in barn swallows (Saino et al., 1997; Saino et al., 2000) as well as in other altricial bird species (Naguib et al., 2004), and was confirmed in the present study along with a lack of effect on body size, suggesting that nestling barn swallows prioritize skeletal growth over allocation to anabolism of soft tissues. In addition, scramble competition among siblings increases as a consequence of reduced nestling satiation (Saino et al., 2000; Romano et al., 2013). Furthermore, increased brood size results in larger abundance of virulent nest-dwelling hematophagous mites (Saino et al., 2002). All of these effects of brood enlargement may concur in determining shorter telomere

Table 1
Summary of linear mixed models of nestling phenotypic traits with brood size treatment and sex as fixed-effect factors

Dependent variable	Effect	Parameter Estimate (SE)	*n*	*F*	df	*P*
RTL	Brood treatment	−0.102 (0.041)	105	6.32	1, 69	0.014
Body mass	Brood treatment	−0.685 (0.317)	97	4.68	1, 64	0.034
Tarsus length	Brood treatment	−0.041 (0.066)	104	0.39	1, 68	0.534
ϑ	Brood treatment	0.015 (0.013)	103	1.41	1, 66	0.239
	Sex[a]	−0.040 (0.013)		9.56	1, 66	0.003
ϕ	Brood treatment	0.014 (0.028)	102	0.27	1, 66	0.607

ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. Pair of broods, brood of origin and brood of rearing were included as random effects. Parameter estimates for fixed effects are given for enlarged broods and for males.
[a]Least square means for males: 0.206 (0.014); females: 0.246 (0.014).

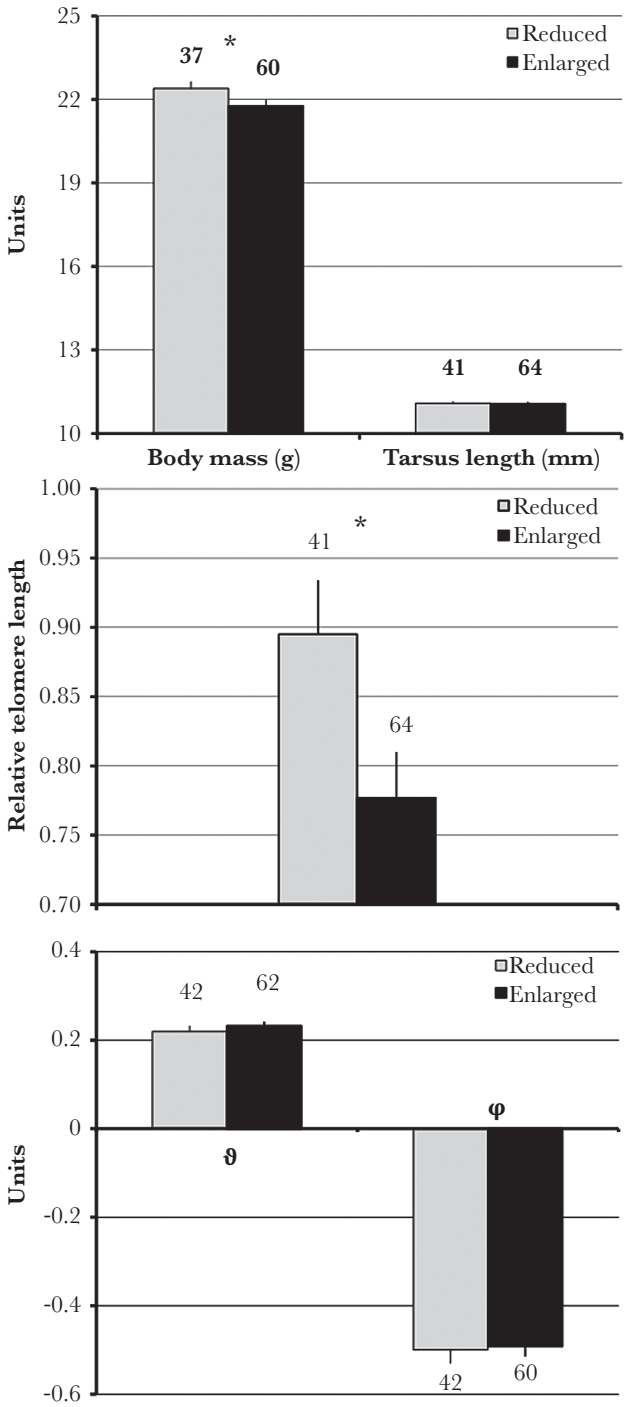

Figure 1
Mean (+SE) phenotypic values of nestlings from reduced (n = 12) or enlarged (n = 12) broods. ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. *the difference between the 2 treatment groups was significant ($P < 0.05$) in linear mixed models controlling for sex (see text). Insert numbers are sample size.

length in enlarged broods. In fact, nutritional and social stress (Kotrschal et al., 2007; Mizutani et al., 2013; Meillère et al., 2015), as well as parasitism (Asghar et al., 2015b), have all been shown to cause telomere shortening in birds. The few previous studies where the effect of brood size manipulation on telomere length has been tested have provided mixed evidence (Voillemot et al., 2012; Boonekamp et al., 2014; Nettle et al., 2015). Idiosyncratic effects of brood size manipulation on telomere shortening in different species may have arisen because of differences in constraints set by food availability on nestling condition or because of differences in age-specific telomere dynamics between species.

Despite the difficulties of estimating the long-term viability consequences of rearing conditions in organisms with large natal dispersal, some studies of trade-offs in birds have demonstrated that being reared in a large brood depresses the odds of local recruitment and survival (e.g. Djikstra et al., 1990; Pettiflor et al., 2001; Tarof et al., 2011), while other studies have shown that offspring body traits that are otherwise known to depend on brood size predict offspring viability, providing indirect evidence for an effect of rearing social environment on viability (e.g. Saino et al., 1997; Soler et al., 2003). Telomere length and rate of shortening have been consistently shown to predict survival prospects of both young and adult mammals and birds (Bize et al., 2009; Heidinger et al., 2012; Barrett et al., 2013). The present evidence of smaller telomere length at growth completion in nestlings from enlarged as compared to reduced broods therefore suggests that telomere dynamics may be one of the ultimate mechanisms that mediate a trade-off between offspring number and viability. The relationships between RTL and tarsus length or body mass were nonsignificant but the correlation coefficient was large and negative, suggesting that lack of statistical significance could arise because of type II statistical error. A negative relationship between RTL and morphological traits could arise because the physiological cost of growing to large body size causes telomere shortening.

In birds, visual communication has a major role in sexual, social and also in parent-offspring relationships (Royle et al., 2012). Offspring plumage and mouth coloration affects parental decisions over food allocation in altricial birds by contributing to food provisioning solicitation displays ("begging"). The present findings provide novel support to the hypothesis that coloration reliably advertises offspring quality, because telomere length was found to covary with plumage coloration and may affect offspring viability. The relationship between telomere length and coloration held at the within- as well as at the between-broods levels. Hence, darker coloration reliably reflects telomere length of individual nestlings relative to their siblings and, in addition, broods with relatively dark coloration can be perceived as consisting of nestlings with on average longer telomeres than broods with relatively pale nestlings. Importantly, in the same barn swallow population where the present study was conducted, we have experimentally shown a preference of parents for feeding darker nestlings (Romano et al., 2016). This is consistent with the expectation, because parents should invest more in more valuable offspring, with longer telomeres.

Importantly, the correlation between telomere length and coloration is unlikely to be the mere consequence of parents investing more resources in darker nestlings. By the age when blood was sampled and RTL was measured (day 12 after hatching), the color of the growing feathers has just (less than 2 days) become visible. Hence, it is highly unlikely that before the age when RTL was measured, parental decisions on allocation of care could be driven by feather coloration, thereby generating a covariation between coloration and condition-dependent telomere length. In fact, we found no covariation between body mass or tarsus length measured at day 6 and also at day 12 (i.e. the time of telomere length measurement) and coloration measured at day 16. However, we cannot discard the alternative interpretation that the positive covariation between telomere length and plumage color was the result of differential

Table 2

Correlation coefficients between relative telomere length (RTL) and nestling phenotypic traits at the between- and within-broods levels as estimated in bivariate linear mixed models

	Grouping	Between-broods				Within-broods			
	Brood	r	n	χ^2	P	r	n	χ^2	P
Correlation of RTL with									
Body mass	Rearing	−0.231	24	0.4	0.527	−0.090	97	0.6	0.439
	Origin	−0.372	24	2.1	0.147	−0.027	97	0.0	0.999
Tarsus length	Rearing	−0.427	24	3.0	0.083	−0.215	103	3.8	0.051
	Origin	−0.468	24	2.8	0.094	−0.161	103	2.1	0.147
ϑ	Rearing	−0.563	24	3.9	0.048	−0.230	103	4.4	0.036
	Origin	−0.479	24	2.7	0.100	−0.267	103	6.0	0.014
ϕ[a]	Rearing	−0.426	24	—	—	−0.222	101	3.9	0.048
	Origin	−0.419	24	—	—	−0.223	101	4.0	0.046

ϑ and ϕ represent the "visible" and the ultraviolet component of the plumage color, respectively. Significance of the correlation coefficients was estimated by likelihood-ratio tests (see also Statistical analyses).
[a]The reduced models testing for the between-broods correlation did not converge.

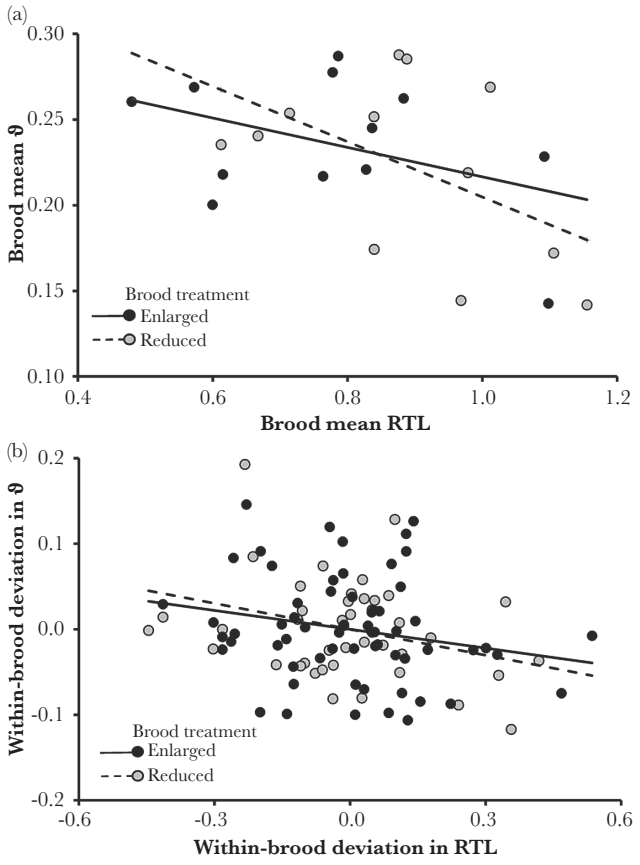

Figure 2

(a) Mean within-brood phenotypic value of the ϑ component of nestling ventral plumage color (larger ϑ indicates paler color) in relation to mean within-brood relative telomere length (RTL) in reduced (n = 12) or enlarged (n = 12) broods. (b) Phenotypic value of the ϑ component of coloration of ventral plumage in relation to RTL of individual nestlings. Phenotypic values are centered to a within-brood mean of 0. In both panels, major-axis regression lines are fitted to better represent the negative trends.

allocation according to another trait correlated with future nestling plumage coloration (e.g. mouth color or begging vocalizations), which could have influenced parental feeding behavior prior to the growth of nestling ventral contour feathers.

The interpretation of the causal pathways that link coloration to telomere length is matter of speculation. A relationship between melanogenesis and telomere dynamics is suggested by the observation that altered telomerase activity affects the expression of tyrosinase, an enzyme involved in early melanogenesis pathways (Bagheri et al., 2006). Although this off-site effect of telomerase was demonstrated in a mouse melanoma cell line, it is tempting to hypothesize that a direct link may exist between telomere length and ventral plumage coloration in the barn swallow. The genes that regulate melanin biosynthesis pleiotropically influence several life-history traits via the melanocortin system (Ducrest et al., 2008). Melanocortins are derivatives of the prohormone encoded by the proopiomelanocortin (*POMC*) gene. Binding of the *POMC* gene products to the melanocortin 1-receptor (MC1R) triggers pheomelanogenesis and eumelanogenesis (Ducrest et al., 2008). However, *POMC* derivatives can also bind to other melanocortin receptors (MC2-5) with regulatory effects on a number of important functions, spanning from energy homeostasis and sociosexual behavior to immunity and stress response mediated by the HPA axis (Ducrest et al., 2008). For example, chronic exposure to glucocorticosteroids can depress the activity of telomerase, that functions to restore telomere length (Choi et al., 2008; Epel et al., 2010). Hence, a covariation between coloration and telomere length might also arise because the genes that control melanogenesis have indirect effects on physiological and behavioral traits whose expression affects telomere shortening.

Brood size manipulation did not affect plumage coloration suggesting that coloration is not influenced by brood size. An alternative, not mutually exclusive, interpretation is that the consequences of brood size manipulation may still not be measurable at the age of 6–7 days, when the distal, colored end of the ventral contour feathers is produced.

This study thus shows that telomere length measured at somatic growth completion in a bird with altricial offspring depends on rearing conditions. Telomere shortening may therefore be one of the core mechanisms mediating the trade-off between offspring number and quality. Our present finding that nestling darkness positively covaries with telomere length is compatible with the idea that the observed parental favoritism for darker nestlings reflects an adaptive parental strategies of preferential allocation of care to the offspring with large expected reproductive value. Future studies should be aimed at assessing the generality of these findings and

to unveil the mechanistic links, if any, between plumage color and telomere length.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Costanzo et al. (2016).

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SUPPORTING INFORMATION

Table S1. Linear mixed models of nestling phenotypic traits with brood size treatment and sex as fixed effect factors. ϑ and φ represent the 'visible' and the ultraviolet component of the plumage color, respectively. Pair of broods and brood of origin and brood of rearing were included as random effects. Covariance parameters for random effects are presented. Parameter estimates for fixed effects are given for enlarged broods and for males.

Dependent variable	Effect	Parameter Estimate (SE)	n	F	df	P
Telomere length	Dyad	0.025 (0.013)				
	Brood of origin	0.001 (0.004)				
	Brood of rearing	0				
	Brood treatment	-0.102 (0.041)	105	6.32	1, 69	0.014
Body mass	Dyad	0.103 (0.379)				
	Brood of origin	0.568 (0.379)				
	Brood of rearing	0.188 (0.248)				
	Brood treatment	-0.685 (0.317)	97	4.68	1, 64	0.034
Tarsus length	Dyad	0.009 (0.012)				
	Brood of origin	0.006 (0.012)				
	Brood of rearing	0				
	Brood treatment	-0.041 (0.066)	104	0.39	1, 68	0.534
ϑ	Dyad	0.001 (0.001)				
	Brood of origin	$8*10^{-5}$ ($4*10^{-4}$)				
	Brood of rearing	0				
	Brood treatment	0.015 (0.013)	103	1.41	1, 66	0.239
	Sex*	-0.040 (0.013)		9.56	1, 66	0.003
φ	Dyad	0.013 (0.008)				
	Brood of origin	0.006 (0.004)				
	Brood of rearing	0.001 (0.002)				
	Brood treatment	0.014 (0.028)	102	0.27	1, 66	0.607

*: least square means for males: 0.206 (0.014); females: 0.246 (0.014)

8. Chapter 3

Telomere length is reflected by plumage coloration and predicts breeding success in the barn swallow.

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ORIGINAL ARTICLE

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Telomere length is reflected by plumage coloration and predicts seasonal reproductive success in the barn swallow

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Abstract

Individuals differ in realized fitness but the genetic/phenotypic traits that underpin such variation are often unknown. Telomere dynamics may be a major source of variation in fitness traits because physiological telomere shortening depends on environmental and genetic factors and may impair individual performance. Here, we showed that, in a population of a socially monogamous, biparental passerine bird, the barn swallow (*Hirundo rustica*), breeding in northern Italy, telomere length (TL) of both adult males and females positively correlated with seasonal reproductive and fledging success, as expected because long telomeres are supposed to boost performance. Telomere length was correlated with sexually dimorphic coloration in both sexes, showing for the first time in any species that coloration reliably reflects TL and may mediate mutual mate choice, leading to the observed positive assortative mating for TL in the barn swallow. Thus, TL appears to be associated with variation in a major fitness trait and may be an ultimate target of mate choice, as individuals of both sexes can use coloration to adaptively choose high-quality mates that possess long telomeres.

KEYWORDS

barn swallow, mate choice, plumage colour, reproductive success, telomeres

1 | INTRODUCTION

Understanding the causes of individual variation in fitness traits is pivotal to the study of ecological evolutionary processes. Variance in complex life history traits, such as breeding success and survival, likely depends on a large number of so-called quality or state traits, some of which, however, may have a disproportionate effect on performance owing to their multifaceted consequences for life histories (Stearns, 1992). Studies of humans and model organisms have suggested that telomere dynamics may determine variation in individual state because of their pervasive effects on organismal function and crucial processes such as senescence (Monaghan & Haussmann, 2006). However, whether the association between telomere dynamics and individual performance actually reflects causation still remains matter of debate, because studies of this relationship are correlational (Simons, 2015).

Telomeres are nucleoprotein structures located at the termini of eukaryotic chromosomes that contribute to maintaining chromosomal integrity (Palm & de Lange, 2008). Telomeric DNA of vertebrates consists of tandem repetitions of the hexamer TTAGGG tightly associated with shelterin complexes, which provide regulation and protection to chromosome ends (Palm & de Lange, 2008). Because DNA polymerase cannot fully replicate linear telomeric DNA, in normal somatic cells telomeres undergo shortening at each cell division. When telomere length (TL) reaches a certain threshold, cells enter either replicative senescence or apoptosis. These situations can

potentially compromise tissue renewal capacity and functioning and, ultimately, performance (Blackburn, 2000; Campisi, Kim, Lim, & Rubio, 2001; Monaghan & Haussmann, 2006). For example, in wild bird populations, short and/or rapidly shortening telomeres negatively predict lifespan (Angelier, Vleck, Holberton, & Marra, 2013; Bize, Criscuolo, Metcalfe, Nasir, & Monaghan, 2009; Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014; Stier et al., 2014; but see Caprioli et al., 2013) and reproductive success (Le Vaillant et al., 2015; Pauliny, Wagner, Augustin, Szep, & Blomqvist, 2006). However, causal links between TL and performance still largely remain to be established (Simons, 2015).

Telomere length and dynamics are partly genetically determined (Asghar, Bensch, Tarka, Hansson, & Hasselquist, 2015; Reichert, Stier, et al., 2014; but see Becker et al., 2015). However, the rate at which telomeres shorten partly depends on exposure to diverse forms of stress (Angelier et al., 2013; Hall et al., 2004; Monaghan, 2014; Monaghan & Haussmann, 2006; Watson, Bolton, & Monaghan, 2015), parasitism (Asghar, Hasselquist, et al., 2015), social environment and competition effects (Costanzo, Parolini, et al., 2017; Nettle et al., 2015; Parolini et al., 2015; Reichert et al., 2015; Stier, Massemin, Zahn, Tissier, & Criscuolo, 2015), parental effort (Bauch, Becker, & Verhulst, 2013; Reichert et al., 2015; Voillemot et al., 2012) and availability of dietary antioxidants (Badás et al., 2015; Noguera, Metcalfe, Boner, & Monaghan, 2015) or oxidative stress (Epel et al., 2004; Houben, Moonen, van Schooten, & Hageman, 2008; von Zglinicki, 2002). All these studies demonstrated that unfavourable conditions are associated with reduced TL and/or increased telomere erosion.

Because telomere dynamics may be a key factor underlying variation in individual "quality" (Monaghan, 2010), they may be relevant to sexual selection processes. Sexual selection theory posits that individuals of the sex that invests more in reproduction are selected to prefer high-quality individuals as mates because this will accrue them fitness benefits (Andersson 1994). In species where both sexes appreciably invest in reproduction, however, mate choice is predicted to be mutual, leading to expect positive assortative mating for quality traits. According to Fisherian-handicap models of sexual selection, preference for highly expressed secondary sexual traits that reliably advertise the genetic/phenotypic quality of their bearer can mediate adaptive choice of high-quality mates (Andersson 1994). The components of variation in individual quality that are the ultimate target of adaptive mate choice are typically difficult to identify. In the present context, we suggest that variation in TL may be a major component of variation in quality and may therefore covary with the expression of sexually dimorphic traits potentially under directional intersexual preference.

The determinants of telomere dynamics and the role of TL in physiology and pathology of humans and model species have been at the focus of intensive research (Monaghan, 2010). However, our knowledge of telomere biology in relation to life histories in wild organisms is at its infancy, and no study has investigated the relationships between telomeres and sexually selected traits. The aims of this correlational study of the barn swallow (*Hirundo rustica*) were thus threefold.

First, we tested whether seasonal reproductive success covaried with TL. If long telomeres are typical of high-quality individuals, we predicted reproductive success to increase with TL.

Melanin-based coloration of the ventral plumage region of barn swallows considerably varies among individuals and subspecies (Turner, 2006), ranging from white to chestnut, and also differs on average between the sexes (Saino, Romano, Rubolini, Teplitski, et al., 2013). In the barn swallow, ventral coloration reflects the absolute and relative concentration of eu- and pheomelanins (Saino, Romano, Rubolini, Teplitski, et al., 2013). Genes involved in melanin-based coloration can have pleiotropic effect on diverse life history traits (Ducrest, Keller, & Roulin, 2008). Therefore, it can be expected that different components of individual quality are reflected by coloration. For example, previous studies of the same barn swallow population have shown that coloration in the human-visible spectrum covaries with immune function and physiological stress responses (Saino, Canova, et al., 2013), with egg-mediated maternal allocation of antibodies to the progeny (Saino, Romano, Rubolini, et al., 2014) and with annual survival (Saino, Romano, Rubolini, Ambrosini, et al., 2013). Moreover, in a previous study of nestlings, it was shown that individuals with darker coloration have longer telomeres (Costanzo, Parolini, et al., 2017). Therefore, our second aim was to test whether ventral plumage coloration reflects TL, predicting a positive relationship between colour darkness and TL.

Third, we analysed the relationship between coloration and seasonal reproductive success. Previous studies of other barn swallow subspecies have documented a relationship between male coloration and annual breeding success, with darker males producing a larger number of biological offspring per breeding season (Safran & McGraw, 2004; Vortman, Lotem, Dor, Lovette, & Safran, 2011; Wilkins et al., 2016; Romano, Costanzo, Rubolini, Saino & Møller, 2017). In the European subspecies, the role of plumage coloration in sexual selection has not been fully elucidated (Romano et al., 2015, 2016, 2017; Costanzo, Ambrosini, et al., 2017). If TL correlates with coloration and predicts breeding success, we also expected coloration to predict seasonal reproductive success, with darker individuals showing higher success.

2 | METHODS

2.1 | Study species and field procedures

The barn swallow is a socially monogamous, semicolonial, passerine bird with biparental care of the progeny (Turner, 2006), breeding in rural buildings. Barn swallows are short-lived birds, with an annual survival rate of 0.3–0.4 (Turner, 2006). Hence, only ca. 3%–6% of the yearling adults reach age 4 years. Females lay 1–3 clutches of 1–7 eggs (modal clutch size = 5 eggs) (Saino et al., 2012). Incubation lasts ca. 14 days and eggs hatch with small asynchrony. In a study area located in northern Italy, close to Milan, we captured, sexed and marked with numbered metal and colour rings all adult barn swallows breeding at 11 colonies (=farms) in the period from 2010 to 2013. In our study population, breeding philopatry is extremely

high (> 99%), whereas natal philopatry is low, with most offspring dispersing to a colony different from their original one to breed. Hence, individuals that have bred in any particular colony do not move to other colonies in the following years. Because in all study years since 2010 we exhaustively captured all adults, we could assign age to breeding adults in 2013. Indeed, newly captured individuals in any year *i* (between 2011 and 2013) that had not been captured as breeding adults in year $i - 1$ (between 2010 and 2012) could be assumed to be one-year-old birds immigrating from another colony. For the birds that, in any year *i*, had been captured in year $i - 1$, age could be assigned based on the first year of capture (see also Saino et al., 2012). For further information about the study organism, see Turner (2006).

In 2013, we sampled focal adults for telomere analyses, coloration and breeding performance. The analyses of TL, coloration and breeding performance variables were only run on samples that were collected in one breeding season (year 2013) from individuals of different ages. Blood for telomere analyses was sampled between 1 May and 15 June, during breeding for all individuals. Because TL of adult barn swallows does not change with age (our unpublished data; see also Section 3), it is unlikely that telomeres considerably shortened during the 45 days over which sampling dates were spread. Blood samples were stored at −80°C within 4 hr of collection. Upon capture, we also collected ca. five contour feathers from the same ventral plumage region from all individuals for later spectrophotometric colour measurement. We assigned adults to nests by observation of individual markings. Nests were regularly (weekly) inspected to record all breeding events during the breeding season. Barn swallow nestlings usually fledge at the age of 20 days. Seasonal clutch size was expressed as the total number of eggs laid by a female. Seasonal reproductive success was expressed as the total number of nestlings that were present in the nest at last visit, when nestlings were at least 12 days old. This is a good proxy of the number of nestlings fledged because in our population nestling mortality during the second half of the nestling period is very low (<5%, N. Saino, unpublished results). Seasonal fledging success was expressed as the ratio between seasonal reproductive success and seasonal clutch size.

2.2 | TL analysis

The methods for TL analysis are fully reported in Parolini et al. (2015). Briefly, genomic DNA was extracted from 10 to 20 μl of red blood cells (RBC) using 1 ml TNSE buffer (10 mM Tris-HCl, 400 mM NaCl, 100 mM EDTA and 0.6% SDS) and a standard phenol–chloroform method. We measured the quantity and quality of the extracted genomic DNAs using a Nanophotometer (IMPLEN). Absorbance ratios for quality and purity of the samples we considered as adequate were OD260/280 = 1.8–2.0 and OD260/230 = 2.0–2.2. Very little degradation was detected in our samples by electrophoresis. In addition, as we previously showed, DNA degradation has no significant effect on the measurement of telomeric repeat content (Parolini et al., 2015). Telomere length was measured by monochrome multiplex quantitative PCR method (MMQPCR) (Cawthon 2009) on a PikoReal 96 thermal cycler (Thermo Scientific). According to this method, TL was measured as the ratio (T/S) of the amount of telomeric repeats (T) to the amount of a single-copy gene (S), relative to a reference sample, indicated as relative telomere length (RTL).

Reactions were performed using 20 ng of genomic DNA as template. Telomeric primers for MMQPCR were previously reported by Cawthon (2009) (telg 5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′; telc 5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′); the single-copy sequence used as control was a fragment from the 12th exon of the swallow CTCF gene (CCCTC-binding factor zinc finger protein) (forward 5′-CCCGCGGCGGGCGGCGCGGGCTGGGCGGCTCCCAATGGAGACCTCAC-3′, reverse 5′-CGCCGCGGCCCGCCGCGCCCGTCCCGCCCATCACCGGTCCATCATGC-3′); these primers are composed of a swallow genomic sequence and a GC-clamp at the 5′ end (underlined). As the melting temperatures of telomeric and CTCF PCR products are different, both primer pairs could be used in the same reaction. PCR were performed using 20 ng of genomic DNA as template, 1× DyNAmo ColorFlash SYBR Green qPCR Master Mix (Thermo Scientific), telomeric and CTCF primers at a final concentration of 1,000 nM and 500 nM each, respectively. Threefold serial dilutions of a barn swallow reference sample (from 5.5 to 150 ng) were included in each plate to produce a four-point standard curve to measure reaction efficiency and to quantify the amount of telomeric repeats and single-copy gene in each sample. As the amount of reference sample DNA was not enough to include it in all the plates, we split the analyses into two different batches and we use two different reference samples. The first group included 22 pairs, while the second group included 43 pairs. To solve any differences in the mean RTL between batches due to the dissimilar reference samples we used, RTL data were standardized within each of two groups of pairs by subtracting the group's mean. All reactions were run in triplicate and six plates containing 25 samples each were performed. Cycling parameters for the PCR were as follows: Stage 1: 15 min at 95°C; Stage 2: two cycles of 15 s at 94°C, 15 s at 49°C; and Stage 3: 35 cycles of 15 s at 94°C, 10 s at 62°C, 15 s at 74°C with signal acquisition, 10 s at 84°C, 15 s at 88°C with signal acquisition. The PIKOREAL Software (Thermo Scientific) was used to calculate the amount of telomeric repeats (T) for each sample by interpolation of the quantification cycle (Cq) into the linear function $y = ax + b$ of the standard curve of the telomeric primers. Similarly, the software calculates the amount of the single-copy gene (S) for each sample. Mean values for T and S for each sample were used to calculate the T/S ratios relative to a reference sample, so TL was indicated as RTL. All reactions were run in triplicate and seven plates containing on average 25 samples each were performed. Four samples were run in each plate. The mean reaction efficiency (±*SD*) for telomere and CTCF amplifications was 78% ± 4% and 81% ± 7%, respectively. The intra- and interplate repeatability of RTL measures, expressed as intraclass correlation coefficient, was 0.76 and 0.79, respectively. The mean intra- and interplate coefficient of variation

(±*SD*) of RTL measures was 9.7% ± 6.8% and 9.4% ± 8.3%, respectively.

2.3 | Spectrometric feather colour analysis

Colour of one ventral feather was quantified by recording the reflectance spectra of its distal end (see Saino, Romano, Rubolini, Teplitski, et al., 2013). Reflectance data were subsequently processed according to the tetrahedral colour space model (Stoddard & Prum, 2008). Feather colour was thus described by three variables: θ and φ, which account for the human-visible and the ultraviolet colour component, respectively, and rA, which accounts for colour saturation. The present method for quantifying coloration provides highly repeatable measures both between repeated measurements of the same feather and between feathers of the same ventral plumage region (Romano et al., 2015; Saino, Romano, Rubolini, Teplitski, et al., 2013). Importantly, we have demonstrated that coloration measured according to the present procedure strongly positively reflects coloration as measured directly on the birds (Romano et al., 2015).

2.4 | Statistical analyses

2.4.1 | Seasonal reproductive performance in relation to RTL

To analyse variation in seasonal clutch size (females only), seasonal fledging success and seasonal reproductive success in relation to RTL, we relied on generalized linear mixed models. In the analysis of seasonal clutch size (i.e., seasonal number of eggs) and seasonal reproductive success, we assumed a Poisson error distribution. In the analysis of seasonal fledging success, we assumed a binomial error distribution. In the models, we included age as a continuous covariate because reproductive success may vary with age, largely because yearling adults have fewer clutches on average than two- or more year-old birds (Møller, 1994; Turner, 2006). Simultaneous inclusion in the models of age and RTL did not raise collinearity issues because age and RTL were very weakly correlated (see Section 3). High correlations existed between age (see Section 3) and also between RTL of mates (r = .384, n = 65, p = .002; Khoriauli et al., 2017). In addition, reproductive success data for males are inherently dependent on reproductive success of their female mates. Because inclusion of highly correlated predictors in linear models can raise multicollinearity issues, and dependence of reproductive success data would violate the assumptions of linear model analyses, we ran separate analyses for males and females.

2.4.2 | Coloration in relation to RTL

Variation in colour components in relation to RTL was analysed in Gaussian linear mixed models including breeding pair as a random factor, while sex, RTL and their interaction as fixed effects. Colour variables of males are only weakly correlated with those of their female mates (correlation coefficients ranged between −0.06 and −0.162 for all colour variables). In these models, we included age as a covariate to control for any age-dependent variation in coloration. Simultaneous inclusion of age and RTL as independent variables did not raise collinearity issues because age and RTL were very weakly correlated (see Section 3). The interaction terms between sex and RTL and the main effect of age were initially included in the models. When both of them were nonsignificant, they were simultaneously excluded from the model. When either of them was significant, it was retained and the other was excluded.

2.4.3 | Seasonal reproductive success in relation to coloration

To analyse variation in seasonal reproductive success in relation to coloration, we relied on Poisson generalized linear models. In the models, we included age as a continuous covariate because reproductive success may vary with age (see above). Coloration was generally weakly correlated with age in both sexes (range of the values of the correlation coefficients between age and colour variables for females: −0.16 to 0.049; males: −0.14 to 0.10). Colour variables and age could therefore be included simultaneously as independent variables in models of seasonal reproductive success. However, because age of males is strongly correlated with age of their female mates, and data on reproductive success of either mate of a pair are inherently not independent (see also above), the analysis of reproductive success in relation to coloration were run on individuals of either sex separately. We did not analyse seasonal clutch size or seasonal fledging success in relation to coloration because in the present study we were not interested in investigating the mechanism that may cause an association between reproductive output and coloration, which will be the topic of a further study on much larger sample size over many years (Costanzo, Ambrosini, et al., 2017).

In all above linear models, we included the random effect of colony. Log-likelihood tests based on Gaussian linear mixed models did not show any significant contribution ($p > .05$ in all cases) of colony. However, the random effect of colony was retained in all models to account for any dependency of data from birds belonging to the same colony. All the analyses were run using PROC GLIMMIX in SAS 9.3.

The sample consisted of 65 females and their 65 male mates for RTL and seasonal reproductive performance analyses. The sample for coloration consisted of 62 females and 65 males (feathers for three females were not available).

3 | RESULTS

The age of both males and females ranged between 1 and 3 years (mean [*SD*] for males: 1.54 [0.56]; females: 1.46 [0.56]), was positively correlated between mates (r = .588, n = 65, $p < .001$) and did not differ between males and females (independent-samples t test; t_{64} = 1.22, p = .228). RTL did not differ between males and their mates (independent-samples t test; t_{64} = 1.38, p = .173) and did not

vary with age both in males (r = .01, n = 65, p = .969) and females (r = −.04, n = 65, p = .769).

3.1 | RTL and reproductive performance

Seasonal clutch size was not predicted by RTL of females (Table 1). Seasonal fledging success, reflecting the proportion of eggs that generated a fledged offspring, increased with RTL in both sexes (Table 1). Seasonal reproductive success increased with RTL in both sexes, although in males the relationship was marginally nonsignificant (p = .055) (Table 1; Figure 1). An increase in RTL by two standard deviations could be estimated to translate into an increase in seasonal reproductive success by 1.2 offspring for males and 1.3 offspring for females, corresponding to 24% (males) or 25% (females) of the mean observed seasonal reproductive success. Breeding success increased with age, as expected (Table 1).

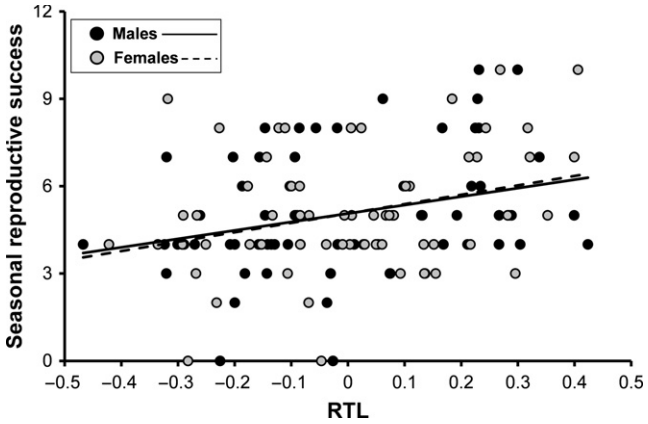

FIGURE 1 Seasonal reproductive success in relation to relative telomere length (RTL) of male or female barn swallows. Seasonal reproductive success is reported as the raw total number of nestlings in the breeding season. The relationship was significantly positive in both sexes

3.2 | RTL and coloration

Males had darker "visible" ventral plumage coloration (θ: paired t test; t_{125} = 4.31, p < .001) and larger ventral plumage saturation (rA: t_{125} = 3.59, p < .001), and their UV coloration differed from that of females (φ: t_{125} = 3.63, p < .001; Figure 2). Because none of the colour components significantly varied with age (see Section 2.4), these analyses were not influenced by age effects.

The relationships of ventral plumage colour components with RTL varied in a complex way between the sexes. Darkness of the "visible" coloration (as reflected by decreasing values of θ) increased with RTL in males, whereas it was not significantly associated with RTL in females, yielding a significant sex by RTL effect (Table 2; Figure 3). The values of the φ (UV) colour component increased with RTL independently of sex (Table 2; Figure 3). In addition, rA significantly decreased with RTL in females, while it nonsignificantly increased with RTL in males, again yielding a significant sex by RTL interaction effect (Table 2; Figure 3). Thus, coloration covaried with RTL, but in a sex-dependent way.

TABLE 1 Generalized linear mixed models of clutch size (females only), seasonal fledging success (proportion of fledged offspring/clutch size in the breeding season) and seasonal reproductive success (total number of offspring in the breeding season) in relation to relative telomere length (RTL) and age for females and males separately. Colony was included in the models as a random factor. For clutch size and seasonal reproductive success, a Poisson error distribution was assumed. For seasonal fledging success, a binomial error distribution was assumed. Estimated β (*SE*) are given. Sample size was 65 females and 65 males

	F	*df*	*p*	β (*SE*)
Seasonal clutch size				
Females				
RTL	0.41	1,53	.525	0.165 (0.258)
Age	7.97	1,53	.007	0.254 (0.090)
Seasonal fledging success				
Females				
RTL	4.02	1,53	.050	1.819 (0.907)
Age	0.08	1,53	.778	−0.093 (0.328)
Males				
RTL	4.99	1,53	.030	2.025 (0.906)
Age	2.22	1,53	.143	−0.492 (0.330)
Seasonal reproductive success				
Females				
RTL	5.08	1,53	.028	0.628 (0.279)
Age	6.85	1,53	.012	0.252 (0.096)
Males				
RTL	3.87	1,53	.055	0.529 (0.269)
Age	3.23	1,53	.078	0.179 (0.010)

3.3 | Coloration and seasonal reproductive success

Seasonal reproductive success differently covaried with ventral plumage coloration in either sex. Darker males had significantly larger seasonal reproductive success, whereas no significant relationship existed between "visible" coloration and seasonal reproductive success in females (Table 3; Figure 4). In addition, seasonal reproductive success of males but not females increased with increasing UV reflectance, whereas it did not covary with colour rA (Table 3; Figure 4).

4 | DISCUSSION

Telomeres have the potential to contribute a major component of variation in life history traits of eukaryotic organisms under both physiological and pathological conditions because their shortening dynamics are sensitive to environmental as well as genetic background, and may have pervasive consequences for organismal function (Monaghan & Haussmann, 2006). In fact, the first main finding

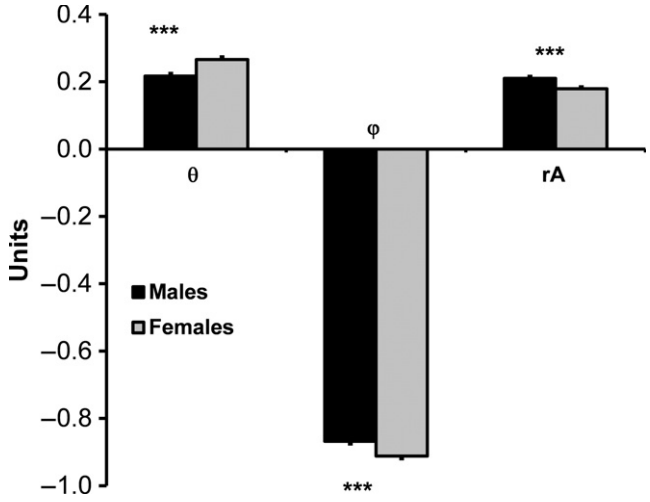

FIGURE 2 Mean (SE bars) tetrahedral colour components of ventral plumage coloration of female barn swallows and of their mates. Sample size is 62 females and 65 males. ***$p < .001$

of our study was that seasonal reproductive success of both male and female barn swallows positively covaried with RTL. These results are by necessity correlational, because experimental manipulation of TL can hardly be devised in the wild (but see Reichert, Bize, et al., 2014 for laboratory experimental approach), and therefore do not conclusively demonstrate causality. However, the relationship between breeding success and RTL was in the expected direction because long telomeres are predicted to enhance general individual performance at a given time, and thus seasonal reproductive success. These relationships were observed while controlling for the potentially confounding effect of individual age. As positive assortative mating for TL exists in the present population of barn swallows ($r = .384$, $n = 65$, $p = .002$; Khoriauli et al., 2017), a proximate explanation for a causal positive relationship between TL and seasonal reproductive success is that high-quality males with long telomeres mate with relatively fecund females which also have long telomeres, and/or that individuals with relatively long telomeres perform better at parental duties. The analysis of seasonal fledging success showed that RTL positively predicted the proportion of eggs that generated a fledged offspring for both adult males and females. However, RTL did not predict clutch size in females. Hence, it appears that larger seasonal reproductive success by individuals with large RTL was attained because individuals with large RTL have egg with larger hatching success and/or better surviving nestlings rather than because females with large RTL are more fecund.

Relative telomere length was found to covary with reproductive success as measured during one breeding season. It may therefore be speculated that RTL dynamics mediate a trade-off whereby individuals with longer telomeres experience higher seasonal reproductive success but also undergo larger telomere attrition and therefore suffer a reduction in residual reproductive value. This hypothesis would be best explored in an experimental framework, whereby reproductive effort during 1-year period is manipulated and the long-term consequences on TL are assessed. Because TL does not change with age in adult barn swallows, however, we consider the possibility that individuals that perform large reproductive investment subsequently undergo larger telomere shortening as unlikely.

Positive assortative mating for RTL (Khoriauli et al., 2017) suggested the existence of traits that reliably signal TL in both sexes, and that assortative mating results from mutual preference for high-quality mates, as expected in a species with extensive biparental reproductive investment. Here, we demonstrated that TL covaries with sexually dimorphic coloration in both sexes and may therefore

	F	*df*	*p*	EMM (*SE*)/β (*SE*)	
θ					
Sex	19.35	1,118	<.001	Males: 0.217 (0.008)	Females: 0.266 (0.008)
RTL	2.49	1,101	.118		
Sex × RTL	3.92	1,120	.049	Males: −0.102 (0.039)**	Females: 0.010 (0.042)
Age[a]	2.97	1,122	.087		
φ					
Sex	13.79	1,120	<.001	Males: −0.868 (0.009)	Females: −0.912 (0.009)
RTL	5.35	1,110	.023		
Sex × RTL[a]	0.00	1,120	.966		
Age[a]	0.79	1,119	.376		
rA					
Sex	13.60	1,118	<.001	Males: 0.210 (0.007)	Females: 0.178 (0.008)
RTL	0.05	1,112	.823		
Sex × RTL	6.26	1,120	.014	Males: 0.049 (0.030)	Females: −0.059 (0.033)*
Age[a]	0.01	1,119	.906		

[a]Term removed from final model.
*$.01 < p < .05$; **$p < .01$.

TABLE 2 Gaussian linear mixed models of ventral plumage tetrahedral colour components in relation to sex, relative telomere length (RTL) and age. The effects of age and of the RTL by sex interaction were removed from the models when nonsignificant and their effect before exclusion is presented. The random effects of breeding pair and of colony were always included in the models. Estimated marginal means (EMM) for factors and β (*SE*) for covariates are given. Sample size was 65 males and 62 females for all analyses

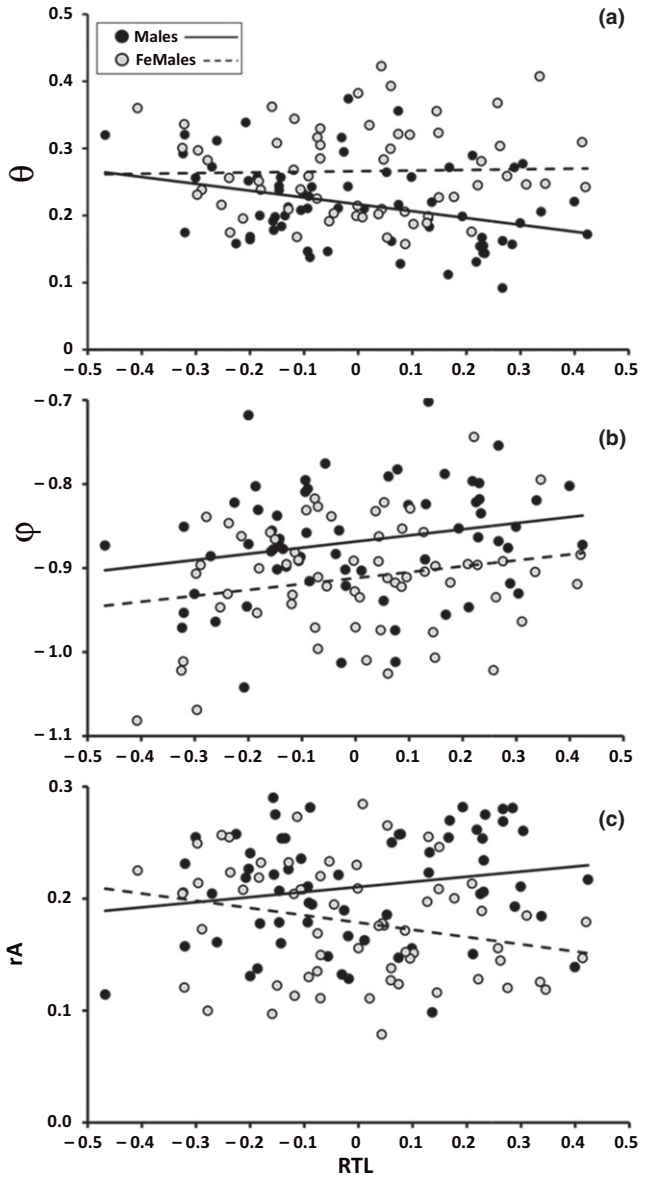

FIGURE 3 Tetrahedral components (θ, ϕ, rA) of the coloration of male and female adult barn swallows in relation to relative telomere length (RTL). Linear regression lines for either sex are shown. (a) θ values significantly decreased with RTL in males, but not in females. (b) φ values significantly increased with RTL in both sexes. (c) rA significantly declined with RTL in females, but did not significantly covary with RTL in males

mediate mutual sexual choice leading to assortative mating. Interestingly, the covariation between RTL and individual components of sexually dimorphic coloration differed between the sexes and in females existed only for the UV component and for colour saturation. The θ colour component of coloration has been shown to covary with survival (Saino, Romano, Rubolini, Ambrosini, et al., 2013) as well as with immune function and physiological stress response (Saino, Romano, Rubolini, et al., 2014) in the same barn swallow population that we studied here, while rA covaries with natal dispersal propensity (Saino, Romano, Scandolara, et al., 2014). In North American and Middle Eastern populations, colour darkness (quantified not using the tetrahedral colour space model) of males has been shown to be under directional sexual preference (Romano et al., 2017; Safran & McGraw, 2004; Vortman et al., 2011; Wilkins et al., 2016). Hence, the present study in combination with previous findings on the same and other barn swallow geographical populations highlights that coloration is involved in mate choice and potentially serves as a multifaceted signal in sexual communication context (but see Costanzo, Ambrosini, et al., 2017). In addition, these studies highlight the importance of considering all colour components in studies of communication in this and potentially also in the majority of the other bird species, which perceive UV wavelengths.

A relationship between coloration and RTL has been documented in adults of tawny owl (Strix aluco; Karell, Bensch, Ahola & Asghar, 2017) and in nestlings from the same barn swallow population we studied here (Costanzo, Parolini, et al., 2017). In barn swallow nestlings, differently from the present results, however, the relationship was not sex dependent (our unpublished results). Because nestling barn swallows show null or small colour sexual dimorphism (Costanzo, Parolini, et al., 2017; Romano et al., 2016), whereas adult males strongly differ in average coloration from females, the sex-dependent association between coloration and RTL appears to be established only in adulthood, when sexual differences in coloration are also established. The mechanisms that link TL to melanin-based plumage coloration are open to speculation. Costanzo, Parolini, et al. (2017) proposed that a link between melanogenesis and telomere dynamics may arise because the activity of telomerase, an enzyme that functions to restore TL (Chan & Blackburn, 2004), affects the expression of tyrosinase, which is involved in early melanin biosynthetic pathways (Bagheri et al., 2006). A general physiological link between melanogenesis and telomere dynamics may also arise via the pleiotropic effects of genes of the melanocortin system, which control melanogenesis and intervene in a number of physiological functions including response to diverse forms of stress and hence telomere dynamics (Ducrest et al., 2008).

The barn swallow is widely distributed across the Holarctic region and morphological variation exists across its range in sexually dimorphic traits, as well as in sexual dimorphism (Møller, 1994; Turner, 2006). In addition, sexual selection on dimorphic traits also seems to vary geographically. Most traits are under directional sexual selection in multiple populations/subspecies, but the strength of selection varies geographically (Romano et al., 2017). Here, we showed for the first time in a European barn swallow population that individual variation in both the "visible" and UV components of ventral plumage coloration predicts male seasonal reproductive success. Interestingly, in the same study population, darker males suffer a viability disadvantage compared to paler males (Saino, Romano, Rubolini, Ambrosini, et al., 2013), suggesting that conflicting fecundity and viability selection may concur in maintaining extensive, genetically based polymorphism in coloration (see also Costanzo, Ambrosini, et al., 2017).

TABLE 3 Poisson generalized linear models of seasonal reproductive success (number of offspring in the breeding season) in relation to ventral plumage tetrahedral colour components and age for females and males separately. Colony was included in the models as a random factor. Estimated β (*SE*) are given. Sample size was 62 females and 65 males

	Females				Males			
	F	*df*	*p*	β (*SE*)	*F*	*df*	*p*	β (*SE*)
θ	1.37	1,50	.248	1.026 (0.878)	4.72	1,53	.034	−2.050 (0.944)
Age	5.78	1,50	.020	0.243 (0.101)	2.24	1,53	.140	0.153 (0.102)
φ	0.01	1,50	.906	−0.102 (0.858)	4.50	1,53	.039	1.798 (0.848)
Age	4.95	1,50	.031	0.224 (0.101)	2.41	1,53	.126	0.157 (0.101)
rA	0.99	1,50	.325	−1.154 (1.160)	0.28	1,53	.600	0.623 (1.167)
Age	5.38	1,50	.025	0.230 (0.099)	2.79	1,53	.101	0.172 (0.103)

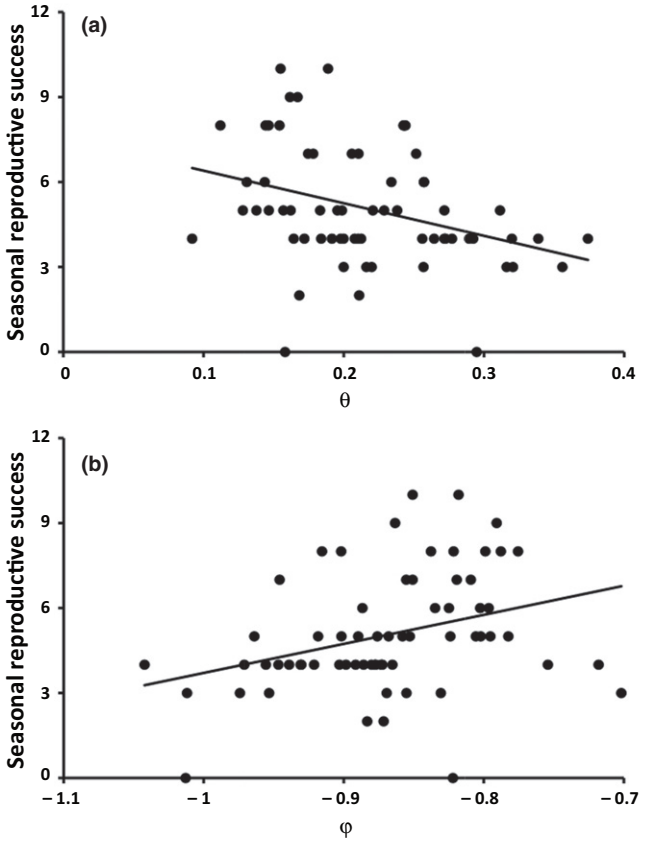

FIGURE 4 Seasonal reproductive success of males in relation to the θ (a) and φ (b) tetrahedral components of their ventral plumage coloration. Seasonal reproductive success is reported as the raw total number of nestlings in the breeding season

In conclusion, we showed for the first time in any bird species that TL covaries with both reproductive success and coloration in both sexes. Because coloration reliably reflects TL, it may mediate the observed positive assortative mating for TL, which may result from adaptive mutual choice between high-quality males and females. Telomere length may therefore be an ultimate target of (mutual) mate choice mediated by the expression of plumage colour traits, an hypothesis that can be tested in several other bird species. Finally, our study highlights the importance of considering all the different components of coloration of birds because these may have different roles in social and sexual communication.

DATA ACCESSIBILITY

The data on which the study is based are available as Supporting Information.

AUTHORS' CONTRIBUTIONS

M.P., A.R., L.C. and N.S. conceived the study; A.R., A.C., D.R. and N.S. collected field data; M.P., L.K., M.S., A.C., S.G.N. and E.G. performed laboratory analyses; A.C. performed colour analyses; M.P., A.R. and N.S. carried out the statistical analyses; M.P., A.R. and N.S. drafted the manuscript. All authors gave final approval for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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9. Chapter 4

Lifetime reproductive success, selection on lifespan and multiple sexual ornaments in male European barn swallows.

Evolution

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Lifetime reproductive success, selection on lifespan, and multiple sexual ornaments in male European barn swallows

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Natural and sexual selection arise when individual fitness varies according to focal traits. Extra-pair paternities (EPPs) can affect the intensity of selection by influencing variance in fitness among individuals. Studies of selection require that individual fitness is estimated using proxies of lifetime reproductive success (LRS). However, estimating LRS is difficult in large, open populations where EPPs cause reallocation of biological paternity. Here, we used extensive field sampling to estimate LRS in a population of barn swallows (*Hirundo rustica*) to estimate selection on lifespan and ornamental traits of males. We found selection on lifespan mediated both by within- and extra-pair fertilization success and selection on tail length mediated by within- but not extra-pair fertilization success. In addition, we found selection on tail white spots via extra-pair fertilization success after controlling for selection on other traits. These results were not confounded by factors that hamper studies of LRS, including nonexhaustive sampling of offspring and biased sampling of males. Hence, natural and sexual selection mediated by LRS operates on lifespan, tail length, and size of the tail white spots in barn swallows.

KEY WORDS: **Barn swallow, lifetime reproductive success, selection, sperm competition.**

Individuals can vary greatly in their relative contribution to the genetic composition of the next generation (Williams 1992). Large variation in individual fitness is most often reflected in the distribution of lifetime reproductive success (LRS) being strongly left-skewed in birds, with most individuals producing few offspring and only a few showing large LRS (Newton 1989; Clutton-Brock 1988). Because variation in individual fitness sets the scope for natural and sexual selection, dissecting the proximate and ultimate sources of variation in LRS is pivotal to our understanding of natural and sexual selection (Williams 1992; Webster et al. 1995; Shuster and Wade 2003).

LRS depends on the combination of the lifetime number of reproductive episodes (e.g., broods) and the average number of viable offspring produced per episode (Newton 1989; McGraw and Caswell 1996; Clutton-Brock 1988). Duration of life can thus be a major source of variation in LRS because it positively affects the lifetime number of reproductive events (Gustafsson 1986; Clutton-Brock 1988; Merilä and Sheldon 2000). However, in some studies, duration of adult lifespan does not strongly predict LRS (Mills 1989; Herényi et al. 2012), suggesting that variations in the number of offspring that are produced in each breeding episode overwhelm lifetime number of breeding episodes in determining LRS.

Variance in male reproductive success can arise in the context of sexual selection processes (Andersson 1994). Sexual selection studies have long sought the proximate and ultimate causes of

variation in male mating success (Kirkpatrick et al. 1990; Owens and Hartley 1998). Female mate preferences are often nonrandomly distributed with respect to the expression of male secondary sexual traits (Jennions and Petrie 1997; Saino et al. 1997; Møller and Ninni 1998; Griffith et al. 2002; Westneat and Stewart 2003; Wong and Candolin 2005). According to Fisherian/honest indicator mechanisms of sexual selection, such ornamental male traits evolve under the effect of directional intersexual selection by females for traits that reliably signal genetic/phenotypic quality of males and/or predict sexual attractiveness of their future offspring (Weatherhead and Robertson 1979; Andersson 1994; Fawcett et al. 2007). In addition, different male ornamental traits (e.g., skin or feather coloration and courtship displays in fish and birds) typically co-occur and are presented to choosy females simultaneously (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994). The selection pressures that lead to the evolution of such "multiple ornaments" and their function in the mate choice process are still contentious issues of debate (Møller and Pomiankowski 1993; Candolin 2003). Importantly, however, conspecific populations may differ in the strength of selection on individual ornaments and this may be a mechanism causing population divergence and eventually speciation (Møller and Cuervo 1998; Panhuis et al. 2001; Van Doorn et al. 2009).

In species where extra-pair paternities (EPPs) occur, variance in reproductive output among males can be affected by the success of males in securing their own (within-pair) paternity of their social progeny (i.e., the offspring generated by their social mate(s)) and in siring extra-pair offspring (EPO) by fertilizing females different from their social mate(s) (Whittingham and Dunn 2005; Lebigre et al. 2012 and references therein). The occurrence of EPPs is most often thought to boost variance in male realized reproductive output because success in siring EPO is not accompanied by a commensurate reduction in the number of within-pair offspring (WPO) (Webster et al. 1995; Sheldon and Ellegren 1999; Vedder et al. 2011). However, depending on the sign of the covariance between within- and extra-pair reproductive success, EPPs may either increase or decrease the variance in reproductive success among males. Hence, competition for paternity via EPP is a potentially double-edged component of sexual selection and can contribute to the evolution of male epigamic traits (Møller and Ninni 1998).

Estimating LRS in iteroparous species is difficult, as it requires long-term studies of populations of individually marked organisms where individuals are monitored during their entire life (Clutton-Brock 1988; Shuster and Wade 2003). The occurrence of frequent EPPs greatly hinders the scope for LRS studies because such studies require collecting exhaustive data on all WPO and EPO sired by individual males. In addition, "edge effects", whereby the study sample reproductively interacts via EPPs with the individuals breeding just outside the study area, can lead to inaccurate LRS estimates due to missed paternity events by the focal males (Webster et al. 1995; Sheldon and Ellegren 1999; Webster et al. 2001). We are unaware of any study of a large, open vertebrate population with frequent EPPs in the wild where the potentially confounding effects of EPP and edge effects could be assumed to have no or negligible role.

The European barn swallow (*H. rustica rustica*) that we studied is a small migratory passerine bird. These socially monogamous birds breed either as pairs or in colonies of two to tens of pairs, typically in farms, spatially isolated from other colonies in our study area. Females lay 1–3 clutches of 1–7 eggs per breeding season, from April to July (Møller 1994a). The proportion of offspring that are sired by a male different from their social father is high, although temporally and spatially variable (see below), as is the frequency of broods where at least one offspring is sired by an extra-pair male (Møller and Tegelström 1997; Saino et al. 1997; Kojima et al. 2009). Barn swallows are short-lived birds, with most adults having only one breeding season (Møller 1994a). Birds older than 3 years are rare as annual survival of adults is low (0.30–0.40; Møller and de Lope 1999). Importantly, barn swallows of both sexes have extremely high breeding philopatry (Møller 1994a). Hence, birds can be followed throughout their reproductive life, and individuals that do not return to the colony where they bred the previous year can confidently be assumed to have died. In addition, males do not apparently fertilize females breeding in other colonies, at least in our study area (see Ambrosini et al. 2012 for a description of the study area). However, since natal dispersal is high (Balbontín et al. 2009, Scandolara et al. 2014), with the vast majority of yearling recruits immigrating from a colony different from their original one, the frequency of mating between close relatives (parents–offspring; siblings) is extremely low (Kleven et al. 2005), implying that barn swallow populations are not affected by the consequences of inbreeding on population genetic structure.

Here, we identified parentage of all offspring produced at three colonies over three years and measured male LRS (including EPPs) to estimate selection differentials and partial selection differentials (i.e., selection gradients controlling for the effect of selection on correlated traits) on lifespan and a number of male secondary sexual traits that have been shown to have a role in sexual selection and competition for genetic parentage in one or more of the geographical populations/subspecies of this species (Romano et al. 2017). Specifically, using LRS as an estimate of fitness we estimated selection on the length and fluctuating asymmetry of the outermost tail feathers, on the size of the white spots on the tail feathers, and on melanin-based coloration of the white to chestnut ventral plumage region. In addition, we quantified selection on "ordinary" (i.e., nonsexually selected) traits including wing length, which is a major trait affecting flight performance, and body size as gauged by tarsus length.

Based on several previous studies (review in Romano et al. 2017), we expected that individuals with longer and more symmetric tails, larger white spots on tail feathers, and darker ventral plumage coloration had larger lifetime number of WPO in the broods where they were the social parents (LRS_{wpo}). In addition, we expected that these individuals also sired a larger lifetime number of EPO in broods other than their social broods (LRS_{epo}) and that, as a consequence, they had larger total LRS (LRS_{tot}, corresponding to $LRS_{wpo} + LRS_{epo}$). In addition, we expected that all indicators of LRS increased with lifespan because the number of breeding events strongly increases with duration of life.

Methods

We studied barn swallows breeding at three colonies (= farms) located west of Milan (Northern Italy) over five years (2012–2016). The colonies were chosen to represent very small (3–6 breeding pairs), medium (12–19 breeding pairs), or large-sized (22–26 breeding pairs) according to recent large-scale censuses (Ambrosini et al. 2012). In all study years, we captured and individually marked with numbered metal and plastic colour rings all the adults breeding in the focal colonies. Thanks to the extremely high breeding philopatry, the individuals that were captured in any year between 2013 and 2015 and had not been captured as adults in the previous year could be assumed to be 1-year-old individuals at their first breeding season immigrating from colonies outside our study area, except in rare cases when they were local recruits (i.e., individuals that were ringed as nestlings at the focal colonies allowing us to directly assess age). We could thus a posteriori identify a set of 79 males that started breeding in 2013–2015 and died before 2016. In 2013–2015, all breeding pairs were identified and breeding activities were monitored. Nestlings from all first, second and third broods were ringed and subjected to blood sampling at the age of 8–12 days for parentage analyses.

Standard morphological measurements were taken on all individuals in all capture years, including length of both outermost tail feathers, chord length of both wings, and tarsus length. Tail length and wing length were expressed as the mean of the left and right character. Tail asymmetry was expressed as the unsigned difference between the length of the left and the right outermost tail feathers. Some contour feathers were also collected from the same region of the white to rufous ventral plumage for later spectrometric colour measurements (see below). The fourth (counting outwards) right rectrix (R4) was plucked and stored flat in individual bags for later measurement of the size of the white spot (Saino et al. 2015). A small blood sample was taken for parentage analyses.

SPECTROMETRIC COLOUR ANALYSIS

Reflectance of one, randomly chosen, ventral feather, was recorded by means of an Avantes DH-2000 spectrometer equipped with a deuterium-tungsten halogen light source in a dark chamber and over a black background, as described in Saino et al. (2013a,b). Coloration was quantified by processing reflectance data according to the tetrahedral colour space model (Goldsmith 1990) using TetraColorSpace program (Version 1a; Stoddard and Prum 2008) implemented in MATLAB 7 (MathWorks, Natick, MA), assuming UVS cone type retina and adopting the spectral sensitivity of the blue tit (*Cyanistes caeruleus*). Each colour vector in the tetrahedral colour space was then converted into the spherical coordinates θ, φ, and rA (Stoddard and Prum 2008). θ and ϕ represent the human visible and the ultraviolet components of chroma, while rA reflects colour saturation. In the range of colours of barn swallow ventral feathers increasing θ values indicate paler, whitish coloration (Saino et al. 2013a,b). Repeatability of the three coloration variables as estimated by measuring twice the same feather was high ($r > 0.73$, $n = 45$ individuals). Similarly, among-feathers repeatability of the three colour variables estimated by measuring two different feathers from the same region was also high ($r > 0.74$, $n = 10$ individuals) (Saino et al. 2013a). Moreover, as demonstrated in Romano et al. (2015), the reflectance measurement of one ventral feather strongly correlates with the measurements taken on three overlapping feathers and well as directly on the bird's body. The correlation coefficients between the colour variable values obtained by the three measurements were: θ: $r > 0.88$, $n = 14$ individuals; ϕ and rA: $r > 0.91$, $n = 14$ individuals.

TAIL WHITE SPOT AREA MEASUREMENT

The R4 feathers were sellotaped to a cardboard backing across the shaft and scanned. Using ImageJ 1.46r software (rsbweb.nih.gov), for each feather we measured the area of the white spot (Saino et al. 2015). Importantly, in an additional sample of birds from which we also plucked the outermost rectrix (R6) (Saino et al. 2015), there was a positive correlation between the area of the white spot on R4 and on the sixth tail feather (R6) ($r = 0.72$, $n = 17$, $P < 0.05$), indicating that the size of the white spot is correlated within individuals across tail feathers.

GENETIC PARENTAGE ANALYSIS

DNA was extracted from blood samples by alkaline lysis and diluted to a final concentration of 50 ng/μL, according to Saino et al. (2008). Genotyping of adults and nestlings was performed on a total of five loci (see Supporting Information for detailed PCR reaction conditions). Three of them were highly polymorphic microsatellite loci previously developed for barn swallows (Hir7, Hir17, Hir20 (Tsyusko et al. 2007)), one was a microsatellite in the 3′ untranslated region (UTR) of the Adcyap1 gene

(adenylate cyclase-activating polypeptide 1; original primers Steinmeyer et al. 2009), and one a polymorphic region within the gene for proopiomelanocortin (POMC). POMC primers were designed by the authors using *H. rustica* genomic sequences kindly supplied by Dr. Anne-Lyse Ducrest and Prof. Alexandre Roulin (University of Lausanne, Switzerland; pers. comm. 2015). Either forward or reverse primers were fluorescently labeled (see Table S1). Polymorphism was determined using a commercial fragment analysis service (Macrogen Inc., Seoul, Republic of Korea) (Bazzi et al. 2015). Fragment lengths were scored for each individual using GeneMarker® version 2.4.2 software (Softgenetics). In total, 1046 individuals (235 adults and 811 nestlings) were genotyped at all five loci and 22 (four adults and 18 nestlings) at four loci. Parentage assignment was performed using Cervus version 3.0.3 software. Eight hundred twenty-one of 829 (99.03%) of the genotyped nestlings could be assigned to their genetic parents (see Supporting Information for details regarding Cervus parentage assignment). The presence of EPP was defined when the genetic father identified by parent pair analyses differed from the social father identified during behavioural observations. In no case did we identify instances of brood parasitism, that is, a nestling that did not genetically match with the social mother.

STATISTICAL ANALYSES

The phenotypic value of some traits (e.g., plumage traits that are molted annually) in barn swallows can change with age. To obtain an average estimate of the expression of any *i*-th trait for any *j*-th male that bred over more than one year, we first computed the difference (x_{ijt}) between the phenotypic value of the *i*-th trait recorded for the *j*-th individual at age *t* and the mean population-level phenotypic value of the *i*-th trait recorded on all individuals at age *t*. The phenotypic value pertaining to any *j*-th individual at the *i*-th trait was then computed as the mean (X_{ij}) of all the x_{ijt} values recorded for that individual during its entire life. These mean values were then used for the selection analyses detailed below. This procedure to obtain a synthetic phenotypic value while accounting for age effects requires that age-corrected values are significantly repeatable within individuals. In fact, analyses on the raw values of individual traits showed that the intraclass correlation coefficients, reflecting repeatability of traits that are renewed annually, as estimated from variance components of linear mixed models including age as a fixed effect, were large for all traits including tail length ($r = 0.74$), size of white spots on tail ($r = 0.53$), wing length ($r = 0.86$), and plumage tetrahedral colour components (θ: $r = 0.42$; φ: $r = 0.67$; rA: $r = 0.58$) (likelihood-ratio test comparing the model including vs. excluding the random effect of individual identity: all $\chi^2 \geq 4.63$, $P \leq 0.03$) (see Table 1 for sample sizes). Repeatability of tail asymmetry was estimated in a linear mixed model not including the effect of age, because tail asymmetry did not change with age in the present sample (Kendall's $\tau = -0.03$, $P = 0.72$, $n = 98$) as well as in other samples from the same population (our unpubl. results). Tail asymmetry had repeatability similar in magnitude to that of the other variables, but the statistical test showed that it was marginally nonsignificantly different from 0 ($r = 0.61$, $\chi^2 = 3.60$, $P = 0.06$) (see Table 1 for sample size). Hence, individuals were consistent in their expression of most of the focal phenotypic traits at different ages and their associated phenotypic value could be estimated as the mean of the deviations from the age-specific population means. An exception is represented by tail asymmetry, whose repeatability among years was statistically marginally nonsignificant. The results of the analyses on tail asymmetry should therefore be considered with this caveat in mind. For tarsus length, no adjustment for age was required because this trait does not vary in adulthood, and its phenotypic value at age 1 was therefore used.

To calculate selection gradients, we followed the method by Arnold and Wade (1984). In linear regression analyses of selection on LRS, X_{ij} vectors (or tarsus length at age 1) were standardized to a mean = 0 and variance = 1. Lifespan was also standardized to mean = 0 and variance = 1. Relative fitness, in terms of total number of offspring produced during life by any individual was expressed as the ratio ($rLRS_{tot}$) between the total offspring fathered divided by the mean number of offspring fathered by the 79 males in the sample. Similarly, the number of WPO or EPO sired was expressed as the ratios ($rLRS_{wpo}$ or $rLRS_{epo}$, respectively) between the WPO or EPO sired by an individual and the mean WPO or EPO sired by the males in the sample. Regression coefficients of rLRS variables on standardized phenotypic traits therefore reflect the proportional change in fitness relative to the population mean caused by a one standard deviation change in the phenotypic trait (Arnold and Wade 1984). The residuals of the regression in some cases did not meet the condition of normality. To test for robustness of the statistical tests, we therefore also applied nonparametric Spearman's correlation analysis. In all cases, the results of regression analyses were qualitatively confirmed by nonparametric correlation analyses, meaning that the tests that were significant with the former approach remained such also with the latter.

Tests for stabilizing/disruptive selection were performed in regression analyses of LRS on second-order polynomial terms on standardized phenotypic traits.

The amount of variance explained by the relationships between rLRS indicators and phenotypic traits was estimated by computing r^2. Correlation coefficients were compared by *z*-tests.

In the analyses of selection gradients, the significance values of the tests of $rLRS_{tot}$, $rLRS_{wpo}$, or $rLRS_{epo}$, respectively, were corrected according to the false discovery rate procedure (Benjamini and Hochberg 1995).

Table 1. Selection differentials on lifespan, morphological, and colour traits of male barn swallows for total lifetime reproductive success ($rLRS_{tot}$), and number of within- ($rLRS_{wpo}$) or extra-pair ($rLRS_{epo}$) offspring.

		$rLRS_{tot}$				$rLRS_{wpo}$				$rLRS_{epo}$			
	N	Coefficient (SE)	r^2	*t*	*P*	Coefficient (SE)	r^2	*t*	*P*	Coefficient (SE)	r^2	*t*	*P*
Lifespan	79	1.11 (0.09)	0.67	12.39	<0.01*	1.05 (0.09)	0.62	11.44	<0.001*	1.55 (0.29)	0.28	5.42	<0.01*
Morphological traits													
Tarsus length	79	0.13 (0.12)	0.02	1.17	0.27	0.09 (0.11)	0.01	0.80	0.43	0.40 (0.25)	0.03	1.60	0.11
Wing length	79	0.12 (0.13)	0.01	0.92	0.36	0.13 (0.12)	0.01	1.02	0.31	0.07 (0.28)	0.00	0.24	0.81
Tail length	79	0.35 (0.12)	0.10	2.87	0.01*	0.39 (0.12)	0.13	3.33	<0.01*	0.10 (0.28)	0.00	0.34	0.73
Tail asymmetry	77	−0.15 (0.13)	0.02	−1.14	0.26	0.15 (0.13)	0.02	−1.19	0.24	−0.14 (0.28)	0.00	−0.48	0.63
Tail white spots	79	0.13 (0.13)	0.01	0.99	0.33	0.05 (0.12)	0.00	0.39	0.70	0.66 (0.27)	0.07	2.49	0.02
Tetrahedral feather colour traits													
θ	79	−0.10 (0.13)	0.01	−0.76	0.45	−0.11 (0.12)	0.01	−0.85	0.40	−0.04 (0.28)	0.00	−0.15	0.88
φ	79	0.02 (0.13)	0.00	0.12	0.90	0.01 (0.12)	0.00	0.11	0.92	0.03 (0.28)	0.00	0.12	0.91
rA	79	0.14 (0.13)	0.02	1.13	0.26	0.16 (0.12)	0.02	1.27	0.21	0.06 (0.28)	0.00	0.23	0.82

The variance explained by the relationships (r^2) and sample size for number of males is reported. For two individuals, tail asymmetry could not be measured due to breakage of either outermost tail feather. Uncorrected *P*-values are reported. Asterisks indicate the *P*-values that remained significant after correction according to the false discovery rate procedure (see Statistical analyses).

In just one case (lifespan by farm effect on $rLRS_{epo}$, see Results), the interaction effect between farm and a phenotypic trait was found to significantly predict rLRS variables after correction of the significance values according to the false discovery rate procedure (Benjamini and Hochberg 1995). In addition, none of the breeding success variables was found to differ among colonies and in all cases the inclusion of the fixed effect of farm in the analyses did not qualitatively alter the results, meaning that the effects that were significant remained such after inclusion of the effect of farm in the model (Table S4). Thus, in all analyses males from the three colonies were pooled.

The analyses were run using PROC GLM in SAS 9.3 or SPSS13. Type III sum of squares was always used. Residuals from regression analyses were visually inspected to assess normality.

Survival in relation to tail length was analyzed in a Cox proportional hazards regression model. In this model, we allowed for time dependency of tail length as a covariate because tail length changes with age. To account for tied event times, we adopted the procedures implemented by PROC PHREG (TIES = EXACT) in SAS 9.3, which is based on the probability of the union of the partial likelihoods for all possible orderings of tied events.

Statistical parameters are reported with their associated standard error (SE).

Results

We measured LRS (LRS_{tot}, LRS_{wpo}, LRS_{epo}) for the 79 males that completed their life-cycle within the study period at the three focal colonies. We assessed parentage of >99% of the 829 nestlings that were produced and reached sampling age at the study colonies over the study period. The 79 focal males were found to have sired on average 5.38 (0.66 SE; range: 0–23) WPO nestlings and 0.78 (0.21 SE; range: 0–12) EPO nestlings at the end of their life, yielding a mean total realized reproductive success of 6.16 (0.78 SE; range 0–30) offspring. Thus, of the 487 nestlings that were found to have been sired by the 79 focal males, 425 (87.3%) were WPO while 62 (12.7%) were EPO. The percentage of broods of the 79 focal males where at least one nestling was found to be extra-pair was 34.7% (58/167 broods). Twenty-one of the 79 males did not produce any WPO nestlings. Ten of these were unmated while 11 males lost their clutch or brood before blood sampling. In three cases, a male that did not produce any WPO produced at least one EPO.

SELECTION DIFFERENTIALS ON LIFETIME REPRODUCTIVE SUCCESS

Selection on lifespan was large and highly significantly different from 0 for $rLRS_{tot}$ (Fig. 1) and also for $rLRS_{wpo}$ and $rLRS_{epo}$, implying that lifespan is a major determinant of the total number of offspring that male barn swallows sire over their life, and that LRS increases with lifespan both as a result of a larger number of WPO and of a larger number of EPO (Table 1). For $rLRS_{epo}$, a statistically significant effect of the interaction between lifespan and farm was detected after false discovery rate correction ($F_{2,73} = 7.14$, $P < 0.01$). The relationship was significantly positive ($P < 0.05$) in the two largest colonies and nonsignificant in the smallest colony. This result suggests that the effect of extra-pair fertilizations on LRS increases with colony size possibly because of more opportunities for EPPs in large colonies.

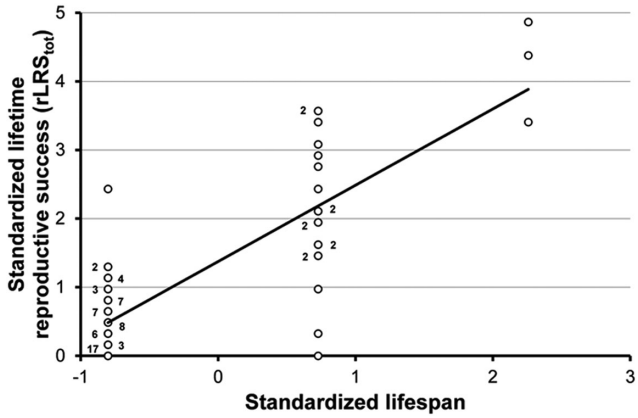

Figure 1. Lifetime reproductive success ($rLRS_{tot}$) expressed as the ratio between the total number of offspring produced by individual males ($n = 79$) throughout their life and the population mean in relation to lifespan standardized to mean = 0 and a variance = 1. The slope of the regression line represents the selection differential. The number of overlying data points is indicated. The line is the linear regression line.

Selection differentials on nonsexually selected morphological traits (wing length and tarsus length) were not significantly different from 0 for all rLRS components (Table 1). Selection on tail length was positive and strong for the total number of offspring sired (Table 1; Fig. 2) and also for the number of WPO, but not for the number of EPO (Table 1). To test if the slopes of the relationships between $rLRS_{wpo}$ or $rLRS_{epo}$ and tail length differed we analyzed LRS estimates in a linear model with tail length as a covariate and indicator of LRS ($rLRS_{wpo}$ or $rLRS_{epo}$) as a classification factor. The interaction effect between indicator of LRS and tail length was not statistically significant ($t = 0.97$, df = 154, $P = 0.33$), implying that the slopes of the relationship between LRS and tail length did not differ between $rLRS_{wpo}$ and $rLRS_{epo}$. However, the correlation coefficients between either indicator of LRS and tail length significantly differed ($z = 2.05$, $P = 0.04$) (Table 1), implying that the relationship with tail length was stronger for $rLRS_{wpo}$ than for $rLRS_{epo}$. However, the increase in either component of fitness per unit increase in tail length did not differ significantly. Importantly, tail

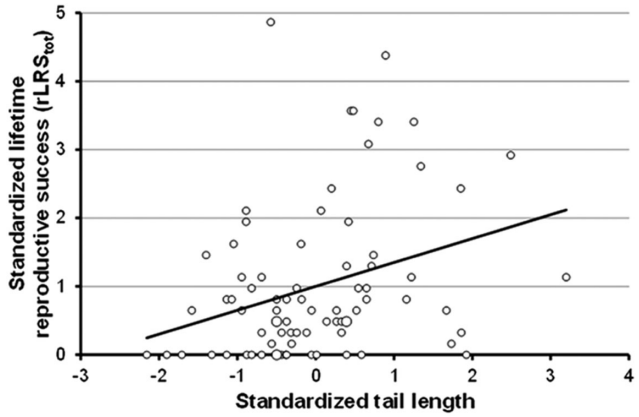

Figure 2. Lifetime reproductive success ($rLRS_{tot}$) expressed as the ratio between the total number of offspring produced by individual males ($n = 79$) throughout their life and the population mean in relation to tail length corrected for age (see Methods) and standardized to mean = 0 and a variance = 1. The slope of the regression line represents the selection differential. Larger dots indicate two overlaying data points. The line is the linear regression line.

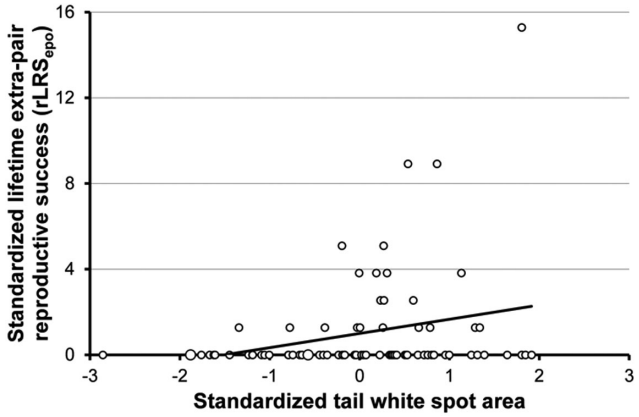

Figure 3. Lifetime extra-pair reproductive success ($rLRS_{epo}$) expressed as the ratio between the total number of extra-pair offspring produced by individual males ($n = 79$) throughout their life and the population mean in relation to size of the white spot on the R4 tail feather corrected for age (see Methods) and standardized to mean = 0 and a variance = 1. The slope of the regression line represents the selection differential. Larger dots indicate two overlaying data points. The line is the linear regression line.

length was expressed as the within-individual mean of the residuals from the age-specific mean phenotypic value (see Methods). Tail asymmetry did not significantly predict LRS components (Table 1).

Selection differentials on ventral colour plumage components were weak and not significantly different from 0 for all rLRS components (Table 1). Selection differentials on white tail spots were not significantly different from 0 for $rLRS_{tot}$ and $rLRS_{wpo}$. Selection differential on white tail spots was marginally nonsignificantly larger than 0 for $rLRS_{epo}$ ($P = 0.07$ after false discovery rate correction) meaning that there was a marginally nonsignificant trend for individuals with larger white spots on the tail feathers to sire a larger lifetime number of EPO (Table 1; Fig. 3). The slopes of the relationships between $rLRS_{wpo}$ or $rLRS_{epo}$ and white spots area significantly differed ($t = 2.09$, $df = 154$, $P = 0.04$) whereas the correlation coefficients did not ($z = 1.46$, $P = 0.15$). Hence, the strength of these relationships did not differ whereas the slopes did so.

Regression analyses of LRS on second-order polynomial terms of phenotypic traits showed no significant effect of the quadratic term after correction of the significance values for false discovery rate. Thus, there was no evidence for stabilizing selection on the traits that we measured.

SELECTION GRADIENTS ON TOTAL LIFETIME REPRODUCTIVE SUCCESS

We first estimated partial selection differentials (i.e., selection gradients) in multiple regression analyses of $rLRS_{tot}$ on lifespan, morphological traits, and θ colour component. We avoided entering all three tetrahedral colour components in the same model due to large correlation between the standardized θ and the rA components ($r = -0.64$, $n = 79$, $P < 0.01$). The correlations between the other traits were relatively small (unsigned r always smaller than 0.32) suggesting that their simultaneous inclusion in the model did not raise multicollinearity issues. We thus ran separate models where we included only one colour component at a time. The selection gradient was large and significantly larger than 0 for lifespan, showing that longer lived individuals accrue larger fitness independent of their phenotypic traits (Table 2). In addition, the selection gradient was significantly larger than 0 for tail length (Table 2). This implies that long-tailed individuals produce more biological offspring independently of any effect of tail length on survival. Further, tail length was found to positively predict survival in a Cox proportional hazard regression model (coefficient: −0.05 (0.02), $\chi^2_1 = 6.93$, $P < 0.01$). Regression gradients were not significantly different from 0 for tarsus and wing length, tail asymmetry, and the θ colour component (Table 2). A multiple regression model of $rLRS_{wpo}$ confirmed the positive effect of lifespan (Table 2). A multiple regression model of $rLRS_{epo}$ disclosed a significant positive effect of tail white spots area (Table 2).

Alternative multiple regression models in which we included the φ or rA components of feather coloration were consistent with the model on the θ component in disclosing significant effects of lifespan and of tail length and in showing nonsignificant effects of the other independent variables on both $rLRS_{tot}$ and $rLRS_{wpo}$ (other details not shown). In addition, they showed a significant effect of tail white spot area on $rLRS_{epo}$.

Table 2. Selection gradients on lifespan, morphological and "visible" plumage chroma for total lifetime reproductive success ($rLRS_{tot}$).

	$rLRS_{tot}$			$rLRS_{wpo}$			$rLRS_{epo}$		
	Coefficient (SE)	*t*	*P*	Coefficient (SE)	*t*	*P*	Coefficient (SE)	*t*	*P*
Lifespan	1.05 (0.09)	11.89	<0.01	0.98 (0.09)	11.11	<0.01	1.50 (0.29)	5.13	<0.01
Tarsus length	−0.05 (0.07)	−0.46	0.51	−0.09 (0.08)	−1.25	0.21	0.26 (0.25)	1.04	0.30
Wing length	0.00 (0.08)	0.02	0.98	0.01 (0.08)	0.14	0.89	−0.06 (0.26)	−0.23	0.82
Tail length	0.23 (0.08)	2.88	0.01	0.28 (0.08)	3.57	0.001	−0.16 (0.26)	−0.60	0.55
Tail asymmetry	−0.14 (0.08)	−1.77	0.08	−0.12 (0.08)	−1.61	0.11	−0.22 (0.26)	−0.85	0.40
Tail white spots	0.14 (0.07)	1.93	0.06	0.07 (0.08)	0.94	0.35	0.63 (0.24)	2.62	0.01
θ	−0.13 (0.07)	−1.74	0.09	−0.13 (0.07)	−1.82	0.07	−0.08 (0.24)	−0.34	0.76

The sample included 77 males for which complete phenotypic information was available.

Discussion

Estimating the intensity of selection on lifespan and sexually selected traits is a key step in the analysis of the evolution of life histories and of sexual selection. Here, based on information on LRS we analyzed selection on lifespan and on secondary sexual and nonsexual morphological traits in male barn swallows while accounting for the effects of EPP. We found evidence for strong LRS directional selection on lifespan and length of the outermost tail feathers while accounting for age-dependent variation in ornamentation. Selection on tail length was independent of the effect of tail length on annual survival. Selection differentials on other sexually selected traits or body size and wing length did not differ significantly from zero. Significant selection based on LRS on tail length was detectable when the within-pair but not the extra-pair component of LRS was considered. The relationship with tail length was stronger for $rLRS_{wpo}$ than for $rLRS_{epo}$. This implies that selection for larger tail ornaments is significantly different from 0 and is stronger when it is mediated by the number of biological offspring that males can secure in the broods where they are the social fathers rather than via extra-pair fertilizations.

Overall, strong selection existed on lifespan both via the number of WPO and the number of EPO. Interestingly, selection mediated by the number of EPO was significantly larger in the largest colonies. This result was obtained based on a small sample of just three colonies, and should therefore be considered with this caveat in mind. However, it may suggest that selection on lifespan mediated by extra-pair fertilizations is larger in larger colonies, where opportunities for extra-pair fertilizations may be larger and/or more variable among males. Selection on the size of the white spots on the tail was stronger via the lifetime number of EPO compared to WPO. Significant estimates of selection on tail length and size of white spots were not the spurious result of the indirect effect of selection on other traits, including body size and wing length, as implied by the significant selection gradients controlling for these potentially confounding effects.

Selection on lifespan was expected based on the fact that in the barn swallow variance in clutch and brood size is small, fledging success is extremely high and broodedness (i.e., the number of broods per breeding season) varies markedly between yearlings and older individuals (Møller 1994a). Thus, the number of breeding events and, consequently, of offspring produced, markedly increases with the number of breeding seasons that individuals experience (Saino et al. 2012). Notably, there may be exception to the observation of positive selection on lifespan even among short-lived small birds, as suggested by the study of an archipelago population of house sparrows *Passer domesticus*, where lifespan did not strongly predict realized LRS (Jensen et al. 2004).

While sexual selection has been intensively studied in the barn swallow, including in the present population, for many years (reviews in Romano et al. 2017), this is the first study in which LRS incorporating the effects of extra-pair fertilization has been quantified and estimated for sexual ornaments. Previous studies have shown clearly significant geographical variation in current sexual selection including EPP on individual male ornaments (i.e., melanin-based coloration and tail length) (Møller et al. 2003; Eikenaar et al. 2011; Vortman et al. 2013; Safran et al. 2016; Wilkins et al. 2016; Romano et al. 2017). The present results indicate that in Western Palearctic populations, contrary to what apparently occurs in other Eurasian (Vortman et al. 2011; Hasegawa and Arai 2013) and most Nearctic populations (Safran and McGraw 2004; but see Kleven et al. 2006), tail length is under selection. This observation is consistent with selection due to variance in annual reproductive success (ARS) on tail length, as observed in several Western Palearctic barn swallow populations (e.g., Møller et al. 1998) also while accounting for EPPs (Saino et al. 1997). The area of the white spots on tail feathers was apparently under positive selection mediated by the success in extra-pair fertilizations after controlling for selection on other traits. This finding is consistent with the observation that in Northern European population white tail spots are targeted by directional

sexual selection (Kose et al. 1999), although no previous study has investigated their role in competition for genetic parentage. Conversely, plumage melanin-based coloration, which has been suggested to be under intersexual selection in other subspecies (Romano et al. 2017 and references therein), seems not to be under directional selection mediated by reproductive success in this Italian population. In fact, selection differentials on "human visible" and UV chroma and on colour saturation were far from being statistically significant, and selection gradients controlling for several traits and for lifespan were also statistically nonsignificant. These results are therefore consistent with meta-analytic evidence and individual studies indicating that different plumage ornaments are differently selected in distinct barn swallow subspecies (summarized by Romano et al. 2017). Selection due to variation in LRS on tail length was also accompanied by viability selection, as long-tailed males had larger annual survival, consistent with previous studies (Møller 1994b; Saino et al. 2011).

Selection on tail length may cause evolution in tail length if the prerequisite condition of nonzero additive genetic variation in the trait is met. Previous studies adopting diverse approaches have led to relatively large estimates of heritability in tail length. For example, parent–biological offspring regression analysis has led to narrow sense heritability (h^2) estimates of 0.39 while regression of EPO phenotype on the phenotype of the social (but nonbiological) father have disclosed low and statistically nonsignificant social parent–offspring resemblance ("heritability" estimate: −0.07) (Saino et al. 2003). Such comparison between father and biological versus nonbiological offspring resemblance suggests no significant maternal and environmental effects on father–offspring resemblance and, hence, that large additive genetic variation in tail length exists. In addition, genetic correlations between tail length and other morphological traits including wing length and body size are generally low suggesting that genetic correlations are not expected to constrain the evolutionary change of tail length. Moreover, selection on male tail length has been shown to be consistent in time within populations, though variable in strength among populations (Møller et al. 2006). Significant heritability, weak genetic correlations with other traits, and consistent selection across generations leads to expectations of evolution in male tail length, although no such change seems to have occurred over 25 years (our unpubl. data). This may suggest that environmental effects may mask evolution. For example, deterioration of the ecological conditions in the sub-Saharan wintering quarters of our study population may cause both its current marked demographic decline and an environmental effect consisting in a reduction in the expression of the tail ornaments, which are produced at the time of the single annual molt during winter in Africa (Saino et al. 2004). Indeed, tail length of barn swallows may depend on individual general state at the time of molt (Møller 1994a; Turner 2006), and deterioration of ecological conditions during wintering may hinder the ability of males to grow a long tail. Thus, apparent evolutionary stasis may result from evolutionary increase in tail length being confounded by negative effects of ecological conditions on physiological state and thus on tail growth. Yet an alternative interpretation is that offspring viability negatively covaries with paternal lifespan or LRS. We deem this explanation unlikely, however, because in a recent study we found no relationship between paternal and offspring longevity (Romano et al. 2016; our unpubl. data), and in previous studies we could find no evidence for phenotypic differences at traits that are likely to affect postfledging survival between WPO and EPO (our unpubl. data).

Estimating selection based on variation in LRS in the wild is challenging, because it requires that reproductive performance of individuals is monitored throughout their lives (Lebigre et al. 2012). In addition, one potential pitfall of LRS studies is nonrandom sampling of individuals, because unmated individuals or individuals that fail their breeding attempt early in the breeding cycle may be more likely to go undetected and may not represent a random sample of the population with respect to phenotypic/genetic quality (Sheldon and Ellegren 1999; Webster et al. 2001; Lebigre et al. 2012). In addition, quantifying selection based on variation in LRS is particularly problematic in species where the occurrence of EPPs causes a reallocation of paternity among individuals with respect to the apparent, social mating pattern (Webster et al. 1995). Because variation in success in sperm competition is typically nonrandom with respect to quality of individual males, as reflected for example by their sexual ornaments (Saino et al. 1997; Safran et al. 2005), neglecting the consequences of EPPs on realized LRS can result in biased estimates of selection on sexual ornaments. In the present study, we exhaustively sampled all the offspring and the adults that were present in any study colony during the study period, thereby assessing parentage of more than 99% of all the offspring produced in the colonies during the study and paying special attention not to exclude individuals that did not breed successfully either because they failed to acquire a social mate or because their breeding attempt failed. Because the genetic father (and mother) could always be identified among the adult males belonging to the focal colonies, extra-pair fertilizations seem not to occur among different colonies, as expected. We can therefore also exclude that our data were confounded by "edge" effects, that is, by missed paternity events by individuals from the focal colonies that fertilized females from other colonies, and be confident that all the biological offspring of the focal males that reached blood sampling age were identified, thereby providing unbiased estimates of realized LRS.

Admittedly, however, our study did not include four- or more years-old individuals because the study spanned five years (2012–2016), the first year (2012) served to identify the individuals that were 1-year-old recruits in 2013 while the last year (2016) served

to identify the individuals that had their last breeding season in 2015. However, relatively old (four or more years) individuals are relatively rare (Romano et al. 2016) and in the present sample no males that reached age four years (i.e., were still alive in 2016) were included. While the present results should be considered with this caveat in mind, we are confident that this feature of our data did not markedly bias the results and certainly did not produce spurious evidence of selection for larger lifespan or ornament size because LRS is expected to increase with lifespan and covary positively with tail length.

Notably, the frequency of EPO as estimated by the proportion of offspring sired in broods from other pairs relative to the total number of offspring sired was considerably smaller than in previous studies of the same geographical population (Saino et al. 1997; Møller et al. 1998). We speculate that this could result from a combination of factors. First, barn swallow populations have declined by as much as 50% during the last decade (Ambrosini et al. 2012), potentially reducing the scope for sperm competition, if sperm competition increases with breeding density (Westneat and Sherman 1997; Møller and Ninni 1998). It should also be noticed that previous EPP estimates were mostly obtained from colonies settled in large cowsheds with a large number of breeding pairs in the same room and typically little physical isolation between nests. This could have boosted the frequency of extra-pair paternity by increasing promiscuity among breeding pairs. Currently, such large colonies have considerably declined in number and no such type of colony is represented in the present sample. It should be emphasized, however, that the colonies where the present study was carried out do not represent exceptions, but, rather, the rule in terms of size and topographical scatter of breeding pairs among farm rooms (Ambrosini et al. 2012), and we deliberately choose the three colonies to represent the whole spectrum of variation between small and relatively large colonies. Second, in a declining population like our focal one the scope for sexual selection may be reduced if decline in population size also entails erosion of additive genetic variance in male quality, thereby reducing the scope for adaptive differential female preference for particular males. Extensive, long-term analysis of variation in the variance in the size of male tail ornaments will provide a clue as to whether reduction in the frequency of EPP is linked to a reduction in the variance in male sexual attractiveness.

In conclusion, we showed that selection currently exists on lifetime and length of the ornamental tail feathers and size of the white spots on the tail but not on other ornaments including ventral plumage coloration or nonsexual traits in a population of the socially monogamous barn swallow. Extra-pair fertilization analysis showed that selection is mediated by certainty of paternity of own social offspring rather than by success in fertilizing extra-pair females. These results on selection due to variance in LRS were obtained while controlling for several potentially confounding factors, including "edge effects" and nonrandom sampling of the study individuals.

AUTHOR CONTRIBUTIONS

Conceived the study: AC LG NS. Collected data in the field: RA AC MC MP AR DR NS. Performed feather colour and white spot area measurements: AC NS. Performed parentage analyses: AC MC EG LG MP. Analyzed the data: RA AC NS. Wrote the manuscript: LC AC LG NS.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Sequences and labelling of primers used to genotype adults and nestlings.

Table S2. Statistics for microsatellite loci used to determine paternity in barn swallows.

Table S3. Combined non-exclusion probability for first and second parent calculated for each year and each colony.

Table S4. Selection differentials on lifespan, morphological and colour traits of male barn swallows for total lifetime reproductive success ($rLRS_{tot}$), and number of within- ($rLRS_{wpo}$) or extra-pair ($rLRS_{epo}$) offspring.

SUPPORTING INFORMATION

PCR reaction condition:

The five loci used for the genotyping were amplified in a single multiplex reaction. PCR amplification was performed using a commercial kit (Qiagen, Multiplex PCR Kit) in a final volume of 25 µL with 12.5 µL 2× QIAGEN Multiplex PCR Master Mix, 2.5 µL 10× primer mix (0.5 µL of each primer) (final concentration 0.2 µM), 2 µL RNase-free water (for genomic DNA extracted from blood only), 5 µL 5× Q-Solution and 3 µL of DNA solution (5 µL for DNA extracted from feather samples). PCR amplification profile was: 95° C for 15 min, 35 cycles at 94° C for 30 s, 56° C for 90 s, 72° C for 60 s, and a final extension at 60° C for 30 min.

Cervus parentage assignment

The observed (H_{obs}) and expected (H_{exp}) heterozygosity, polymorphic information content (PIC) and frequency of null alleles (F(Null) were calculated using Cervus version 3.0.3 (Field Genetics Ltd.) (Kalinowski et al. 2007; Table S2). The combined non-exclusion probability of the marker set was always above 1.06×10^{-3} for the first parent and above 1.07×10^{-4} for the second parent (for a detailed analysis of combined non-exclusion probability, see Table S3).

Since all social pairs were assigned to their own nest during behavioural observation, and the sex of each parental individual is ascertained, we carried out parent pair analysis (by computing log-likelihood statistics for all possible offspring and candidate parent pairs (hereafter LOC)) in order to distinguish between within- and extra-pair paternity. We conservatively assumed that 99% of breeding females and 95% of males were sampled in each year and colony. Significance of parentage assignment was determined by the observation of Delta statistics value (LOC difference between the most likely and second most likely parental pair). When Delta value was above 95%, indicating full compatibility in the genotype comparison between offspring and parental pair or one mismatch (in most cases due to inconsistences in the alleles of the Adcyap1 gene, see Steinmeyer et al. 2009), the best candidate mother and father were considered the genetic parents of the nestling. We refrained to assign a nestling to the genetic parents in case of 2 or more mismatch between the nestling and the most likely pair. Thus, 8 out of 829 nestlings genotyped over 3 years in the 3 colonies could not be assigned to their biological parents and were thus excluded from the analyses.

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Table S1. Sequences and labelling of primers used to genotype adults and nestlings. Size indicates the range of observed alleles in bp.

Locus		Labelling	Primer sequence 5′−3′	Size (bp)
POMC	Forward	6FAM	GCTGGAACCGCTTCGGAC	190–220
	Reverse		ATGGAGTACGAGCGCTTCC	
Adcyap1	Forward	6FAM	GATGTGAGTAACCAGCCACT	160–180
	Reverse		AGATAACACAGGAGCGGTGA	
Hir20	Forward		GAAGTTGGAGAAAGATTAG	250–300
	Reverse	6FAM	TTATTGCTCTGGGTATGT	
Hir7	Forward	HEX	CTTGCGCAGAAAGTAT	190–246
	Reverse		GCTCTGGGATCTCTAG	
Hir17	Forward	TAMRA	ATGCCATGCTTCAGAT	200–300
	Reverse		CTGTCATGCCTAAGTATCA	

Table S2 Statistics for microsatellite loci used to determine paternity in barn swallows. K: number of alleles; N: number of genotyped individuals; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity; PIC: polymorphic information content; F_{null}: frequency of null alleles.

Locus	K	N	H_{obs}	H_{exp}	PIC	F_{null}
POMC	8	1042	0.807	0.799	0.769	-0.0048
Adcyap1	17	1061	0.845	0.835	0.818	-0.0050
Hir20	35	1067	0.839	0.850	0.834	0.0052
Hir7	34	1065	0.950	0.935	0.931	-0.0085
Hir17	28	1067	0.910	0.920	0.914	0.0052

Tab S3. Combined non-exclusion probability for first and second parent calculated for each year and each colony.

Colony		2013	2014	2015
Large sized	First parent	1.28×10^{-3}	1.13×10^{-3}	1.16×10^{-3}
	Second parent	1.37×10^{-4}	1.18×10^{-4}	1.19×10^{-4}
Medium sized	First parent	1.59×10^{-3}	1.16×10^{-3}	1.06×10^{-3}
	Second parent	1.75×10^{-4}	1.19×10^{-4}	1.07×10^{-4}
Small sized	First parent	1.15×10^{-3}	1.68×10^{-3}	2.54×10^{-3}
	Second parent	1.20×10^{-4}	1.85×10^{-4}	3.05×10^{-4}

Table S4. Selection differentials on lifespan, morphological and colour traits of male barn swallows for total lifetime reproductive success ($rLRS_{tot}$), and number of within- ($rLRS_{wpo}$) or extra-pair ($rLRS_{epo}$) offspring. In the models we included the fixed effect of colony. Uncorrected P-values are reported. Asterisks indicate the P values that remained significant after correction according to the false discovery rate procedure (see Statistical analyses)

	N	$rLRS_{tot}$				$rLRS_{wpo}$				$rLRS_{epo}$			
	F	df	P	Coefficient (SE)		F	df	P	Coefficient (SE)	F	df	P	Coefficient (SE)
Lifespan	151.24	1,75	<0.001*	1.113 (0.090)		126.44	1,75	<0.001*	1.042 (0.093)	31.47	1,75	<0.001*	1.598 (0.285)
Farm	0.92	2,75	0.404			0.74	2,75	0.482		1.68	2,75	0.193	
Morphological traits													
Tarsus length	1.23	1,75	0.272	0.133 (0.120)		0.72	1,75	0.398	0.099 (0.116)	2.02	1,75	0.159	0.366 (0.257)
Farm	0.64	2,75	0.531			0.97	2,75	0.385		0.40	2,75	0.669	
Wing length	0.54	1,75	0.465	0.097 (0.133)		0.75	1,75	0.390	0.111 (0.128)	0.00	1,75	0.985	0.006 (0.288)
Farm	0.48	2,75	0.619			0.79	2,75	0.459		0.61	2,75	0.545	
Tail length	7.81	1,75	0.007*	0.345 (0.124)		10.89	1,75	0.002*	0.387 (0.117)	0.05	1,75	0.829	0.061 (0.280)
Farm	0.53	2,75	0.588			0.96	2,75	0.386		0.61	2,75	0.548	
Tail asymmetry	0.93	1,73	0.338	-0.127 (0.132)		0.98	1,73	0.325	-0.126 (0.127)	0.22	1,73	0.642	-0.134 (0.287)
Farm	0.66	2,73	0.521			0.92	2,73	0.404		0.66	2,73	0.519	
Tail white spots	1.42	1,75	0.237	0.162 (0.136)		0.49	1,75	0.488	0.092 (0.132)	4.98	1,75	0.029	0.639 (0.286)
Farm	0.86	2,75	0.426			1.09	2,75	0.341		0.15	2,75	0.864	
Tetrahedral feather colour traits													
θ	0.61	1,75	0.437	-0.100 (0.128)		0.75	1,75	0.391	-0.107 (0.124)	0.04	1,75	0.846	-0.054 (0.278)
Farm	0.65	2,75	0.523			0.94	2,75	0.397		0.65	2,75	0.526	
φ	0.09	1,75	0.767	0.039 (0.130)		0.10	1,75	0.752	0.040 (0.126)	0.01	1,75	0.914	0.031 (0.281)
Farm	0.67	2,75	0.516			0.96	2,75	0.387		0.64	2,75	0.530	
rA	1.26	1,75	0.266	0.143 (0.128)		1.64	1,75	0.204	0.158 (0.123)	0.02	1,75	0.877	0.043 (0.279)
Farm	0.63	2,75	0.535			0.95	2,75	0.391		0.63	2,75	0.537	

10. Chapter 5

Extra-pair fertilizations vary with female traits and pair composition, besides male attractiveness in barn swallows.

Animal Behaviour

Extra-pair fertilizations vary with female traits and pair composition, besides male attractiveness in barn swallows

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Abstract

Reproductive promiscuity, whereby females are fertilized by extra-bond mates, is common. The frequency of extra-bond fertilizations depends on at least three sources of variation. First, females may differ in their proneness to being fertilized by extra-bond males. Second, males may differ in traits that affect realized promiscuity of female. Third, extra-bond fertilizations decisions depend on the combined effects of the identity of both social mates. Here, we rely on extensive genetic parentage analysis of the offspring of a socially monogamous bird, the barn swallow (*Hirundo rustica*), to assess which of the above sources of variation predict the occurrence of extra-pair fertilizations (EPFs). EPFs covaried with female feather morphological and coloration traits while controlling for pair composition and social mate sexual attractiveness. As expected, females mated with highly ornamented, long-tailed males had fewer EPFs. The composition of the breeding pair also accounted for variation in EPFs, implying that the ability of individual males to secure genetic parentage varies between female mates. These results show that females differ in promiscuity and female phenotypic traits that are sensible to males are associated with promiscuity, potentially serving as signals to prospecting males. Hence, contrary to common interpretations of the negative relationship between male sexual attractiveness and female promiscuity, larger genetic parentage by highly ornamented males may result from their ability to secure the less promiscuous social mates rather than from female decision of being less promiscuous when mated to them. In addition, our study shows that EPFs also depend on the composition of the social pair, as expected if a component of female promiscuity decisions depends on genetic compatibility with the social male mate. Our study emphasizes that female promiscuity and its phenotypic correlates, and composition of the social pair, deserve closer attention in studies of sexual selection mediated by extra-bond fertilizations.

Keywords: barn swallow, female phenotype, male attractiveness, promiscuity, sperm competition.

Highlights

- birds are rarely sexually monogamous
- females can differ in promiscuity
- female phenotypic traits may covary with promiscuity
- barn swallow (*Hirundo rustica*) were monitored during multiple breeding events
- promiscuity covaried with female traits and depended on pair composition

Introduction

Reproductive promiscuity is the rule, rather than the exception, in many plant and animal taxa. Even in animals with strong (monogamic or polygamic) socio-sexual bonds between the sexes, a batch of ova from a single female may be fertilized both by her social and by extra-bond males (Petrie and Kempenaers, 1998; Griffith, Owens and Thuman, 2002; Westneat and Stewart, 2003). Reciprocally, a single male may fertilize ova from multiple females, including extra-bond ones, during a single breeding episode (i.e. season) (Petrie and Kempenaers, 1998; Griffith, Owens and Thuman, 2002).

Promiscuity is adaptive for males, as long as their advantages of enhanced individual reproductive output via extra-bond fertilizations are not overwhelmed by any costs of paternity loss of the own social progeny or by the costs associated with extra-bond mating behaviour (e.g. search costs; infection by horizontally transmitted parasites) (Birkhead and Møller, 1992). Variation in the frequency of extra-bond fertilizations and in paternity (i.e. the proportion of social offspring that are also biological offspring of a focal male) is often found to be non-random with respect to male phenotypic and genetic traits, depending on the expression of male sexual ornaments (Jennions and Petrie, 1997; Wong and Candolin, 2005), position in the social dominance hierarchy (Smith, 1988; Qvarnström and Forsgren, 1998) or timing of emergence at the breeding sites (Stutchbury, 1998; Griffith, Owens and Thuman, 2002). Such variation has consequences for sexual selection as well as for population genetic variability ultimately because asymmetric competition for genetic parentage among males can affect the variance in realized reproductive success (Partridge, 1989; Clutton-Brock, 1988).

A number of adaptive evolutionary explanations of female promiscuity have been proposed. These hypotheses posit that females acquire either direct benefits (Birkhead and Møller, 1992; Sheldon, 1994; Nakamura, 1998) or indirect genetic benefits for their progeny, if the extra-bond male is of superior genetic quality compared to the social mate (Yasui, 1998; Møller and Ninni, 1998, Jennions and Petrie, 2000). An alternative view is that females choose as extra-bond mates those

that carry ‘compatible’ genes (Tregenza and Wedell, 2000; Colegrave et al., 2002; Mays et al., 2008). For example, females may be expected to choose males that are genetically dissimilar to them because this will assure them fitness benefits in terms of offspring heterozygosity at genes where heterozygosity enhances fitness (Griffith, Owens and Thuman, 2002; Griffith and Immler, 2009; for a review, see Jennions and Petrie, 2000), like the MHC genes (Juola and Dearborn, 2012). Extra-bond fertilizations may therefore be a tool to circumvent the social constraints on optimal choice of social mates that are either of superior genetic quality or carry compatible genes to those of the choosy female. If female decision on promiscuity depends on ‘absolute’ quality of the social mate, all females should be less promiscuous when mated to a male displaying reliable phenotypic signals of superior quality, like large sexual ornaments (Petrie and Kempenaers, 1998; Griffith, Owens and Thuman, 2002). If, on the other hand, female decisions on promiscuity depend on genetic compatibility, independent of sexual ornamentation of their mate, females should differ in fidelity to different males and, reciprocally, a given male should experience different promiscuity by different female mates.

However, many studies have failed in identifying any obvious net advantage to females arising from reproductive promiscuity (Arnqvist and Kirkpatrick, 2005; Griffith, 2007). This has led to speculate that female promiscuity can also arise as a consequence of genetic constraints on female sexual behaviour (Forstmeier et al., 2014). Such constraints may operate at the between-sexes level, with genes that promote adaptive sexual promiscuity in male offspring also having positive pleiotropic effects on promiscuity in daughters (Forstmeier et al., 2011), or at the within-sex level, with females that are genetically more responsive to courtship by their social mate being also more responsive to courtship by extra-bond males (Patrick et al., 2012).

The hypotheses on the evolution of female promiscuity rest on the implicit assumption that females can differ in promiscuity (Forstmeier, 2007), i.e. that there are females that are more prone to engage in extra-bond copulations/fertilizations than others, and such individual variation is the target of selection driven by the benefits and costs of extra-bond copulations/fertilizations and of the

genetic constraints on female mating behaviour (Forstmeier et al., 2014). However, the evidence that females differ in *realized* promiscuity is typically confounded by the effects of the (largely undocumented) variation in female proneness to engage in extra-bond fertilizations with social effects such as variation in mate quality, which are both expected to affect the frequency of extra-bond fertilizations (e.g. Dyrcz et al. 2002). One approach to test if individual females differ in promiscuity is to analyse the covariation between realized female promiscuity and female phenotypic traits while controlling for social effects such as those of the identity and of the ornamental traits of their male social mates that may affect availability of females to extra-bond fertilizations.

Variation in female promiscuity, in turn, is likely to have a major impact on several evolutionary processes. First, it can affect the outcome of male-male competition for genetic parentage by affecting paternity and the access of males to extra-pair fertilizations, and the consequent sexual selection processes (Birkhead, 2000). Second, particularly in species with relatively large paternal investment in reproduction, males may prefer females with low promiscuity as social mates because of reduced risks of incurring the fitness costs of loss of paternity in their social broods (Sheldon and Ellegren, 1998). Third, reliable signals of female promiscuity may evolve in species where females actively solicit extra-pair copulations, as females that reliably signal high promiscuity will have easier access to preferred extra-bond male mates (Bouwman and Komdeur, 2005; Whittingham and Dunn, 2010). These signals, in addition, may be the target of adaptive male choice of females with low promiscuity as social mates. Finally, at the population level, variation in female promiscuity will be a major determinant of population parameters that depend on individual variation in realized reproductive success. Variation in female promiscuity can thus be expected to have pervasive effects on sexual selection and population genetic processes. Yet, the extent of variation in female promiscuity and the traits of females that covary with it are only very sparsely known.

129 Some studies have hinted at small consistency in female 'fidelity' across breeding episodes (e.g.
130 Weatherhead, 1999; Forstmeier, 2007). Those studies however did not control for potentially
131 confounding social effects and therefore do not allow inferences on propensity of females to engage
132 in extra-bond fertilizations. In a study of coal tits (*Parus ater*), for example, females were not
133 consistent in their level of promiscuity across breeding episodes, except in cases where mate
134 retention occurred, suggesting that promiscuity depended on the combined effects of female and
135 male identity (Dietrich et al., 2004). In a manipulative study of blue tit (*Cyanistes caeruleus*) (Jong
136 et al., 2017), female promiscuity was lower in females that were treated with testosterone compared
137 to controls. Because testosterone levels may differ among females, this study provides a
138 mechanistic explanation for individual-level consistency in promiscuity of females (Jong et al.,
139 2017). In the pied flycatcher (*Ficedula hypoleuca*), females with longer wings had smaller
140 proportion of extra-pair offspring in their brood while controlling for the effects of age and male
141 coloration, providing evidence for an association between promiscuity and wing morphology
142 independent of confounding effects (Moreno et al., 2015). Interestingly, in a study of the aquatic
143 warbler (*Acrocephalus paludicola*) the opposite pattern was found, with females with longer wings
144 being more promiscuous (Dyrcz et al., 2002). In the same study, females with relatively short bills
145 were also found to be more promiscuous. However, the results for the aquatic warbler were
146 obtained without controlling for potentially confounding social effects. In a study of great tits
147 (*Parus major*), extra-pair fertilizations were found not to be related with exploratory behaviour of
148 females, suggesting that variation in female promiscuity does not depend on genetic linkage with
149 other, potentially related behavioural traits (Patrick et al., 2012). Female promiscuity has also been
150 shown to vary with age (Røskaft, 1983; Stutchbury et al., 1997), although the proximate causes in
151 terms of social effects (e.g. age-dependent variation in mate quality), ontogenetic variation of
152 female behavioural traits related to mating (e.g. ability in escaping/resisting forced copulation
153 attempts) or viability selection mediated for example by horizontal parasite transmission remain
154 unclear.

In the present study we capitalize on extensive genetic parentage analysis of a population of the socially monogamous barn swallow (*Hirundo rustica*) with moderate frequency (ca. 15% of the nestlings; 29% of the broods) of EPFs (Costanzo, unpublished data; see Results) and where a moderate proportion (ca. 30%, see Results) of individuals change mate between breeding episodes/breeding seasons, to address the following questions.

First, do phenotypic traits of females exist that are correlated with their promiscuity? Evidence for such association while controlling statistically for the identity and sexual ornamentation of the male mates would lend support to the hypothesis that females differ in promiscuity and suggest that female traits exist that may reveal promiscuity to prospecting males. Because very little empirical information on the female traits that may covary with female promiscuity exists, we intend the present study as an exploratory exercise aimed at generating rather than at testing hypotheses. We therefore explore the relationships between the frequency of EPFs and a number of female traits including size-related traits, coloration and the expression of sexually dimorphic traits currently under directional sexual selection in males. We had no explicit predictions on the direction of any such relationship and we therefore interpreted any statistically significant relationship *a posteriori*.

Second, does female promiscuity that individual males experience vary among their social female mates in different breeding episodes? Evidence for such variation would suggest that promiscuity is an attribute of the social breeding pair rather than of the female alone, i.e. that promiscuity depends on the combination of male and female identity, lending support to the genetic compatibility hypothesis (Tregenza and Wedell, 2000; Colegrave et al., 2002; Mays et al., 2008).

Third, does realized promiscuity of females depend on sexual ornamentation of their social mate? Previous studies of the same population (Saino et al. 1997; Møller et al., 1998a) have shown that males are more likely to be the biological fathers of their social offspring when they possess large tail ornaments. In this respect the present analyses are therefore a confirmatory test of previous evidence.

Methods

We studied three barn swallow colonies located in farms west of Milan (Northern Italy) from 2012 to 2015. The three colonies varied in size from 3 to 26 breeding pairs depending on the year. In all years, we captured and individually marked with numbered metal and plastic colour rings all the breeding adults and with metal rings all nestlings when they reached an age of approximately 10 days. Because barn swallows in our study population as well as in other European areas have extremely high breeding philopatry (Møller, 1994), the individuals that were captured in any year between 2013 and 2015 and had not been captured as adults in the previous year could be assumed to be 1-year-old individuals at their first breeding season that immigrated from other colonies (except in rare cases when they were individuals that were ringed as nestlings at the focal colonies allowing us to directly asses age). We identified within- and extra-pair offspring in all first (60.9%), second (38.1%) and third (1%) broods from 2013-2015 (see *Genetic parentage analysis*). Adults were assigned to their breeding pair and nest by behavioural observation.

From all individuals some contour feathers were collected from the same region of the white to rufous ventral plumage for later spectrometric colour measurements and a small blood sample was taken for parentage analyses.

In all capture years, we measured a number of morphological traits. Specifically, for the purposes of the present study, as phenotypic traits of the mother we considered:

1) Age, dichotomously classified as yearling (i.e. a female born in the previous calendar year and thus at her first breeding season) or ‘older’ (i.e. at her second or later breeding season);

2) Bill length, measured (in mm × 100) as the distance between the start of the feathering to the anterior tip of the bill;

3) Keel length, as a proxy for skeletal body size, measured (in mm × 100) as in Caprioli et al. (2013);

4) Tail length, expressed (in mm) as the mean of the outermost rectrices of either side measured from the insertion to the posterior tip. When either outermost rectrix was missing or broken, the length of the other was used;

5) Chord of both wings, wing span, length of the 9 primary wing feathers (thus excluding the outermost, vestigial one), length of the outermost secondary remex, length of the right innermost tail feather. Wing chord, wing span and length of the innermost tail feather were measured (in mm) as in Saino et al. (1997). Length of the primary and secondary remiges was measured as in Saino et al. (2015). A synthetic index of the length of the flight feathers (wing and tail), excluding the two outermost rectrices (see 3 above) that are subject to sexual selection in males, was obtained as the scores on the first principal component (PC1) from a PCA run on these variables (see also *Statistical analyses*);

6) The coloration in the 'human visible' (400-700 nm) and in the UV (300-400 nm) bands, expressed as ϑ and, respectively, φ components according the tetrahedral colour space model (see *Spectrometric colour analysis*).

In the present study population as well as in other European populations, tail length of males has been shown to predict their paternity of their social offspring as well as their likelihood of fertilizing extra-pair females (Saino et al. 1997; Møller et al., 1998b; Costanzo et al., unpublished data). We therefore considered tail length of the social mate, measured as for females (see point 4 above), as a covariate in some analyses. We refrained from including other male phenotypic traits besides length of the ornamental tail in the analyses in order to reduce the number of statistical tests because previous studies of the same population have shown that morphological and colour traits do not predict paternity or lifetime number of within-pair offspring (Saino et al. 1997, Costanzo et al., unpublished data).

Spectrometric colour analysis

We recorded the reflectance of one randomly chosen ventral feather by the means of an Avantes DH-2000 spectrometer (Saino et al., 2013a, b). Reflectance data were then processed according to the tetrahedral colour space model (Goldsmith, 1990) using TetraColorSpace program (Version 1a; Stoddard and Prum, 2008) implemented in MATLAB 7 (MathWorks, Natick, MA). Each colour vector in the tetrahedral colour space was subsequently converted into the spherical coordinates ϑ, representing the "human-visible" colour component, φ, representing the ultraviolet colour component and rA, representing colour saturation (Stoddard and Prum, 2008). In the barn swallow, higher ϑ values represent a paler colouration. Repeatability of colour variables determined by measuring twice the same feather is high, as well as repeatability obtained by measuring two different feathers taken from the same region (Saino et al., 2013a). Moreover, the colour components measured on one ventral feather, on three ventral feathers overlapped and on the bird's body are highly correlated (Romano et al., 2015). Being ϑ and rA values highly correlated ($r = -0.602$, $n = 200$, $P < 0.001$), we did not include rA in the analyses in order not to further inflate the number of tests.

Genetic parentage analysis

DNA was extracted from blood samples by alkaline lysis and subsequently diluted to a final concentration of 50 ng/µL (Saino et al., 2008). Genotyping of adults and nestlings was performed in a single multiplex reaction using a commercial kit (Qiagen, Multiplex PCR Kit) on a total of five loci: 3 highly-polymorphic microsatellite loci previously developed for barn swallows (Hir7, Hir17, Hir20), a polymorphic region within the proopiomelanocortin gene (POMC) and a microsatellite in the 3′ untranslated region (UTR) of the Adcyap1 gene. Either forward or reverse primers were fluorescently labelled. PCR amplification was performed in a final volume of 25 µL with 12.5 µL 2× QIAGEN Multiplex PCR Master Mix, 2.5 µL 10× primer mix (0.5 µL of each primer) (final concentration 0.2 µM), 2 µL RNase-free water (for genomic DNA extracted from blood only), 5 µL

5× Q-Solution and 3 µL of DNA solution (5 µL for DNA extracted from feather samples). PCR amplification profile was: 95° C for 15 min, 35 cycles at 94° C for 30 s, 56° C for 90 s, 72° C for 60 s, and a final extension at 60° C for 30 min. Polymorphism was determined using a commercial fragment analysis service (Macrogen Inc., Seoul, Republic of Korea) (see Bazzi et al., 2015). GeneMarker® version 2.4.2 software (Softgenetics) was used to score fragment lengths of each individual. In total, 239 adults and 829 nestlings were genotyped. Cervus version 3.0.3 (Field Genetics Ltd.) (Kalinowski, Taper and Marshall, 2007) was used to calculate the observed (H_{obs}) and expected (H_{exp}) heterozygosity, polymorphic information content (PIC) and frequency of null alleles (F(Null)). The combined non-exclusion probability of the marker set was always above 1.06×10^{-3} for the first parent and above 1.07×10^{-4} for the second parent. Parentage assignment was performed using Cervus version 3.0.3 software. Since the sex of each parental individual is ascertained, and all social pairs were assigned to their own nest during behavioural observation, we performed parent pair analysis (by computing log-likelihood statistics for all possible offspring and candidate parent pairs (hereafter LOC) in order to distinguish between within- and extra-pair paternity. We conservatively assumed that, in each year and colony, 95% of breeding males and 99% of females were sampled. When LOC difference between the most likely and second most likely parental pair was above 95%, indicating full compatibility or one mismatch in the genotype comparison between parental pair and offspring, the best candidate father and mother were considered the genetic parents of the nestling. The presence of extra-pair paternity was defined when the genetic father identified by parent pair analyses differed from the social father identified during behavioural observations.

Statistical analyses

To analyse paternity data, i.e. the proportion of offspring in the brood that were sired by the social father, we mainly relied on generalized linear mixed models (GLMM) assuming a binomial error

279 distribution. For comparisons of nested models differing in the random effects, likelihoods were
280 estimated by applying Laplace approximation, which provides reliable likelihood estimates for
281 binomial GLMM (Bolker et al., 2009). The comparison between nested models that differed for
282 random effects was performed by likelihood ratio tests.

283 The main aims of the study were to test if variation in paternity depended on identity of the social
284 father, of the social mother or on a combination of paternal and maternal identity (i.e. on pair
285 composition) and, in addition, to test for a covariation between paternity and maternal
286 morphological traits or paternal tail length. Before testing for the effects of these individual sources
287 of variation, we scrutinized the data on paternity for other sources of variation, including year and
288 colony. The strategy to test the effect of these factors was to compare a model including the random
289 effects of maternal-ID, paternal-ID, pair-ID, year (3-levels) and colony (3-levels) with models from
290 which the random effects of year or colony were removed one at a time. However, simultaneous
291 inclusion of all five random factors caused convergence problems in likelihood estimation. We
292 therefore tested the effects of year or farm by comparing models including male-ID, female ID,
293 pair-ID and year or farm with models from which the effect of year or farm, respectively, was
294 excluded. Because year and farm provided null contribution to the fit of the models (see Results),
295 these effects were subsequently neglected in all analyses.

296 The tests of the effect of individual maternal traits and paternal tail length on paternity were
297 performed in binomial GLMM always including the random effects of paternal-ID, maternal-ID and
298 pair-ID. Importantly, no over-dispersion was observed in these models (Pearson χ^2/df always <
299 1.00), and variance estimates associated to parameter coefficients therefore required no correction
300 for over-dispersion. A multiple binomial GLMM of paternity data was then run while including all
301 the maternal traits and paternal tail length that had either significant or marginally non-significant
302 ($P \leq 0.10$) effects in the separate GLMM, while again including paternal-ID, maternal ID and pair
303 ID as random effects. The effects of paternal-ID, maternal-ID and pair-ID were estimated by

likelihood tests comparing the model including all random effects with models were individual effects were removed one at a time.

Because of missing information for some females, the sample size for the analyses of the different traits slightly varied between 198 and 201.

The information on the size of flight feathers was summarized by applying Principal Component Analysis to all measurements available for all females in all years and breeding events simultaneously. Thus, PCA was run on female morphological data for 198 breeding events (information on female morphology for 3 broods was not available). The PC1 explained a very large fraction (73.1%) of the variance in flight feather length, and all measures had very large positive loadings (0.638 – 0.915) on the PC1 (see Supplementary Online Material for the loadings of the variables on PC1). Thus, we used PC1 scores as a synthetic index of size of flight feathers length (excluding tail length; see above).

The linear mixed model analyses were run by PROC GLIMMIX in SAS 9.3 using the events/trial syntax. Because the study was an exploratory exercise and running multiple tests on the same set of data may lead to an inflation of the risk of type I statistical errors, P values were corrected using the false discovery rate approach according to Benjamini and Hochberg (1995).

Statistical parameters are reported with their associated standard error.

Ethics statement

The study was authorized by Funzione Caccia, Pesca, Parchi e Gev, Corpo Polizia Provinciale, Provincia di Novara (Settore Agricoltura, Determina n. 55/2015, issued on January 20, 2015). The study was conducted in private lands, and land owners gave us the permission to access their farms. No approval by Animal Ethics Committee was required for the present experimental protocol.

Results

The study included 201 breeding events (i.e. first, second, and third broods) by 91 females and their 89 social male mates over three years. Twenty-six (28.6%) females and 26 (29.2%) males bred with more than one mate in the same and/or in different breeding seasons, whereas the remaining individuals only had one social mate. Mate switching generated 119 different pair combinations. Thus, because a non-negligible proportion of females had more than one mate during the study and the reciprocal was also true for males, we had the opportunity of disentangling the effect of individual from that of the breeding pair composition on paternity. Genetic parentage was assessed for 765 nestlings, 111 (14.5%) of which were found to be sired by a male different from their social father. One or more extra-pair offspring were present in 58 (28.9%) of the broods. No cases of brood parasitism were observed, meaning that the social and the biological mother of the offspring always coincided.

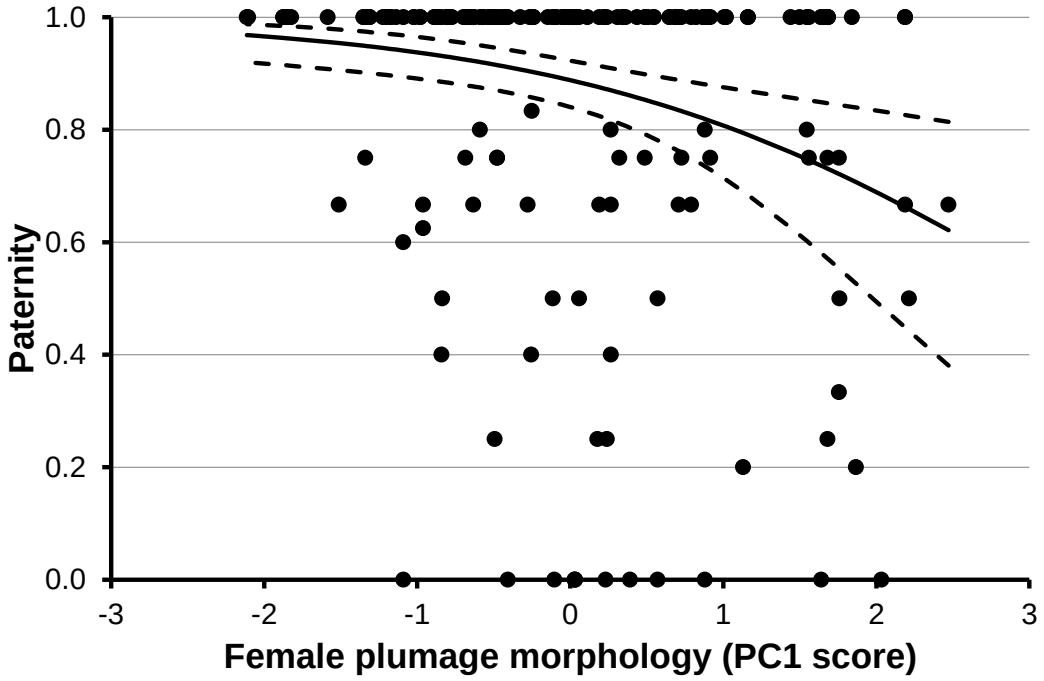
In binomial GLMM of paternity that also included paternal-ID, maternal-ID and pair-ID, the covariance estimates associated with the random effects of year or colony were 0 and likelihood ratio tests for the effects of these factors were therefore statistically non-significant ($\chi^2 = 0.00$, $P > 0.99$) in both cases (see also *Statistical analyses*). Because of the null contribution to the fit of the models of paternity and the fact that their inclusion in the models caused convergence problems, the random effects of year and colony were not considered in the subsequent analyses.

The statistical effect of individual female traits on paternity was first analysed in separate binomial GLMM with paternal-ID, maternal-ID and pair-ID as random effects. Paternity significantly declined with the size of flight feathers of females, as reflected by PC1 (see *Statistical analyses*) after adjusting the P values for the false discovery rate (Table 1; Fig. 1). Bill length and UV-coloration showed a marginally non-significant ($P \leq 0.10$) negative effect on paternity, whereas the effect of other female traits on paternity was weaker (Table 1).

Table 1. Binomial linear mixed models of paternity (proportion of offspring in the brood that were sired by the social father) in relation to phenotypic traits of the mother. Identity of the mother, of the social father and of the pair were included as random effects in the model. The analyses are based on 198-201 breeding episodes (see Methods). The maximum number of offspring included was 765, 111 of which were extra-pair. *: $P < 0.05$ after correction for false discovery rate.

	F	df	P	Coefficient (SE)
Age (yearling vs older)	1.16	1,81	0.284	-0.374 (0.347)
Bill length	3.22	1,81	0.077	-0.008 (0.004)
Keel length	1.84	1,81	0.179	-0.005 (0.003)
Tail length	0.04	1,81	0.851	-0.006 (0.034)
Flight feathers size (PC1)	10.66	1,81	0.002*	-0.638 (0.195)
'Human visible' color (θ)	0.09	1,80	0.761	0.814 (2.667)
UV color (φ)	2.73	1,80	0.103	-3.634 (2.200)

368 Figure 1



369

370 Figure 1. Paternity (proportion of offspring in a brood that were sired by the social father) in
371 relation to the size of the flight feathers (wing and tail feathers) of the mother. The solid line shows
372 the fitted function not using the predictors of the random effects in computing the residuals. The
373 dashed lines are 95% confidence intervals.

374

In our barn swallow study population, tail length of the social father positively predicts paternity in its social brood (Saino et al. 1997). This was confirmed in the present dataset, in a binomial GLMM with paternal-ID, maternal-ID and pair-ID as random effects and tail length of the social father as the independent variable (effect of tail length: $F_{1,81} = 7.69$, P = 0.007, coefficient: 0.062 (0.022); Fig. 2).

Figure 2

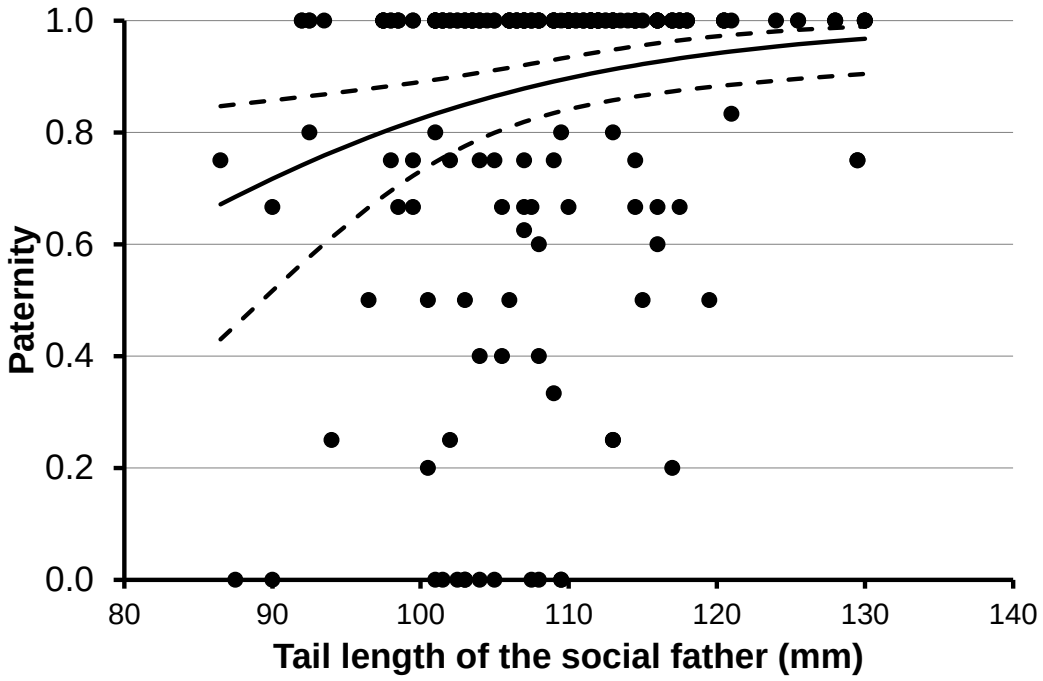


Figure 2. Paternity (proportion of offspring in a brood that were sired by the social father) in relation to tail length of the social father. The solid line shows the fitted values not using the predictors of the random effects in computing the residuals. The dashed lines are 95% confidence intervals.

The statistical effect of female flight feathers size on paternity was independent of the effect of ornamentation of the social mate on paternity, as shown by a multiple binomial GLMM while controlling for the significant positive effect of tail length of the social male mate (Table 2). In

addition, this model showed a significant negative effect of UV-coloration and a marginally non-significant negative effect of bill size on paternity.

Importantly, a comparison between the model in Table 2, which included the random effects of paternal-ID, maternal-ID and pair-ID, with models from which these random effects were removed one at a time showed that the effect of pair-ID was statistically significant (likelihood ratio test; χ^2 = 8.09, df = 1, P = 0.004), whereas the effect of paternal-ID or maternal-ID was nihil (χ^2 = 0.00, df = 1, P > 0.99). This result implies that after controlling statistically for the effect of traits of both social mates that affect paternity, EPFs depend on the specific composition of each breeding pair. Hence, the ability of individual males to secure their paternity depends on the identity of their social female mate and, reciprocally, individual females differ in promiscuity depending on the identity of their social male mate, independently of its sexual attractiveness.

Table 2. Multiple binomial linear mixed model of paternity (proportion of offspring in the brood that were sired by the social father) in relation to flight feathers size (PC1), bill size, intensity of the UV ventral plumage colouration of the mother and tail length of her social male mate. The random effects of paternal-ID, maternal-ID and pair-ID were included in the model. Collinearity between independent variables was weak (unsigned correlation coefficients < 0.20 in all cases).

	Covariance estimate	F	df	P	Coefficient
Paternal-ID	0				
Maternal-ID	0				
Pair-ID	1.887 (0.514)				
Fligh feathers size (PC1)		8.26	1,77	0.005	-0.564 (0.196)
Bill length		3.61	1,77	0.061	-0.008 (0.004)
UV color (φ)		4.57	1,77	0.036	-4.852 (2.271)
Tail length of the male mate		7.71	1,77	0.007	0.062 (0.023)

Discussion

The first main finding of our study was that the proportion of offspring that were sired by their social father (paternity) in barn swallow broods not only positively covaried with sexual ornamentation of the social father, as expected based on previous studies (Saino et al., 1997), but also with feather morphological and coloration traits of the mother. The second main finding was that, after controlling for the effects of the parental traits that significantly predicted paternity, the composition of the breeding pair, rather than the identity of either social parent, predicted paternity.

The existing information on variation in promiscuity of females in relation to their own morphological traits is very sparse (Forstmeier et al., 2014). Because female barn swallows apparently cannot be forced to copulate (Møller, 1988, 1994), our study suggests that female barn swallows varied in their proneness to being fertilized by extra-pair mates according to their own morphological and coloration traits, after accounting for sexual attractiveness and identity of their mate as well as the composition of the breeding pair. Variation in female promiscuity can have major consequences for sexual selection processes because it can affect the variance in reproductive success realized by males. Whether female promiscuity will boost or conversely reduce the variance in male reproductive success will depend on any assortative mating according to female promiscuity and male traits that affect success in male-male competition for genetic parentage. We therefore feel that male-biased perspective in extra-pair paternity studies, which have mostly focused on the traits of males that affect their success in securing genetic parentage, has led to an over-looking of variation in female promiscuity as major complementary phenomenon in determining the outcome of sexual selection processes.

We had no *a priori* expectations on which female traits are related to female promiscuity in barn swallows and we therefore interpret the present findings *a posteriori*. Female traits related to flight feathers size were potential candidates based on the evidence of an association of promiscuity with wing length in two passerine species (Stutchbury et al., 1997, Moreno et al., 2015), although the

direction of the association was opposite in either study. Here, a component of female body size mainly consisting of flight feathers size positively predicted promiscuity, as small within-pair paternity was associated with larger size. Females with relatively large wing and tail feathers may be in better general state because of condition-dependent plumage growth during wintering in Africa and carry-over effects of conditions during wintering on breeding performance (Saino et al., 2013c). Males perceiving high mate promiscuity may be less prone to invest in parental care because of the viability costs associated with parental effort (Sheldon and Ellegren, 1998; Saino et al., 2002; Neff, 2003). Large females may be more prone to engage in extra-pair copulations and fertilizations because any reduction in parental investment by the social mate due to uncertainty of paternity (Gowaty, 1996; Whittingham and Dunn, 2001) could have less severe negative effects on offspring phenotype because of better maternal performance. In addition, large females in relatively good conditions may be preferentially targeted by males prospecting for extra-pair fertilizations than relatively small females in poorer state. Notably, female skeletal body size as gauged by keel length did not predict paternity. Hence, promiscuity was selectively predicted by the plumage rather than the skeletal size component of females. Bill length of females also marginally non-significantly predicted paternity, with larger bills being associated with larger proportion of extra-pair offspring. This trend is opposite to that observed in the only other study showing a relationship between bill length and promiscuity we are aware of where, in the aquatic warbler, females with shorter bill were found to be more promiscuous (Dyrcz et al., 2002). Hence, both flight feather size and bill size seem to be associated with promiscuity, but the sign of the relationships changes among species.

In addition, we observed that females with larger UV reflectance of their ventral plumage coloration were also more promiscuous. The proximate determinants of variation in UV coloration are unknown in barn swallows. Again, this finding may be interpreted along the same lines as for flight feathers size if brighter UV coloration positively reflects physical state and females in prime condition are more prone to sustain any costs of extra-pair copulations/fertilizations and/or they are more frequently targeted by prospecting males. The human-visible component of coloration did not

predict paternity. Hence, this study also corroborates the importance of considering the UV, besides the human-visible component in the analyses of sexual selection processes in birds, most of which perceive UV-wavelengths.

Several studies focusing on variation in success in competition for genetic parentage among males have revealed that males differ in paternity of their social offspring and have disclosed the proximate mechanisms that generate such variation (Williams, 1992; Webster et al., 1995; Shuster and Wade, 2003). The present study confirms that male barn swallows differ in paternity in their broods, and that sexual ornamentation enhances paternity. Because female barn swallows apparently cannot be forced to copulate (Møller, 1988, 1994), the present results imply that females are less promiscuous when mated with a sexually attractive mate and/or that the ability of fertilizing the own social mate positively covaries with male sexual ornamentation.

In barn swallows, mutual mate choice between the sexes has been shown to occur, as expected in a socially monogamous species where both mates contribute substantial, quantitatively similar parental effort (Cuervo, de Lope and Møller, 1996; Safran and McGraw, 2004; Khorialuli et al. unpublished data). Males can benefit from choosing females with low promiscuity as social mates because this will increase their within-pair reproductive success. In addition, males may benefit from seeking extra-pair copulations with the most promiscuous females because this will enhance their extra-pair fertilization success. We may therefore expect males to show a preference for females with traits that are associated with low or high promiscuity as social or, respectively, extra-pair mates, an hypothesis that could be tested experimentally, at least for traits that are amenable to experimental manipulation like coloration. A general implication of variation in female promiscuity and of the existence of traits that covary with it in barn swallows as well as in other species is that the interpretation of the association between male genetic/phenotypic quality and paternity can be partly reframed. It is commonly suggested that the association between male sexual ornamentation and female fidelity is proximately determined by females expressing lower promiscuity when mated

to males of high genetic/phenotypic quality (Westneat and Stewart, 2003). An alternative interpretation, however, is that only high quality males are successful in securing females that signal low promiscuity as social mates, thereby generating the positive covariation between male sexual ornamentation and high paternity in their broods. We are not aware of any study where this hypothesis has been presented and future research may provide empirical tests of it.

The second main finding of our study was that part of the variation in paternity depends on the specific composition of the breeding pair, meaning that males differ in their ability to secure paternity of their social offspring depending on the identity of the female to which they are mated. Hence, after controlling for sexual attractiveness of their social mate, females are differently promiscuous depending on the male to which they are mated. One interpretation of this finding is that some other, yet unidentified trait varies among males that makes individual males differently susceptible to lose paternity. This interpretation is not supported by the observation that the effect of male identity per se did not significantly contribute to the models of paternity, which indicates that males are not consistent in their paternity when mated to different females, after controlling for the effect of sexual ornamentation. The effect of pair composition on paternity is rather consistent with the mate compatibility hypothesis (Colegrave et al., 2002; Tregenza and Wedell, 2000; Mays et al., 2008) because it suggests that proneness of individual females to engage in EPFs varies depending on the specific male to which they are mated, independent of sexual ornamentation. However, which genetic traits are responsible for differential decisions on promiscuity by females to acquire 'compatible' genes remains to be elucidated.

Thus, the present study shows that engagement of females in EPFs covaries with their own morphological and colour traits, indicating that females differ in promiscuity independently of the identity of the social mate and of the positive effect of sexual ornamentation of the social male mate on paternity. Hence, obvious phenotypic female traits exist that can reliably reflect female promiscuity and may therefore affect male choice of social as well as of potential extra-pair mates

520 In addition, the present results suggest that EPFs depend on the specific composition of the
521 breeding pair, lending support to the hypothesis that female realized promiscuity depends on
522 compatibility with the social male mate.

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SUPPORTING INFORMATION

Loadings of the flight feathers measurements on the first principal component, which explained 73.1% of the variance in flight feathers length data. P1-P9 indicate the length of the nine primary wing feathers (excluding the outermost, vestigial one). Primaries are counted inwards. S1 is the outermost secondary wing feather.

	Loading
Right wing chord	0.885
Left wing chord	0.866
Wingspan	0.792
Innermost rectrix length	0.638
P9	0.870
P8	0.908
P7	0.909
P6	0.915
P5	0.909
P4	0.874
P3	0.874
P2	0.859
P1	0.828
S1	0.800

11. Chapter 6

Geographical and seasonal variation in the intensity of sexual selection in the Barn swallow *Hirundo rustica*: a meta-analysis.

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Geographical and seasonal variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*: a meta-analysis

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ABSTRACT

Sexual selection arises from competition among individuals for access to mates, resulting in the evolution of conspicuous sexually selected traits, especially when inter-sexual competition is mediated by mate choice. Different sexual selection regimes may occur among populations/subspecies within the same species. This is particularly the case when mate choice is based on multiple sexually selected traits. However, empirical evidence supporting this hypothesis at the among-populations level is scarce. We conducted a meta-analysis of the intensity of sexual selection on the largest database to date for a single species, the barn swallow (*Hirundo rustica*), relying on quantitative estimates of sexual selection. The intensity of sexual selection was expressed as the strength (effect size) of the relationships between six plumage ornaments (tail length, tail asymmetry, size of white spots on tail, ventral plumage colour, throat plumage colour and throat patch size) and several fitness proxies related to reproduction, parental care, offspring quality, arrival date from spring migration, and survival. The data were gathered for four geographically separated subspecies (*H. r. rustica*, *H. r. erythrogaster*, *H. r. gutturalis*, *H. r. transitiva*). The overall mean effect size ($\bar{Z}_r = 0.214$; 95% confidence interval = 0.175–0.254; $N = 329$) was of intermediate magnitude, with intensity of sexual selection being stronger in males than in females. Effect sizes varied during the breeding cycle, being larger before egg deposition, when competition for access to mates reaches its maximum (i.e. in the promiscuous part of the breeding cycle), and decreasing thereafter. In addition, effect sizes from experiments were not significantly larger than those from correlative studies. Finally, sexual selection on different sexually dimorphic traits varied among subspecies. This last result suggests that morphological divergence among populations has partly arisen from divergent sexual selection, which may eventually lead to speciation.

Key words: barn swallow, *Hirundo rustica*, mate choice, meta-analysis, multiple sexually selected traits, population divergence, secondary sexual traits, sexual selection, speciation, subspecies.

CONTENTS

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I. INTRODUCTION

Sexual selection arises from mating or fertilization advantages that individuals of the chosen sex, usually males, gain from bearing attractive ornaments (inter-sexual mate choice) or other traits that enhance success in competition for mates or limiting resources that attract mates (intra-sexual mate competition; Andersson, 1994). The adaptive function of mate preferences, which may involve direct or indirect genetic benefits, is a controversial issue in evolutionary biology (Andersson, 1994; Prum, 2010), and it is the subject of considerable debate and empirical and theoretical research efforts (Johnstone, 1995; Andersson & Simmons, 2006).

Sexual selection has long been recognized as a crucial force driving evolutionary processes within populations (Andersson, 1994). However, more recently it has also been invoked as a major evolutionary promoter of divergence among populations, ultimately leading to speciation (Møller & Cuervo, 1998; Panhuis *et al.*, 2001; Turelli, Barton & Coyne, 2001; Ritchie, 2007; Van Doorn, Edelaar & Weissing, 2009; Safran *et al.*, 2013). Sexual selection mediated by mate choice promotes the evolution of conspicuous ornaments, which can be involved in mate recognition, thus favouring pre-zygotic isolation (Lande, 1981, 1982). This would particularly be the case for species in which female choice is based on multiple sexually dimorphic traits (Møller & Pomiankowski, 1993; Iwasa & Pomiankowski, 1994), which can be differently selected in distinct populations. Variation in mate preference should therefore rapidly lead to the evolution of pre-zygotic barriers and reproductive isolation, as also predicted by mathematical models of speciation (Lande, 1981, 1982; Kirkpatrick, 1982; Kirkpatrick & Ravigné, 2002; Prum, 2010). However, with few exceptions (e.g. Uy & Borgia, 2000; Masta & Maddison, 2002; Rundle & Nosil, 2005), experiments have generally failed to provide strong evidence in support of the possibility that divergence between populations/subspecies can arise as a consequence of variation in sexual selection (Rice & Hostert, 1993). In addition, comparative analyses testing for positive correlations between extant species diversity and proxies of the intensity of sexual selection have provided conflicting results (Barraclough, Harvey & Nee, 1995; Mitra, Landel & Pruett-Jones, 1996; Møller & Cuervo, 1998; Arnqvist *et al.*, 2000; Katzourakis *et al.*, 2001; Gage *et al.*, 2002; Morrow, Pitcher & Arnqvist, 2003; Stuart-Fox & Owens, 2003; Isaac *et al.*, 2005; Seddon, Merrill & Tobias, 2008; Kraaijeveld, Kraaijeveld-Smit & Maan, 2011; Seddon *et al.*, 2013).

Ever since Darwin (1871), sexual selection has been examined in a huge range of species (Andersson, 1994; Andersson & Simmons, 2006; Zuk *et al.*, 2014). Among vertebrates, birds are the most studied group (Zuk *et al.*, 2014). However, only few species have been subject to repeated studies of the consequences of variation in sexually dimorphic traits for mate choice and competition for mates, and even fewer allow for investigation of patterns of inter-population divergence according to geographical variation in sexual selection (Andersson & Simmons, 2006; Møller *et al.*, 2006). This is unfortunate because replication of observations and experiments is a crucial requisite for determining whether reported effects in a given study reflect 'accidents' or 'true' biological phenomena (Palmer, 2000; Kelly, 2006). In fact, analyses of poorly studied species may not serve as reliable proxies for generalizing biological phenomena because they are more likely to report both statistically significant findings (Møller & Jennions, 2001; Jennions & Møller, 2002) and large effect sizes (*sensu* Jennions & Møller, 2002; Ioannidis, 2005; Forstmeier & Schielzeth, 2011), thus inflating the risk of overestimating the effects. A possible but so far neglected approach to improve our knowledge of sexual selection in birds (and even for all other biological phenomena) is to deeply scrutinize the few well-studied species by considering all available published information, independently of their consistency with theoretical expectations and previous findings. Such well-studied species may contribute to reject erroneous hypotheses, to provide highly valuable data for testing general ecological and evolutionary hypotheses, and therefore to suggest a research agenda which can be applied to other species.

A powerful approach to summarize any information concerning a biological phenomenon is meta-analysis (e.g. Arnqvist & Wooster, 1995). Meta-analysis of sexual selection in birds has a relatively long-standing tradition. In a general study on the intensity of sexual selection on both morphological and chromatic traits in birds, Gontard-Danek & Møller (1999) showed a mean effect size of 0.30, which is a relatively large effect compared to those generally found in meta-analyses of eco-evolutionary data (Møller & Jennions, 2002*b*; Jennions & Møller, 2003). In addition, despite the fact that specific issues concerning sexual selection in birds have been addressed by previous meta-analyses (Møller & Ninni, 1998; Møller & Thornhill, 1998; Møller & Alatalo, 1999; Møller, Christe & Lux, 1999; Jennions, Møller & Petrie, 2001; Meunier *et al.,* 2011), most studies focused on the general literature of sexual selection, while meta-analytic syntheses within single species are scarce (Parker & Ligon, 2003; Simons & Verhulst, 2011), especially for wild organisms (Parker, Barr & Griffith, 2006; Nakagawa *et al.,* 2007; Parker, 2013).

Recently, Parker (2013) analysed sexual selection on plumage colouration in the blue tit (*Cyanistes caeruleus*) in depth, concluding that there was little or no evidence of significant independent effects of different colour traits on fitness components. Following Parker (2013), the main aim of the present study was to provide a quantitative review of all the published results concerning sexual selection in the barn swallow (*Hirundo rustica*), a classical species for the study of sexual selection since the end of 1980s (Møller, 1994*c*; Turner, 2006), and one of the most commonly represented species in the literature on sexual selection in birds. Several barn swallow populations belonging to four subspecies (*H. r. rustica*, *H. r. erythrogaster*, *H. r. gutturalis*, and *H. r. transitiva*; see Section II.1 for details) have been studied and are currently under investigation, making sexual selection studies on this species among the most frequently replicated in behavioural ecology, providing a rare opportunity to analyse variation in the intensity of sexual selection among populations and plumage ornaments.

The barn swallow is a socially monogamous but sexually promiscuous species, with extra-pair paternity being common (Møller, 1994*c*; Turner, 2006; Hubbard, Jenkins & Safran, 2015). The adults show small to moderate sexual dimorphism in several traits, including the length of the outermost tail feathers (hereafter, tail length) and their fluctuating bilateral asymmetry (hereafter, tail asymmetry), the size of the white spots on the tail feathers (hereafter, white spots on tail), the colour of ventral contour feathers (hereafter, ventral colour), as well as the size and colour of the chestnut throat patch (hereafter, throat patch size and throat colour, respectively; Møller, 1994*c*; Turner, 2006). Several studies have suggested that the expression of one or more of these traits in males is related to mating opportunities, realized reproductive success and sperm competition (e.g. Møller, 1994*c*; Turner, 2006; Scordato & Safran, 2014). However, sexual dimorphism, as well as epigamic signals and life-history traits, differ considerably among subspecies (Turner, 2006; Dor *et al.,* 2010; Scordato & Safran, 2014; see Section II.1 for details). Despite accumulating evidence suggests ongoing divergence among geographical populations, only a recent qualitative review has summarized the potential difference in sexual selection patterns among them (Scordato & Safran, 2014).

In the present study, we examined the intensity of sexual selection on plumage ornaments, which have been shown to vary greatly among individuals within each sex and have previously been suggested to be relevant in intra- and inter-sexual interactions. The intensity of sexual selection was expressed as the magnitude of the relationships between the aforementioned plumage ornaments (tail length, tail asymmetry, white spots on tail, ventral plumage colour, throat plumage colour and throat patch size) and several fitness proxies concerning reproduction, parental care, offspring quality, arrival date from spring migration, and survival. Our goals were fivefold: (*i*) we analysed whether the intensity of sexual selection varied between males and females, as well as according to age, by comparing yearlings with older individuals; (*ii*) we investigated variation in the intensity of sexual selection during the breeding season, by comparing the magnitude of the effects among different stages of the breeding cycle; (*iii*) we tested for variation in the intensity of sexual selection among different plumage ornaments on all subspecies (e.g. the mean effect size for 'tail length' considering all the subspecies), as well as among subspecies considering all ornaments (e.g. the mean effect size for '*H. r. rustica*' by using data on all plumage ornaments); (*iv*), we investigated whether the intensity of sexual selection on different plumage ornaments varied among subspecies by testing the effect of the interaction between plumage traits and subspecies. A difference in the patterns of sexual selection among geographical populations would be consistent with a role of sexual selection in promoting phenotypic divergence and speciation (Fisher, 1930; Lande, 1981; Prum, 2010). Finally, (*v*) we tested for variation in mean effect size originating from correlative as compared to experimental studies, because the strength of the effects may differ between the two types of studies.

II. METHODS

(1) Study organism

The barn swallow is a small (*ca.* 20 g), insectivorous, mostly migratory passerine bird. It is socially monogamous and it mainly breeds synanthropically or associated with man-made structures. Sexual promiscuity is common, leading to intense sperm competition and high frequency of extra-pair paternity (Saino *et al.,* 1997*b*; Safran *et al.,* 2005; Vortman *et al.,* 2013; but see Hasegawa *et al.,* 2010*a*). The barn swallow is considered to include at least six subspecies, which breed across the Holarctic region and show reciprocal differences in behaviour, morphology and phenology (Turner, 2006; Dor *et al.,* 2010). The nominate *H. r. rustica* breeds in Europe, except the polar region, North Africa and Western Asia,

while *H. r. erythrogaster* breeds across most of North America and Argentina. *H. r. gutturalis* and *H. r. tytleri* are the two Asian subspecies breeding from South to East Asia and in Northwest Asia, respectively. The four aforementioned subspecies migrate to tropical regions during winter. The two remaining subspecies are non-migratory and have a narrower distribution: *H. r. savignii* is found only in Egypt along the Nile River, and *H. r. transitiva* in the Middle East (Israel, Jordan, Lebanon, and Syria). Information on breeding and sexual selection is currently available for *H. r. rustica*, *H. r. erythrogaster*, *H. r. gutturalis* and *H. r. transitiva*.

Depending on subspecies and breeding latitude, females lay 1–3 clutches of 1–7 eggs each breeding season and incubate them for approximately 14 days (Møller, 1994*c*; Turner, 2006). However, in at least two subspecies, *H. r. erythrogaster* and *H. r. gutturalis*, males also contribute to incubation (Ball, 1983; Smith & Montgomerie, 1992; Turner, 2006). Nestlings are usually fed by both parents (with female contribution generally being larger than that of their partner; Turner, 2006), fledge when they are approximately 20 days old, and are attended for some days by both parents after fledging (Møller, 1994*c*; Turner, 2006; Grüebler & Naef-Daenzer, 2008*a*,*b*). Fledging success is usually very high, as mortality in the nest only accounts for approximately 5% of hatched nestlings (excluding rare nest predation events or nest failures due to disappearance of parents). In addition, infanticide is rare (<2%) and also related to the expression of male plumage ornaments, because more-ornamented males are less likely to suffer infanticide from unmated individuals (Møller, 2004).

The first 20 years of studies on sexual selection in the barn swallow were mostly carried out in European populations, and have provided evidence of female directional preference for long- and symmetric-tailed males (reviewed in Møller, 1994*c*; Møller *et al.*, 1998*a*). However, different patterns of sexual selection may exist in other subspecies (Scordato & Safran, 2014). For instance, traits such as ventral plumage colour or size of the throat patch have been shown to predict male fitness including mate choice, sperm competition and realized reproductive success in *H. r. erythrogaster*, *H. r. transitiva*, and *H. r. gutturalis* (e.g. Safran & McGraw, 2004; Hasegawa *et al.*, 2010*b*; Vortman *et al.*, 2011).

(2) Data file preparation

In our analyses we considered the following sexually dimorphic characters: tail length, tail asymmetry, size of white spots on tail, ventral plumage colour, throat patch colour and size. We firstly collected all papers (including any associated supplementary results) focusing on these traits published in scientific journals before September 21st 2015, using *Web of Science*, *Scopus* and *Google Scholar* by combining the key words 'barn swallow*' or '*Hirundo rustica*' with any one of the following: 'tail', 'colo*r', 'plumage' or 'feather*', 'ventral', 'belly', and 'throat'. We also carefully screened the references in each paper to obtain the broadest possible coverage. As a result, 624 papers were retrieved (Fig. 1). Information provided only in books on the barn swallow was discarded

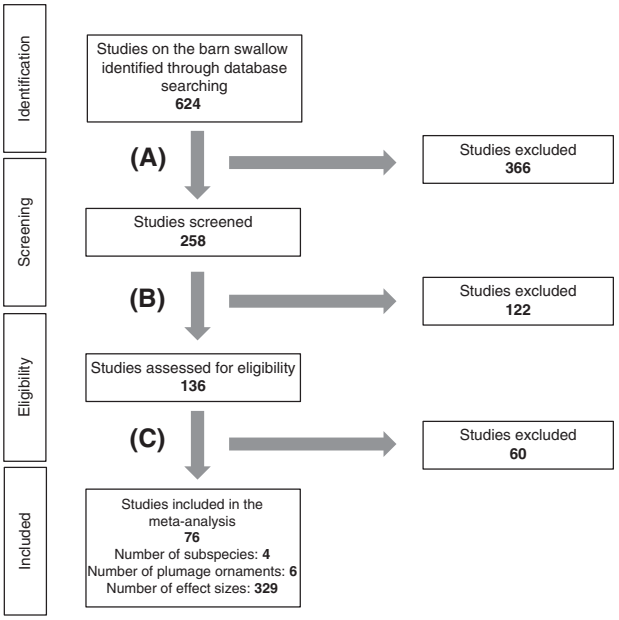

Fig. 1. Flow chart illustrating the different steps of the data file preparation: (A) exclusion of studies on the barn swallow not focusing on plumage ornaments; (B) exclusion of studies focusing on costs or condition-dependent expression of dimorphic traits (i.e. when dimorphic characters were not predictors of fitness proxies); and (C) exclusion of studies focusing on other dependent variables not linked to reproduction, parental care, offspring quality, arrival date or survival (see Section II.2 for details).

(Møller, 1994*c*; Brombach, 2004; Turner, 2006), as well as, if any, the scientific papers published in languages other than English because we could not formulate standard search criteria and because of difficulties in reliable translation.

We then checked all papers and selected only those providing at least one estimate of the relationship between one of the aforementioned plumage ornaments and fitness proxies, grouped into the following main categories: arrival date from spring migration (hereafter, arrival date), reproduction, parental care, offspring quality, and survival (see Table 1). As clarified further below (see Section II.3), these components of fitness constitute broad, inclusive groups of more-specific aspects of individual life-history traits. In our data set we only included the statistical relationships where plumage ornaments were hypothesized to determine fitness-related traits, independently of how the test was designed. Thus, we included the results of analyses where an ornament was included as a predictor of a fitness trait, as well as analyses testing for a difference in ornament expression between groups of individuals with different fitness (e.g. tests comparing tail length of mated *versus* unmated individuals). Importantly, we did not include tests of condition-dependence of the expression of ornaments (e.g. tests of variation in ornament expression according to parasite load, physiological parameters or environmental conditions) nor tests of the potential costs of ornaments, because these were not the focus of the study (Fig. 1).

Table 1. Predicted sign of the relationship between expression of plumage ornaments (TL = tail length; TA = tail asymmetry; WS = size of white spots on tail; VC = ventral colour; TC = throat colour; TP = throat patch size) and each fitness proxy. When the statistical relationship reported in a given study matched these expectations, the effect size was assigned a positive sign. Fitness proxies denoted by the same superscript letter were pooled in the analyses of variation in effect size during the breeding season because they occur simultaneously. The chronological sequence of fitness proxies during the breeding season is also shown. Information on laying date, incubation, breeding success, care provisioning and offspring quality are available for first and second broods

		Males						Females					
Fitness proxy	Chronological sequence	TL	TA	WS	VC	TC	TP	TL	TA	WS	VC	TC	TP
Reproduction													
Mating success	2	+	−	+	+	+	+	+	−	+	+	+	+
Mating date	3	−	+	−	−	−	−	−	+	−	−	−	−
Paternity	4	+	−	+	+	+	+						
Laying date	5	−	+	−	−	−	−	−	+	−	−	−	−
Breeding success	7	+	−	+	+	+	+	+	−	+	+	+	+
Overall reproductive success		+	−	+	+	+	+	+	−	+	+	+	+
Parental care													
Female incubation[a]	6	+	−	+	+	+	+	−	+	−	−	−	−
Male incubation[a]	6	−	+	−	−	−	−	+	−	+	+	+	+
Female care provisioning[b]	8	+	−	+	+	+	+	−	+	−	−	−	−
Male care provisioning[b]	8	−	+	−	−	−	−	+	−	+	+	+	+
Offspring quality													
Offspring size[c]	9	+	−	+	+	+	+	+	−	+	+	+	+
Offspring physiology[c]	9	+	−	+	+	+	+	+	−	+	+	+	+
Arrival date	1	−	+	−	−	−	−	−	+	−	−	−	−
Survival		+	−	+	+	+	+	+	−	+	+	+	+

However, we considered sex (males, females or both sexes pooled), as well as age (yearling *versus* older individuals) as potential sources of heterogeneity in effect sizes.

We included the results of both correlative and experimental studies. In addition, we included effect sizes from studies reporting individual-level data (e.g. variation in fitness traits according to variation in plumage ornaments in a breeding season), and within-individual variation among years (e.g. the difference in fitness traits of individuals between consecutive breeding seasons in relation to the difference in the expression of plumage ornaments; e.g. Bradley *et al.*, 2014). In one study (Møller, 1993*c*), statistical relationships on both within- and inter-individual variation in fitness were available for the same fitness proxies and plumage ornaments: both were included in the data set, because they provide different information (see online Table S1).

In the data set we included results both from univariate and multivariate models. When the independent variable of interest was included in a two-way interaction term with one or more fixed factors, we included only the main effect in our analyses. If an alternative analysis without the interaction term was presented in the paper, we selected the effect from this analysis instead of the effect from the analyses including interaction terms. The cases in which an interaction term was included represented only a very small proportion of all the statistical relationships included in the dataset (*ca.* 1%), and their exclusion from the analyses did not affect the conclusions (details not shown). After these steps were completed, the number of remaining studies suitable for the meta-analysis was 76 (Fig. 1; see online Table S1).

For each statistical relationship we recorded: (*i*) the dependent variable (fitness proxy; see Section II.3 and Table 1); (*ii*) the independent variable (plumage ornament); (*iii*) the sex and age (yearlings, older individuals or unspecified age) to which the data referred; (*iv*) the publication year; (*v*) the barn swallow subspecies on which the study was performed; (*vi*) the test statistic, the mean values ± S.D. of the fitness proxy under investigation for different groups of individuals displaying a different expression of plumage ornament (e.g. mean brood size of individuals with experimentally elongated or shortened tail), or the mean values ± S.D. of the plumage ornament under scrutiny for different groups of individuals with different fitness (e.g. mean tail length of mated *versus* unmated individuals); (*vii*) the *P* value; and (*viii*) the degrees of freedom and sample size (see online Table S1). When any of this information was missing, it was inferred from other information reported in the paper, whenever possible. This information was used to compute standardized effect sizes (r and Z_r; see below).

To avoid 'duplication of study' effects (Wood, 2008), a single effect reporting the statistical relationship between a plumage ornament and a particular fitness variable (see Section II.3 for details) in the same set of individuals was included per study by adopting the following criteria: (*i*) effects that were estimated while controlling for confounding variables, such as age or other morphological traits, were preferred over other relationships (e.g. partial correlation coefficients were preferred to correlation coefficients); (*ii*) if a given study reported multiple similar measures from the same set of individuals (e.g. tail length predicting

number and proportion of extra-pair nestlings reared), we computed a mean effect size among them for inclusion in the meta-analysis; and (*iii*) when the sample size between two or more tests on the same statistical relationship differed, we included the effect size based on the largest sample size. The latter criterion was also adopted when a paper presented results for the same statistical relationship on individuals of age classes combined, and then separately between yearlings and/or adults: in this case, we used the effect size for all data combined. However, the analyses concerning age-related variation in effect size were performed on a different data set including this information (see online Table S1). Furthermore, in one study (Safran & McGraw, 2004) where ventral colour was measured in three different body regions (breast, belly and vent) we chose to use the mean effect size for these plumage regions. In another study (Saino *et al.,* 2013) where plumage colour was converted into three different colour variables, we chose to use the mean effect size computed for ϑ, indicating reflectance in the 'visible' spectrum, and rA, which is a proxy of colour saturation [i.e. the variable φ, indicating the ultraviolet (UV) wavelength reflectance, was excluded because it would represent the only data about plumage UV reflectance in the data set]. In addition, Safran *et al.* (2005) evaluated the overall effect of the manipulation of both throat and ventral colour on reproduction: in this case, we included the effect size of both throat and ventral colour.

Finally, we excluded from the data set two recently published papers (Wilkins *et al.,* 2015; Hasegawa, Watanabe & Nakamura, 2016). In one case, as clearly stated by the authors, the experimental manipulation of plumage colour simulated colouration of female barn swallows from a different subspecies (Hasegawa *et al.,* 2016). Wilkins *et al.* (2015) analysed the combined effect of tail length, ventral colour and song features by using Principal Components Analysis making it impossible to discern the effect of either trait on fitness. At the end of this step, the number of effect sizes suitable for meta-analysis was 329 from 76 different studies (Fig. 1; see online Table S1). The file containing additional information about age included 351 effect sizes (see online Table S1).

Like most meta-analyses in evolutionary studies, the focus was on the strength of the relationship between ornaments and fitness proxies, rather than on their slope (i.e. the effect of a unit change in ornament expression on fitness). All statistical effects were converted into Fisher's $\mathcal{Z}$ ($\mathcal{Z}_r$). $\mathcal{Z}_r$ was easily computed from Pearson product-moment correlation coefficients (r) (Rosenberg, Adams & Gurevitch, 2000; Nakagawa & Cuthill, 2007; Koricheva, Gurevitch & Mengersen, 2013). When r (or r^2) was unavailable in the text, it was derived for parametric analyses by using standard formulae that combine information on test statistic, degrees of freedom and sample size (Rosenberg *et al.,* 2000). Kendall τ correlations were converted to r using the formula suggested by Walker (2003). When studies only reported information about mean, standard deviation and sample size of two groups of individuals, they were used to compute r (Rosenberg *et al.,* 2000). For statistics that lack an established method of converting the test statistic into r (e.g. several non-parametric tests, and F-tests with numerator degrees of freedom >1), we first converted the P value to a $\mathcal{Z}$-score, representing the standard normal deviate, which was subsequently converted to $\mathcal{Z}_r$ (Rosenberg *et al.,* 2000). When no exact P value was available, for statistically significant results we conservatively computed $\mathcal{Z}_r$ from the maximum P value provided in the text. For example, in cases of $P < 0.0001$, we assumed $P = 0.0001$. Some studies describing comparisons between three or more experimental groups (e.g. individuals with elongated, shortened and unmanipulated tail) reported neither exact statistics nor P-values, but included information about mean, standard deviation and sample size for all the experimental groups. In those cases, the correlation coefficient was computed by comparing the mean values of the extreme groups only (e.g. individuals with elongated and shortened tail length). If this was the case for a statistical relationship reported in a table, for consistency this procedure was applied to all the other statistical relationships included in the same table, even if they were reported with sufficient information to compute effect size (e.g. Table 1 in de Lope & Møller, 1993).

We then assigned a sign to each value of $\mathcal{Z}_r$ according to whether the given statistical relationship was consistent or not with the *a priori* expectation (see Section II.4 and Table 1). In the analyses including three or more experimental groups the sign of the relationship was determined by considering only the extreme groups.

Some papers reported insufficient information to compute the effect size (most commonly reporting the unsigned value of the statistic, or that the effect was 'not significant'). When no other information about the statistical relationship between a plumage ornament and a fitness proxy was available, we asked for the relevant information directly from the authors. We could recover the vast majority of missing effect sizes. The few effect sizes ($N = 17$; from five studies) that we could not obtain because authors did not reply or the original data were no longer available, were excluded from the analyses. Since these effect sizes represent a very small fraction (<5%) of those included in our data set, their exclusion should not have affected our conclusions.

(3) Explanatory variables

The meta-analytic approach involves combining statistical effect sizes from conceptually similar relationships across studies which may or may not have used different types of analyses. An important decision is thus how to identify the effect sizes which are sufficiently similar to be included in the same category. In a first step, we pooled effect sizes referring to the same fitness proxy.

Briefly, data on *reproduction* were analysed by comparing relationships between each plumage ornament and both male and female reproductive output by separately considering the following fitness proxies, which refer to different phases of the breeding cycle (details in Table 1): (*i*) mating success, which comprised the probability of obtaining a social mate, and the time elapsed between arrival date to

the breeding site and reproduction; (*ii*) mating date; (*iii*) success in paternity, as gauged by gaining extra-pair and within-pair offspring, as well as by successfully engaging in extra-pair copulations (e.g. Møller, 1992*a*); (*iv*) laying date; (*v*) breeding success, including clutch size, brood size, and fledging success for any breeding attempt; and (*vi*) overall reproductive success, considering the number of broods, all eggs produced, and total number of offspring sired (but not when total number of eggs/offspring was weighted by the number of broods; e.g. Cuervo & Møller, 2006) during the entire breeding season. We note that data on clutch and brood size were pooled because in the barn swallow both the number of unhatched eggs and mortality rate during the nestling period are normally very low (Møller, 1994*c*; Turner, 2006). In practice, the number of eggs laid and the number of nestlings fledged are highly correlated.

Data on *parental care* were divided into two categories, corresponding to different phases of the breeding period: (*i*) incubation period, including the duration of incubation and the (absolute or relative) time spent by females or males in incubating eggs; and (*ii*) care provisioning of nestlings, including feeding rate, number of prey brought to the nest, and duration of the nestling period. Importantly, we distinguished between parental care provided by the mother and the social father of the nestlings (Table 1).

We then identified two categories of *offspring quality*: (*i*) offspring size, including skeletal size (e.g. tarsus length) and body mass measurements of nestlings; and (*ii*) offspring physiology, accounting for immune function and other physiological variables.

Finally, we also recorded effect sizes concerning the associations between arrival date or survival/mortality and plumage ornaments.

Importantly, data regarding laying date, incubation, breeding success, care provisioning and offspring quality were categorized according to the breeding attempt to which they referred, because barn swallows often lay more than one clutch per breeding season, and the intensity of sexual selection may vary during the breeding season. We thus considered separately the results concerning first or second broods (no data were available for subsequent broods, as very few females lay more than two clutches in a breeding season; Turner, 2006; Table 1). Data on paternity may refer to first brood only or to both broods pooled (e.g. Eikenaar *et al.*, 2011*b*; see online Table S1).

Because the fitness proxies could be ordered chronologically, we could test whether the intensity of sexual selection varied among different phases of the breeding cycle. We note that 'breeding success', indicating clutch and brood size, was placed before 'care provisioning' because it mainly reflects parental decisions on initial clutch size rather than subsequent adjustments of brood size (see above). Analysis of variation in the effect size among different phases of the breeding cycle was limited to first broods because of the small number of effect sizes (and breeding stages) concerning second broods (however, mean effect sizes for each breeding stage in second broods are shown in Section III.3 for completeness). In addition, because only high-quality breeding individuals (i.e. the more ornamented ones) usually lay a second clutch, during second broods smaller inter-individual variability in ornament expression compared to the first broods is expected. This difference in ornament variation between first and second broods may therefore reduce mean effect size during the breeding season.

(4) Attribution of sign to effect sizes

The direction (sign) of each effect size was established based on the hypothesis that plumage ornaments are honest indicators of individual quality (e.g. Møller, 1990*a*, 1991*b*, 1994*e*; Møller & de Lope, 1994; Saino & Møller, 1996; Saino, Bolzern & Møller, 1997*a*; Kose & Møller, 1999; Hasegawa *et al.*, 2014*b*; Saino *et al.*, 2015) and are under sexual selection *via* mate choice (e.g. Møller, 1990*b*, 1992*b*, 1993*a*; Saino *et al.*, 1997*b*; Safran & McGraw, 2004; Safran *et al.*, 2005; Hasegawa & Arai, 2013). We therefore expected a 'positive' effect of plumage ornament expression (but 'negative' for tail asymmetry) on the fitness proxies by considering the direction of the sexual dimorphism in these ornaments. For instance, in the case of tail length (larger in males compared to females), we expected a positive association between ornament expression and fitness. In addition, because studies could differ in colour-measurement methods (see e.g. Safran *et al.*, 2005; Hasegawa *et al.*, 2010*b*), we always considered darker colouration as 'more exaggerated' compared to paler colouration, irrespective of the procedure adopted for measuring or quantifying it. When the statistical relationships matched the sexual selection expectation, 'positive' signs were assigned to effect sizes, while the opposite was the case for statistical relationships whose sign was contrary to expectations. Table 1 provides detailed information about the predicted sign of the relationships between expression of plumage ornaments and fitness proxies included in the analyses.

Importantly, the signs of the relationships reported in Table 1 were consistent irrespective of the mechanism of sexual selection that was assumed to act (Andersson, 1994). For example, highly ornamented individuals should produce larger broods, have larger reproductive success, as well as mate and start egg laying earlier, irrespective of whether the ornaments are directionally selected because they are individual quality indicators, confer advantages in intra-sexual competition for acquiring a mate or a better reproductive territory, or are preferred because of a sensory bias. In addition, the fitness proxies shown in Table 1 also accounted for the effect of intra-sexual social interactions, such as same-sex interactions to acquire a high-quality breeding territory, or infanticide, which have not been explicitly considered in our analyses. Indeed, the outcome of such intra-sexual social interactions may be predicted by plumage ornaments (see e.g. Møller, 1992*a*, 2004; Hasegawa *et al.*, 2014*a*; Wilkins *et al.*, 2015) and can ultimately affect mating or breeding success, respectively.

On the other hand, the expected relationships between parental care and ornamentation may differ depending on

assumptions of which sexual selection mechanism operates. First, in both sexes, parental care is expected to increase with increasing level of ornamentation of the mate (Horváthová, Nakagawa & Uller, 2012; Hegyi, Kötel & Laczi, 2015), especially in species with frequent extra-pair paternity (Møller & Thornhill, 1998), as is the case in the barn swallow (Saino *et al.*, 1997*b*; Hubbard *et al.*, 2015). We therefore assigned a positive sign to effect sizes showing an increase in mate parental care with increasing levels of ornamentation. Second, because highly ornamented males are expected to invest less in parental care, while their social mate should increase their efforts (Burley, 1985; Møller & Thornhill, 1998), as repeatedly shown in the barn swallow (e.g. Møller, 1994*e*; Maguire & Safran, 2010; Hasegawa *et al.*, 2014*a*; Hasegawa & Arai, 2015), we assigned a positive sign to effect sizes showing a decrease in male parental care (including incubation) with increasing levels of male ornamentation. Along the same line of reasoning, we assumed the same pattern for the association between female parental care and female ornamentation. However, since some disagreement exists concerning the sign of the relationship between female parental care and female ornamentation, because females may be expected to either increase ('good parent hypothesis'; Hoelzer, 1989) or decrease ('compensation hypothesis'; Winkler, 1987) their parental effort depending on their own level of plumage ornamentation, we re-ran all the analyses removing the data on female parental care in relation to female ornamentation. Because all the results remained qualitatively unchanged (details not shown), we retained the analyses performed on the entire data set.

(5) Meta-analysis

Because our data set included multiple effect sizes from the same study (mean ± S.D. number of effect sizes per study: 4.33 ± 3.76), we accounted for non-independence of effect size estimates by adopting a random-effects hierarchical linear mixed modelling approach as advocated by Van den Noortgate *et al.* (2013, 2015). Random-effects models were chosen because they account for both true random components, as well as sampling error potentially affecting effect sizes (Borenstein *et al.*, 2010). Variation in weighted effect size ($\widetilde{Z}_r$ weighted by sample size − 3) was thus analysed by hierarchical linear mixed models (HLM models hereafter), including two random effects: 'study identity', which accounts for non-independence of multiple effect sizes extracted from the same study, and 'effect size identity' (as usually used in random-effects meta-analyses) nested within 'study identity', while assuming equal variances among studies (see Van den Noortgate *et al.*, 2013, 2015, for details). Parameters and their 95% confidence interval (CI) were estimated using REML by means of SAS 9.3 PROC MIXED (Van den Noortgate *et al.*, 2013, 2015).

To test for differences in effect size according to study type (correlational *versus* experimental studies), sex (male *versus* female), age (yearling *versus* older), subspecies (*H. r. rustica*, *H. r. erythrogaster*, *H. r. gutturalis*, *H. r. transitiva*), and plumage ornament (tail length, tail asymmetry, white spots on tail, ventral colour, throat colour, throat patch size) we ran univariate HLM models. Significance was tested by likelihood ratio tests (LR tests). Individual levels of a given moderator were discarded if less than two effect sizes were available. The few effect sizes referring to pooled male and female data were omitted from all the analyses in which 'sex' was used as a predictor.

In addition, we tested if the intensity of sexual selection varies during the breeding cycle as follows. We first assigned each effect size for first broods to its pertaining 'breeding stage' and breeding stages were ordered in chronological sequence according to their occurrence during the breeding season (Table 1). Because fitness proxies referred to breeding stages of different nature or duration, we could not include their chronological sequence as a continuous covariate in the models. We therefore used breeding stage as a factor in a univariate HLM model to test for variation in mean effect size among different breeding stages. Inspection of mean effect sizes (see Section III.3) showed that a maximum occurred at intermediate breeding stages. Because, to the best of our knowledge, no non-parametric methods have been devised that test for non-linear relationships, to account for variation in the strength of the relationship along the breeding cycle we ran non-parametric (Spearman) correlation analyses on mean effect sizes over five consecutive breeding stage 'intervals' at a time. In the first analysis we tested the correlation between mean effect size and ranked breeding stages 1–5. In the second analysis we tested the correlation for breeding stages 2–6 and so forth for subsequent intervals. Five breeding stages per interval were considered to be the best compromise between 'resolution' of the analysis of variation of mean effect size along the breeding cycle and statistical power of each test. However, analyses performed on intervals of four breeding stages provided qualitatively similar results (details not shown). The pattern of variation in mean effect size during the breeding cycle was inferred by looking at the sign and magnitude of the Spearman correlation coefficients obtained for the different intervals. These analyses focused on first broods, because of the small number of effect sizes and breeding stages for second broods (see also Section II.3). However, for descriptive purposes a similar univariate HLM model was used on data for second broods only. The difference in mean effect size between first and second broods was estimated in a HLM model including a dichotomous factor (brood) indicating if effect sizes referred to first or second broods. In this analysis, we included only breeding stages for which data were available for both first and second broods (i.e. from laying date to offspring quality).

We then ran HLM models including multiple moderator variables (sex, plumage ornament, subspecies, and study type) as fixed effects to assess the independent effect of any single variable while taking into account the concomitant effects of the other moderators. The effect of moderator variables was tested by LR tests. Finally, we tested if the intensity of sexual selection on any specific plumage ornament varied among subspecies in multiple HLM models where we included,

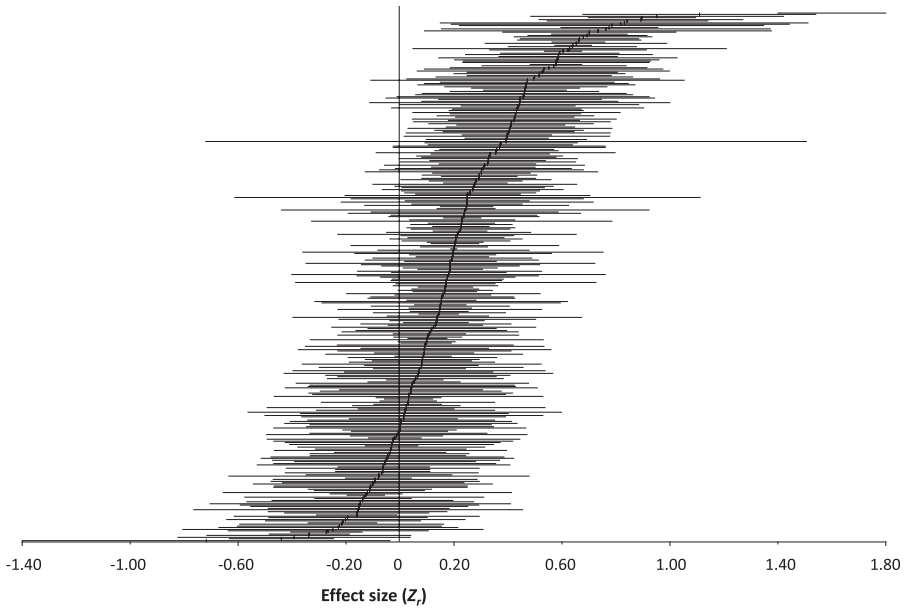

Fig. 2. Plot of the 329 effect size estimates (from 76 studies) of the relationship between expression of plumage ornaments (tail length, tail asymmetry, size of white spots on tail, throat colour, ventral colour, throat patch size) and fitness proxies, ordered by increasing effect size. Effect sizes are Z-transformed Pearson product-moment correlation coefficients (Z_r) with their associated 95% confidence intervals. The vertical line indicates an effect size of zero. The overall mean effect size is 0.214 (CI = 0.175–0.254).

besides the above moderators, the interaction term between subspecies and plumage ornament. Unfortunately, not all the plumage ornaments that we considered have been studied in all subspecies, making any test of differential variation in the intensity of sexual selection among all traits and across all subspecies impractical. Hence, the trait-by-subspecies interaction was tested in different models restricting the analyses to pairs of subspecies for which at least two effect sizes for the same two or more ornaments were available. Data from different subspecies and plumage ornaments could also refer to different breeding stages. In all the analyses of interaction between plumage ornament and subspecies we therefore included 'breeding stage' (including 'survival' and 'overall reproductive success'; see online Table S1) as a fixed factor to account for this additional source of heterogeneity in the data. Finally, trait-by-subspecies interaction analyses were also limited to the breeding stages for which at least one effect size was available for one or more plumage ornaments shared by the two subspecies of interest (including 'survival' and 'overall reproductive success'; see online Table S1). As we performed multiple analyses to test the same hypothesis, we adjusted the α level by using the sequential Bonferroni correction for each set or subset of data.

Because sexual selection is expected to be stronger in males than in females (Andersson, 1994; see also Section III.2), all analyses were also run on male data only. Importantly, sexual and natural selection may act in opposite directions because large expression of sexually selected traits may impose costs in terms of survival to their bearers, while still providing reproductive advantages. All analyses (including those on males only) were therefore repeated by excluding data on survival to account for the potentially negative effect of plumage ornaments on survival. Moreover, because of variability in the specific type of effect sizes available for individual subspecies, plumage ornaments and sexes (e.g. experimental studies were mostly performed in certain subspecies, plumage ornaments, and males; data for second broods were not available for all subspecies), we re-ran all analyses by removing the data from experimental manipulations, as well as excluding data on second broods. In summary, the effects of moderator variables, including the interaction between plumage ornament and subspecies, were tested on the following sets or subsets of effect sizes: (*i*) all (329 effect sizes); (*ii*) males only (240 effect sizes); (*iii*) excluding survival (ES, 281 effect sizes); (*iv*) excluding experiments (EE, 262 effect sizes); (*v*) excluding second broods only (ESB, 299 effect sizes); (*vi*) excluding survival on males only (ESM, 212 effect sizes); (*vii*) excluding experiments on males only (EEM, 176 effect sizes); and (*viii*) excluding second broods only on males only (ESBM, 218 effect sizes).

Effect sizes and their 95% CIs reported throughout the results were computed according to unstructured, random-effects models separately for each subset (e.g. mean weighted Z_r and CIs for males and females were computed from sex-specific models).

(6) Tests of heterogeneity in effect sizes and publication bias

As a measure of heterogeneity in effect sizes, we used I^2 (Higgins *et al.*, 2003), which represents the proportion of observed variation in the data that is not attributable

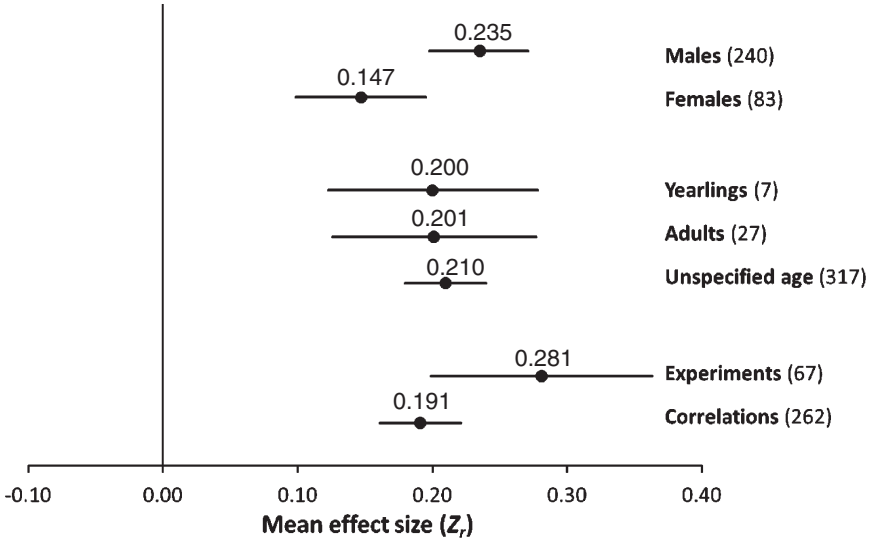

Fig. 3. Forest plots showing mean effect size and 95% confidence intervals for males versus females, age classes, and experimental *versus* correlational studies. Numbers above the bars indicate the mean effect sizes for each category. Numbers in parentheses indicate the numbers of effect sizes included to compute mean effect sizes. Vertical line denotes an effect size of zero.

to random error. I^2 spans between 0 and 100%, with larger values indicating increasing heterogeneity (Higgins *et al.*, 2003).

Publication bias in the scientific literature is considered to be common because 'negative' effects are expected to be under-represented in the published papers compared to 'positive' ones. However, publication bias seems less strong in ecology and evolutionary biology, as demonstrated by previous studies (Koricheva, 2003; Møller, Thornhill & Gangestad, 2005*b*). However, we conducted five indirect tests for publication bias. This was done by computing the Rosenthal fail-safe number, which coincides with the number of studies with effect size equal to zero that are required to make the mean effect size not significantly different from zero (Rosenberg *et al.*, 2000). In addition, we estimated the Kendall rank-order correlation coefficient between effect sizes and sample sizes (Begg & Mazumdar, 1994), and the Egger regression to test for asymmetry in the distribution of our data (Egger *et al.*, 1997). We also performed a 'trim-and-fill' analysis, which assumes that a plot of effect sizes on sample sizes should be symmetric around the 'true' effect size in case of absence of publication bias (Duval & Tweedie, 2000*a*,*b*). We therefore estimated the number of apparently 'missing' studies and their effect that may have potentially caused asymmetry in the distribution of effect sizes, by using the L_0 parameter in the 'trim and fill' test (Duval & Tweedie, 2000*a*,*b*). The mean effect size was then re-calculated after the addition of values for those putative 'missing' studies. All the analyses of heterogeneity and publication bias were performed with the R package *metafor* (Viechtbauer, 2010).

Finally, we tested for the existence of the potential effect of 'fading' over time of the statistical effect sizes for published studies on a given topic (Møller & Jennions, 2001; Jennions & Møller, 2002). This was tested by a HLM model including year of publication as a fixed effect.

III. RESULTS

(1) General results and tests for publication bias

The overall mean effect size of the association between fitness proxies and the expression of all the dimorphic traits was $\bar{z}_r = 0.214$ (CI = 0.175–0.254; $N = 329$ effect sizes; Fig. 2). This value differed significantly from zero and accounted for approximately 4–5% of total variance, corresponding to an intermediate biological effect (Cohen, 1988).

Rosenthal's fail-safe number associated with the mean effect size was very large (113911 studies). Rank correlation analysis (Begg & Mazumdar, 1994) between effect size and the corresponding sample size suggested that no significant publication bias was present in our data (Kendall $\tau = 0.035$, $P = 0.35$). Egger regression testing the symmetry of the funnel plot of all the effect sizes indicated that no significant asymmetry occurred in the distribution of our data ($\bar{z} = -0.049$, $P = 0.96$). In addition, the 'trim and fill' method led to an estimate of 0 (S.E. = 9.94) 'missing' studies on the side of relatively small effect sizes (i.e. left side of distribution of mean effect size on sample size). Moreover, the mean effect size decreased only slightly with the year of publication [estimate = −0.007 (0.002), $P = 0.0024$].

Across the entire data set there was large heterogeneity in effect sizes ($I^2 = 81.89\%$; Cochran test for heterogeneity: $Q = 1892.35$, d.f. = 328, $P < 0.0001$). Such large heterogeneity persisted in a random-effect model including study identity as a categorical moderator variable (Cochran test for residual heterogeneity: $Q_E = 1880.56$, d.f. = 327,

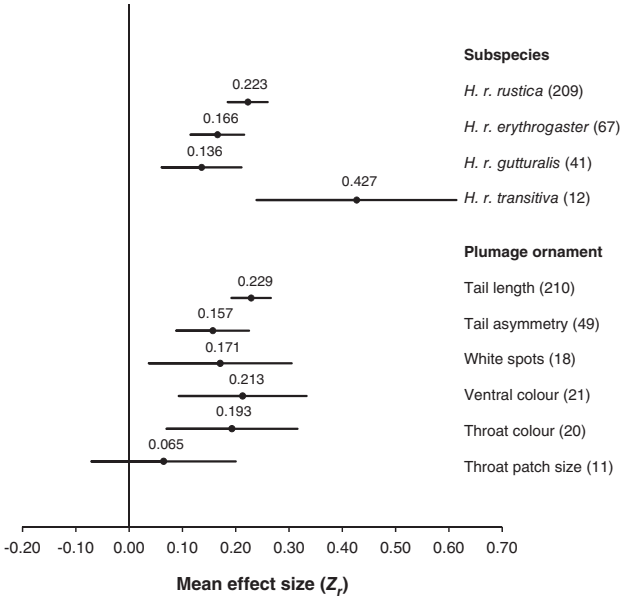

Fig. 4. Forest plots showing mean effect size and 95% confidence intervals for each subspecies and plumage ornaments. Numbers above the bars indicate the mean effect sizes for each category. Numbers in parentheses indicate the numbers of effect sizes included to compute mean effect sizes.

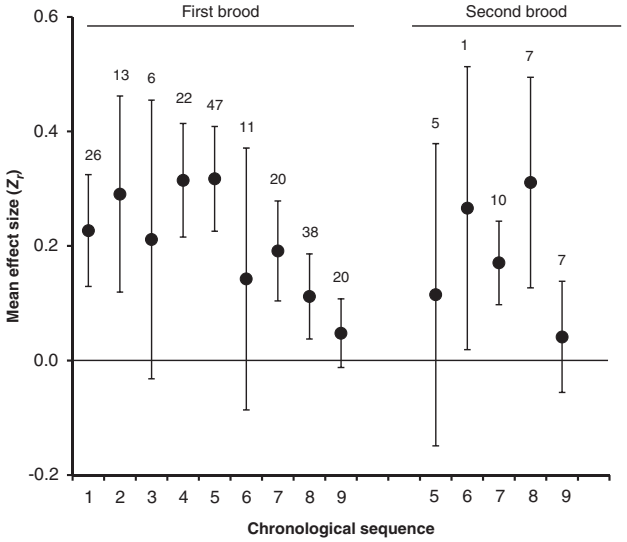

Fig. 5. Mean effect size and 95% confidence intervals for each 'breeding stage' in first and second broods. Increasing numbers on the *x* axis indicate later position in the chronological sequence of fitness proxies during the breeding cycle: 1 = arrival date; 2 = mating success; 3 = mating date; 4 = paternity; 5 = laying date; 6 = incubation; 7 = breeding success; 8 = care provisioning; and 9 = offspring quality; see Table 1). The horizontal line denotes an effect size of zero. Numbers above bars indicate the number of effect sizes included in each breeding stage.

$P < 0.0001$). Hence, other effects besides study identity should explain the observed variability in effect sizes.

(2) Descriptive statistics and univariate comparisons

Effect sizes did not vary according to study type, although the mean effect size computed from experimental studies was larger than that from correlational ones (Fig. 3; see online Table S2 for details on analyses on ES, EE and ESB subsamples of effect sizes). A large, significant difference in mean effect size existed between the sexes, with mean effect size for males being almost twofold larger than that for females (Fig. 3; see online Table S2). Interestingly, the mean effect size was significantly greater than zero in both sexes. On the other hand, there was no difference in mean effect size between age classes (Fig. 3).

The mean effect size according to plumage ornaments showed an effect that was significantly larger than zero for all but one trait (throat patch size), although differences between plumage ornaments were not significant (Fig. 4; see online Table S2). In addition, no significant difference existed when pooled effect sizes for plumage colour variables (ventral colour, throat colour, throat patch size; $\bar{Z}_r = 0.195$, CI = 0.109–0.280, $N = 52$) were compared to pooled effect sizes for tail feather variables (tail length, tail asymmetry, white spots on tail; $\bar{Z}_r = 0.218$, CI = 0.177–0.259, $N = 277$) (LR test: $\chi^2 = 0.3$, d.f. = 1, $P = 0.58$). This was also the case on males only (ventral colour, throat colour, throat patch size; $\bar{Z}_r = 0.210$, CI = 0.112–0.308, $N = 42$; tail length, tail asymmetry, white spots on tail; $\bar{Z}_r = 0.245$, CI = 0.196–0.294, $N = 198$; LR test: $\chi^2 = 0.5$, d.f. = 1, $P = 0.48$). Furthermore, effect sizes for all plumage ornaments pooled were significantly larger than zero in all subspecies, and no statistically significant difference in mean effect size emerged among subspecies (Fig. 4; see online Table S2).

Qualitatively similar results were obtained on effect sizes when analysing data from males only and on ESM, EEM and ESBM subsamples (see online Table S2).

(3) Effect size variation during the breeding cycle

When limiting the analysis to first-brood data, mean effect size differed significantly among breeding stages (LR test: $\chi^2 = 20.7$, d.f. = 8, $P = 0.008$; $N = 203$), with laying date being associated with the largest effect (Fig. 5).

Spearman correlations performed on intervals of five consecutive breeding stages showed that the mean effect size increased between the first and the fifth breeding stage although not significantly so (Spearman ρ: 0.700, $P = 0.19$), did not change between breeding stage 2 and 6 (ρ: −0.100, $P = 0.87$), and then declined at increasing pace in the following intervals (3–7: ρ: −0.500, $P = 0.39$; 4–8: ρ: −0.800, $P = 0.10$; 5–9: ρ: −0.900, $P = 0.037$). Thus, the Spearman correlation coefficients from the five intervals that were tested declined along the breeding cycle (ρ: −1.00, $P < 0.01$, $N = 5$) turning from positive for early breeding stages to strongly negative for the latest intervals. On the whole, these analyses indicated that mean effect size slightly increased in the initial part of the first broods (from arrival

Table 2. Multiple HLM model of variation in weighted effect size between plumage ornaments and fitness proxies according to sex, plumage ornament, subspecies and study type (correlation *versus* experiment). The effect of any single moderator variable was computed by full *versus* reduced model comparisons *via* LR tests (see Section II.5 for details). Sample size is 323 effect sizes from 72 studies for both sexes and 240 effect sizes from 67 studies for males

Moderator variable	Estimate (S.E.)	χ^2	d.f.	P
Sexes pooled				
Study type	0.033 (0.049)	0.7	1	0.40
Sex	0.118 (0.039)	9.1	1	0.003
Plumage ornament	—	5.3	5	0.38
Subspecies	—	4.8	3	0.19
Males				
Study type	0.042 (0.055)	0.9	1	0.34
Plumage ornament	—	3.8	5	0.58
Subspecies	—	6.7	3	0.08

date to laying date), and then steeply declined thereafter (from laying date to offspring quality).

Mean effect size did not differ significantly among breeding stages in second broods (LR test: $\chi^2 = 7.3$, d.f. $= 4$, $P = 0.12$; $N = 30$), and between first and second broods (LR test: $\chi^2 = 0.8$, d.f. $= 1$, $P = 0.37$, $N = 166$, Fig. 5).

(4) Multiple HLM model and variation in effect size of different traits among subspecies

The multiple HLM model revealed a statistically significant sex difference in mean effect size, while no other moderator variable significantly predicted variation in mean effect size (Table 2; Figs 3 and 4). Qualitatively similar results were obtained when the above analyses were repeated on males only (Table 2) and on EE, ES, ESB, EEM, ESM, and ESBM data subsets (details not shown), with the only exception of a slight significant difference in mean effect size among subspecies in the ES subset of data (LR test: $\chi^2 = 8.3$, d.f. $= 3$, $P = 0.040$; $N = 278$).

HLM models testing the interaction between plumage ornament and subspecies showed a significant effect of the interaction term in models including, respectively, *H. r. rustica* and *H. r. erythrogaster*, or *H. r. rustica* and *H. r. gutturalis* in analyses including data on both sexes or on males only (Table 3; Fig. 6). The significant effect of the interaction between plumage ornament and subspecies was also confirmed after using sequential Bonferroni correction for multiple tests (i.e. $k = 5$ on both sexes; $k = 4$ on males only), with the only exception of the comparison between *H. r. rustica* and *H. r. gutturalis* on both sexes. In particular, the comparison between *H. r. rustica* and *H. r. erythrogaster* indicated that tail length, tail asymmetry, ventral colour and throat colour were differently selected in the European and North American subspecies: tail length appears to be more intensely selected in the former, and plumage colouration in the latter (Fig. 6). In addition, the analysis including *H. r. rustica* and *H. r. gutturalis* comprised tail length, size of white spots on tail and throat colour: in *H. r. rustica* tail length, but not size of white spots and throat colour, significantly correlated with fitness traits in males, while the opposite was true for *H. r. gutturalis* (Fig. 6). Interestingly, *H. r. gutturalis* was the only subspecies in which tail length did not significantly correlate with fitness variables (Fig. 6). Very similar results were obtained in all analyses performed on EE, ES, ESB, EEM, ESM, and ESBM data subsets in both pairs of subspecies (see online Table S3). When the analyses were limited to the breeding stages shared by the two subspecies of interest, the effect of the interaction was statistically significant only in the comparison between *H. r. rustica* and *H. r. erythrogaster* (see online Table S3). In the comparison between *H. r. rustica* and *H. r. gutturalis*, the effect of the interaction between subspecies and plumage ornament was marginally non-significant. However, the number of effect sizes included in this analysis was reduced to approximately half of those reported in Table 3, thus considerably reducing the statistical power of the tests.

The models including the remaining pairs of subspecies, *H. r. rustica* and *H. r. transitiva*, *H. r. erythrogaster* and *H. r. gutturalis*, *H. r. erythrogaster* and *H. r. transitiva*, included only two plumage ornaments, and did not reveal statistically significant effects of the interaction between subspecies and plumage ornament in analyses including data on both sexes and males only (Table 3; Fig. 6; see online Table S3). The comparison between *H. r. transitiva* and *H. r. gutturalis* was not feasible because only a single plumage ornament was shared. In addition, the comparison between *H. r. rustica* and *H. r. transitiva* could not be performed on males only because a single effect size (on ventral colour) was available for *H. r. rustica* (see online Table S1).

Finally, the mean effect size for tail length varied among the four subspecies on data on males only (LR test: $\chi^2 = 11.1$, d.f. $= 3$, $P = 0.011$, $N = 149$) and on both sexes (LR test: $\chi^2 = 8.6$, d.f. $= 3$, $P = 0.035$, $N = 210$).

IV. DISCUSSION

We found evidence for sexual selection on several plumage ornaments in the barn swallow, with moderate effect sizes and little variation in mean effect sizes among different ornaments, age classes and subspecies. In addition, sexual selection on individual ornaments was strongly sex-dependent and its intensity changed during the breeding cycle. Importantly, sexual selection on individual ornaments varied among subspecies, suggesting ongoing divergence in sexual selection among barn swallow subspecies. As a cautionary note, because effect sizes for some subspecies (e.g. *H. r. rustica*) and plumage traits (e.g. tail length) are over-represented in our data set (see online Table S1), we emphasize that the results of sex-, age-, and seasonal-related variation in effect sizes might not be easily generalized to all subspecies and ornaments. Hence, some of the results should be considered with this caveat in mind.

Table 3. Variation in weighted effect size between plumage ornaments among subspecies. Analyses carried out on data on both sexes and on males only are presented. Analyses were conducted for every pair of subspecies for which information on at least two shared plumage ornaments (with at least two effect sizes) was available. Full HLM models included sex (for the analysis involving data for both sexes), plumage ornament, subspecies, study type, breeding stage and the interaction between subspecies and plumage ornament as moderators. Reduced models included the same moderators with the exception of the interaction between subspecies and plumage ornament. *P* values were computed by full *versus* reduced model comparisons using LR tests. Information about plumage ornaments included in each analysis is also shown (TL = tail length; TA = tail asymmetry; WS = size of white spots on tail; VC = ventral colour; TC = throat colour). See Fig. 6 for effect size estimates. *N* refers to the number of effect sizes included in each model, and d.f. indicates the difference in the number of degrees of freedom between full and reduced models. Asterisks indicate *P* values significant after sequential Bonferroni correction for multiple tests ($k = 5$ on data on sexes pooled; $k = 4$ on data on males only)

	Plumage ornaments	*N*	χ^2	d.f.	*P*
Sexes pooled					
H. r. rustica × *H. r. erythrogaster*	TL, TA, VC, TC	262	15.2	3	0.0016*
H. r. rustica × *H. r. gutturalis*	TL, WS, TC	184	8.2	2	0.0166
H. r. rustica × *H. r. transitiva*	TL, VC	158	1.8	1	0.18
H. r. erythrogaster × *H. r. gutturalis*	TL, TC	73	1.5	1	0.22
H. r. erythrogaster × *H. r. transitiva*	TL, VC	70	0.3	1	0.58
Males					
H. r. rustica × *H. r. erythrogaster*	TL, TC	142	7.4	1	0.0065*
H. r. rustica × *H. r. gutturalis*	TL, WS, TC	133	10.2	2	0.0061*
H. r. erythrogaster × *H. r. gutturalis*	TL, TC	62	1.3	1	0.25
H. r. erythrogaster × *H. r. transitiva*	TL, VC	56	0.7	1	0.40

(1) Variation in sexual selection intensity on plumage ornaments between males and females

The estimated effect size of sexual selection on dimorphic traits amounted to 0.147 in females and 0.235 in males, and differed significantly between the sexes, corresponding to a proportion of variance explained of 2% in females and 6% in males. Thus, according to Cohen's (1988) classification (see also Nakagawa & Cuthill, 2007), the effect for females was small, whereas it was intermediate for males. This difference implies that, as expected, sexual selection has larger impact on males than on females. However, the fitness of both sexes was positively affected by carrying large sexually selected traits, as the effect sizes for both males and females differed significantly from zero. These findings are in accordance with female tail length and colouration differing from that of homologous traits in juveniles (Møller, 1994*c*; Turner, 2006; Hubbard *et al.*, 2015), and can be interpreted in two non-mutually exclusive ways. Firstly, female conspicuousness may be selected through male directional preference for individuals carrying more expressed ornaments or signalling in female-female contest competition (West-Eberhard, 1983; Amundsen, 2000; Clutton-Brock, 2009). Sexual selection may therefore operate on females in a similar way as it acts on males by favouring conspicuous secondary sexual traits, as previously demonstrated in other vertebrate species, including birds (Jones & Hunter, 1993; Amundsen, Forsgren & Hansen, 1997; Amundsen & Forsgren, 2001; Griggio *et al.*, 2005). Under such circumstances, the sex-related difference in the intensity of sexual selection may ultimately be explained by larger variance in reproductive success in males than in females. Secondly, a genetic correlation in plumage ornaments may exist between the sexes in this species, as occurs in several other organisms (Chapman *et al.*, 2003; Roff *et al.*, 2004). In this case, when sexual selection acts on males, there is an indirect, smaller effect of sexual selection on females. This would be the case regardless of whether female ornaments are favoured by male choice. In the barn swallow, ornament expression reliably reflects individual quality (Møller, 1990*a*, 1991*b*, 1994*e*; Møller & de Lope, 1994; Saino & Møller, 1996; Saino *et al.*, 1997*a*, 2015; Kose & Møller, 1999; Hasegawa *et al.*, 2014*b*) and is heritable (Møller, 1994*c*; Saino *et al.*, 2013; Hubbard *et al.*, 2015; Vortman *et al.*, 2015). It is therefore likely that the most ornamented females would also inherit the high quality of their parents, and thus accrue larger fitness, for example, by advancing laying date and/or producing larger clutches. In addition, while females displaying large ornaments may pay some fitness costs (see e.g. Cuervo, Møller & de Lope, 2003), they should also benefit indirectly from the production of highly ornamented, 'sexy' sons. However, assessing which of these non-mutually exclusive explanations applies to the barn swallow is premature, because previous studies concerning variation in female breeding success according to the expression of dimorphic traits have provided conflicting results (Møller, 1993*c*; Cuervo, de Lope & Møller, 1996), and because no information about genetic mechanisms responsible for the development of plumage ornaments is available.

(2) Variation in sexual selection intensity during the breeding season

A previous meta-analysis on sexual selection intensity in birds (Gontard-Danek & Møller, 1999) reported a mean effect size of 0.301 for mating preference, 0.352 for mating success, 0.282 for reproduction, and 0.203 for paternity. All these

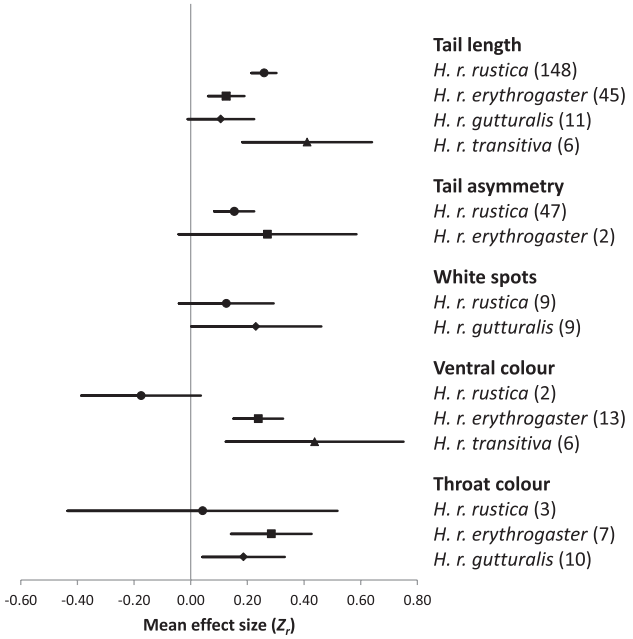

Fig. 6. Forest plots showing mean effect size and 95% confidence intervals for different plumage ornaments in distinct barn swallow subspecies. Throat patch size is not shown because it was measured in a single subspecies. Each symbol denotes one subspecies (circle = *H. r. rustica*; square = *H. r. erythrogaster*; diamond = *H. r. gutturalis*; triangle = *H. r. transitiva*). Numbers in parentheses indicate the numbers of effect sizes included to compute mean effect sizes.

effects were thus of approximately the same magnitude. The present analysis comparing different stages of the breeding cycle, spanning from arrival date from spring migration to parental care of the first brood, showed that the intensity of sexual selection changed significantly during the course of the first seasonal breeding attempt. In particular, our results suggest that fitness benefits of carrying large plumage ornaments decreased in the final part of the breeding season (i.e. after egg fertilization). Such a pattern is not surprising, because mate choice and competition for mates (as well as for paternity) only occurs before the completion of egg laying, which coincides with the promiscuous part of the breeding cycle when the scope for enhancing reproductive success reaches a maximum. This finding is consistent with a previous meta-analysis showing that direct benefits of sexual selection in terms of parental care were only of small magnitude (Møller & Jennions, 2002*a*).

Nevertheless, large effect sizes were also observed for overall reproductive success (both sexes: 0.271, CI = 0.194–0.348, $N = 40$; males: 0.315, CI = 0.231–0.399, $N = 29$), including the total number of eggs and nestlings produced during the entire breeding season, which are largely dependent on the number of breeding attempts per season. This result might be explained by the fact that intense selection in the first part of the breeding season results in earlier arrival from migration, mating and laying for highly ornamented individuals, which therefore have more broods per season than less ornamented ones.

(3) Variation in sexual selection intensity among plumage ornaments and subspecies

The overall mean effect size across all plumage ornaments and subspecies was 0.214, accounting for approximately 4–5% of the variance. Gontard-Danek & Møller (1999) showed a larger mean effect of 0.328 for colour on sexual selection across birds while the effect size for studies of morphological characters was 0.279, accounting for 11 and 8% of the variance, respectively. The present estimates for colour and tail characters in the barn swallow were 0.195 and 0.218, respectively (4 and 5% of the variance). When the analyses were restricted to males, effect sizes for both colour and tail variables increased to 0.210 and 0.245, respectively (4 and 6% of the variance). However, despite this apparent difference in mean effect size, evidence for substantial differences in sexual selection between the two categories of plumage ornaments in all subspecies pooled is lacking.

Across all subspecies, the mean effect size estimates for five out of six plumage ornaments differed significantly from zero, throat patch size being the only exception. However, no statistically significant difference in sexual selection among traits was observed, indicating that the intensity of sexual selection does not differ between different plumage ornaments. Moreover, despite barn swallow subspecies differ considerably in the level of expression of plumage ornaments (Turner, 2006), no difference in the average intensity of sexual selection was found among subspecies when pooling all sexually selected traits. This result was corroborated by a further analysis including only data on reproductive success (breeding success and overall reproductive success), confirming no difference among subspecies (details not shown).

(4) Differential sexual selection on distinct plumage ornaments among subspecies: implications for population divergence

While mean effect size did not vary significantly across subspecies and plumage ornaments, sexual selection on different plumage ornaments varied among distinct subspecies, as shown by comparisons between *H. r. rustica* and *H. r. erythrogaster*, and between *H. r. rustica* and *H. r. gutturalis* males. Interestingly, the significant differences were observed between the phylogenetically less closely related subspecies: a recent study showed that the *H. rustica* species complex is in fact divided into two well-supported clades, including respectively the European-Middle Eastern subspecies *H. r. rustica*, *H. r. transitiva* and *H. r. savignii*, and the American-Asian subspecies *H. r. erythrogaster*, *H. r. gutturalis* and *H. r. tytleri* (Dor *et al.*, 2010). The present findings are thus in accordance with the evidence that substantial gene flow is still present between *H. r. rustica* and *H. r. transitiva* (Dor *et al.*, 2012), despite their apparent differences in morphology and life-history traits.

More importantly, they provide quantitative meta-analytical support for the findings that different ornaments are subject to different selection regimes in different subspecies. This evidence is corroborated by the observation that effect sizes for tail length differed among the four subspecies in both sexes combined and in males only, thus confirming previous studies suggesting that there is a difference in the effect of tail length on sexual selection between Europe, North America and Japan (Scordato & Safran, 2014). Furthermore, an effect of ventral and/or throat colour on sexual selection has been observed in North America and Japan (e.g. Safran *et al.*, 2005; Hasegawa *et al.*, 2010*b*), and of the size of white tail spots in Estonia and Japan (e.g. Kose & Møller, 1999; Hasegawa *et al.*, 2010*b*, 2012*a*). We note however that this finding suggests variation in intensity of sexual selection on particular ornaments among subspecies, but not qualitative differences in sexual selection among subspecies.

On the whole, our results support the hypothesis that sexual selection on different plumage ornaments varies among geographically distinct populations. Geographical variation in sexual selection regimes is expected to promote divergence among subspecies, potentially leading to speciation (Scordato & Safran, 2014), as hypothesized in theoretical and empirical studies (Darwin, 1871; Andersson, 1994; Møller & Cuervo, 1998; Panhuis *et al.*, 2001; Turelli *et al.*, 2001; Ritchie, 2007; Safran *et al.*, 2013). Divergence in sexually selected traits in different populations may have several potential promoters, including sensory drive (Boughman, 2002), disruptive ecological selection favouring adaptation to the local environment (Van Doorn *et al.*, 2009), social competition for mates (West-Eberhard, 1983), or directional mate preference (Fisher, 1930; Lande, 1981, 1982), which may act independently or in concert. Irrespective of the proximate mechanism causing fitness advantages for more-ornamented individuals in different geographical populations, sexual selection has been proved to lead to divergence among populations and thus increase phenotypic diversity within a clade (e.g. Darwin, 1871; Fisher, 1930; Carson, 1978; Kaneshiro, 1980; Seddon *et al.*, 2008, 2013; but see Morrow *et al.*, 2003). Indeed, previous comparative evidence indicated that there are disproportionately more species and subspecies in bird taxa with exaggerated ornamentation compared to less-ornamented sister taxa (Møller & Cuervo, 1998; Seddon *et al.*, 2008, 2013; Kraaijeveld *et al.*, 2011). This was particularly the case in taxa with greater skew in mating success (Møller & Cuervo, 1998). This line of argument may even apply to the evolution of multiple secondary sexual characters (Møller & Pomiankowski, 1993; Iwasa & Pomiankowski, 1994).

Directional mate preference for plumage ornaments has been demonstrated repeatedly in the barn swallow (e.g. Møller, 1990*b*, 1992*b*, 1993*a*; Saino *et al.*, 1997*a*; Safran & McGraw, 2004; Safran *et al.*, 2005; Hasegawa & Arai, 2013). We therefore regard variation among populations in the expression of female (and maybe male) mate preference and male (and maybe female) secondary sexual characters as a possible mechanism causing the different patterns of sexual selection observed here (Lande, 1981, 1982). However, this conclusion should be drawn with caution because the relatively small number of plumage ornaments which have been studied in each subspecies (Fig. 6) prevented us from investigating general patterns of differentiation among subspecies and ornamental traits. As an additional cautionary note, we stress that variation in the intensity of sexual selection on different plumage ornaments was clear only in the comparison between *H. r. rustica* and *H. r. erythroagaster*, which are the phylogenetically less related subspecies. Yet, we emphasize that this is the study of birds where geographical variation in sexual selection has been investigated in the largest number of populations and sexually dimorphic traits so far. In order accurately to define and complete the picture of geographical variation in sexual selection that we have documented here, we encourage greater data-collection efforts on the less-studied geographical populations (i.e. *H. r. tytleri* and *H. r. savignii*) and sexually dimorphic traits (e.g. ventral and throat colour in *H. r. rustica*, tail asymmetry in *H. r. gutturalis*, white spots and throat colour in *H. r. transitiva*), including song features (Møller *et al.*, 1998*b*; Wilkins *et al.*, 2015).

Sexually selected traits may act as pre-zygotic reproductive barriers between some barn swallow subspecies (Vortman *et al.*, 2013; Hasegawa *et al.*, 2016), even if in most cases reproductive isolation is incomplete (Turner, 2006; Scordato & Safran, 2014). Whether geographical variation in sexual selection is driving divergence among subspecies is unknown. The study of assortative mating and evolution of reproductive barriers between sympatric and/or parapatric populations of different subspecies is thus a necessary step forward to identify a possible incipient speciation process in the barn swallow. Finally, it has been shown that multiple plumage ornaments may synergistically contribute to affect mating success and other life-history traits in all the subspecies studies here (e.g. *H. r. gutturalis*: Hasegawa *et al.*, 2010*b*; *H. r. transitiva*: Vortman *et al.*, 2013; *H. r. rustica*: Romano *et al.*, 2015; *H. r. erythrogaster*: Wilkins *et al.*, 2015). Hence, a future challenge to understand better the patterns of sexual selection observed in the present study will be to assess both the independent and combined effects of multiple sexually selected traits on fitness in different subspecies.

(5) The value of experiments

The experimental approach to science has, since the days of Lazzaro Spallanzani (1729–1799), constituted the basis for rapid scientific progress. However, the magnitude of this benefit has rarely been quantified. In a meta-analysis of sexual selection in birds, Gontard-Danek & Møller (1999) showed a mean effect size of experiments on sexual selection of 0.322 while the effect size for correlational studies was 0.289, a non-significant difference. The results from our study were similar. Observations of novel phenomena are often the basis for subsequent experiments, implying that one approach does not replace the other. We stress that, despite apparently not resulting in significantly larger effects, experiments have at least two major advantages over

observations. First, experiments allow drawing inferences about causation. Second, experiments control for potentially confounding variables that may otherwise obscure or weaken underlying patterns, and provide larger statistical effects, especially when the magnitude of biological effects is low.

V. CONCLUSIONS

(1) We conducted meta-analyses of sexual selection in the barn swallow, a classical organism for the study of sexual selection, based on the largest database on sexual selection for a single species. We found mean effect sizes of intermediate magnitude with only small differences among subspecies, plumage ornaments and age classes. As expected, the intensity of sexual selection was stronger in males than in females. However, experiments did not provide larger effect sizes than correlational studies.

(2) We disclosed variation in the intensity of sexual selection during the course of the first brood, indicating that large expression of sexually selected traits provides more fitness advantages in the initial promiscuous part of the breeding cycle, when competition for access to mates reaches its maximum.

(3) Finally, we revealed statistically significant differences in the intensity of sexual selection for different plumage ornaments among barn swallow subspecies. However, these differences emerged particularly between the phylogenetically less related subspecies. This is consistent with sexual selection playing a major role in speciation processes, since sexual selection of different traits may result in divergence among geographical populations and hence differentiation among taxa.

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VIII. SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. All effect sizes included in the meta-analyses. Information about study of origin, study area, year of data collection (Data Year), subspecies, sex (M = males; F = females; M + F = both sexes), age, plumage ornament, study type (experiment or correlation), fitness proxy, brood, breeding stage (see Table 1; Sections II.3 and II.5; Fig. 2 for details), sample size (N), and mean effect size ($\bar{Z}_r$) are provided. The column 'Main Data Set' indicates effect sizes only included in the analysis on age-related variation in intensity of sexual selection.

Table S2. Mean effect sizes and their 95% confidence intervals for each level of moderator variables included in the analyses computed according to unstructured, random-effects models separately for different subsets of data on both sexes pooled and on males only.

Table S3. Variation in weighted effect size between plumage ornaments among subspecies on data for both sexes and for males only on different subsamples of data.

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SUPPORTING INFORMATION

Table S2. Mean effect sizes and their 95% confidence intervals for each level of all moderator variables included in the analyses (study type, sex, subspecies, and plumage ornament) computed according to unstructured, random-effects models separately for different subsets of data on both sexes pooled and on males only: all data; excluding data on survival (ES and ESM); excluding data on experiments (EE and ESM); excluding data on second broods only (ESB and ESBM). Tests for differences in mean effect sizes according to moderator variables were run by univariate hierarchical linear mixed models, and significance was tested by likelihood ratio tests (see main text for details). The number of degrees of freedom differs among moderator variables: study type = 1; sex = 1; subspecies = 3; plumage ornament = 5. Asterisks indicate significant effect of the moderator variable ($P < 0.05$). *N* indicates the number of effect sizes included in each analysis.

Sexes pooled	**All**			**ES**			**EE**			**ESB**		
	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2
Study type												
Experiment	0.281 (0.199–0.363)	67	1.5	0.281 (0.199–0.363)	67	1.1	– – – –			0.297 (0.200–0.394)	52	1.5
Correlation	0.191 (0.161–0.221)	262		0.204 (0.171–0.237)	214		– – – –			0.194 (0.162–0.226)	247	
Sex												
Males	0.235 (0.198–0.271)	240	9.2*	0.241 (0.202–0.279)	212	4.7*	0.213 (0.174–0.252)	176	9.1*	0.242 (0.203–0.280)	218	9.6*
Females	0.147 (0.099–0.195)	83		0.171 (0.117–0.225)	66		0.151 (0.102–0.200)	80		0.139 (0.087–0.191)	75	
Subspecies												
H. r. rustica	0.223 (0.185–0.260)	209	5.1	0.244 (0.203–0.285)	171	6.6	0.199 (0.160–0.239)	156	3.9	0.230 (0.188–0.272)	179	5.1
H. r. erythrogaster	0.166 (0.115–0.216)	67		0.164 (0.111–0.218)	61		0.169 (0.111–0.217)	57		0.166 (0.116–0.216)	67	
H. r. gutturalis	0.136 (0.061–0.211)	41		0.135 (0.044–0.227)	37		0.135 (0.061–0.209)	39		0.136 (0.061–0.211)	41	
H. r. transitiva	0.427 (0.239–0.614)	12		0.427 (0.239–0.614)	12		0.438 (0.237–0.640)	10		0.427 (0.239–0.614)	12	
Plumage ornaments												
Tail length	0.229 (0.192–0.266)	210	5.1	0.237 (0.197–0.277)	183	7.3	0.216 (0.180–0.252)	170	4.1	0.236 (0.196–0.277)	186	4.5
Tail asymmetry	0.157 (0.088–0.225)	49		0.152 (0.076–0.228)	37		0.106 (0.027–0.185)	33		0.155 (0.082–0.229)	43	
White spots	0.171 (0.037–0.305)	18		0.255 (0.106–0.404)	14		0.124 (–0.066–0.315)	12		0.171 (0.037–0.305)	18	
Ventral colour	0.213 (0.093–0.333)	21		0.267 (0.185–0.350)	19		0.206 (0.080–0.332)	19		0.213 (0.093–0.333)	21	
Throat colour	0.193 (0.070–0.316)	20		0.194 (0.054–0.333)	18		0.185 (0.058–0.312)	19		0.193 (0.070–0.316)	20	
Throat patch size	0.065 (–0.071–0.200)	11		0.025 (–0.114–0.163)	10		0.052 (–0.081–0.184)	9		0.065 (–0.071–0.200)	11	

Males	**All**			**ESM**			**EEM**			**ESBM**		
	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2	Effect size (CI)	*N*	χ^2
Study type												
Experiment	0.295 (0.211–0.379)	64	0.7	0.295 (0.211–0.379)	64	1.0	– – – –			0.316 (0.215–0.416)	49	0.7
Correlation	0.213 (0.174–0.252)	176		0.217 (0.175–0.259)	148		– – – –			0.220 (0.180–0.260)	169	
Subspecies												
H. r. rustica	0.261 (0.211–0.310)	140	7.1	0.269 (0.216–0.323)	119	7.8	0.231 (0.174–0.287)	90	4.5	0.277 (0.222–0.332)	118	7.1
H. r. erythrogaster	0.167 (0.110–0.225)	53		0.170 (0.111–0.230)	50		0.165 (0.104–0.226)	43		0.167 (0.110–0.225)	53	
H. r. gutturalis	0.156 (0.079–0.232)	37		0.160 (0.063–0.256)	33		0.155 (0.080–0.230)	35		0.156 (0.080–0.232)	37	
H. r. transitiva	0.485 (0.308–0.662)	10		0.485 (0.308–0.662)	10		0.502 (0.310–0.694)	8		0.485 (0.308–0.662)	10	
Plumage ornaments												
Tail length	0.250 (0.202–0.297)	149	3.8	0.252 (0.201–0.303)	132	6.8	0.230 (0.182–0.277)	112	1.9	0.260 (0.209–0.312)	133	3.3
Tail asymmetry	0.205 (0.119–0.292)	34		0.187 (0.093–0.281)	28		0.151 (0.033–0.269)	18		0.211 (0.115–0.307)	28	
White spots	0.237 (0.099–0.374)	15		0.286 (0.145–0.428)	13		0.229 (0.002–0.456)	9		0.237 (0.099–0.374)	15	
Ventral colour	0.256 (0.106–0.406)	15		0.278 (0.186–0.370)	14		0.251 (0.089–0.413)	13		0.256 (0.106–0.406)	15	
Throat colour	0.202 (0.059–0.344)	17		0.213 (0.059–0.366)	16		0.193 (0.044–0.341)	16		0.202 (0.059–0.344)	17	
Throat patch size	0.090 (–0.048–0.229)	10		0.053 (–0.096–0.202)	9		0.079 (–0.057–0.214)	8		0.090 (–0.048–0.229)	10	

Table S3. Variation in weighted effect size between plumage ornaments among subspecies on data for both sexes and for males only on different subsamples of data. Analyses were conducted for every pair of subspecies for which information on at least two shared plumage ornaments (with at least two effect sizes) was available in each subsample of data. Full models included sex (for the analysis involving data for both sexes), plumage ornament, subspecies, study type, breeding stage and the interaction between subspecies and plumage ornament as moderators. Reduced models included the same moderators with the exception of the interaction between subspecies and plumage ornament. *P* values were computed by full *versus* reduced model comparisons *via* LR tests. *N* refers to the number of effect sizes included in each model, and d.f. indicates the difference in the number of degrees of freedom between full and reduced models. Asterisks indicate *P* values significant after sequential Bonferroni correction for multiple tests ($k = 5$ on data on sexes pooled; $k = 4$ on data on males only). Superscript letters indicate combinations of plumage ornaments included in each analysis.

	Excluding survival				**Excluding experiments**				**Excluding second broods**				**Breeding stages in common**			
	N	χ^2	d.f.	*P*	*N*	χ^2	d.f.	*P*	*N*	χ^2	d.f.	*P*	*N*	χ^2	d.f.	*P*
Sexes pooled																
H. r. rustica × *H. r. erythrogaster*	173[a]	9.2	1	0.0024*	205[b]	16.2	3	0.0010*	232[b]	14.5	3	0.0023*	230[b]	14.4	3	0.0024*
H. r. rustica × *H. r. gutturalis*	158[c]	7.1	2	0.0287	147[c]	9.8	2	0.0074*	160[c]	7.9	2	0.0193	119[c]	5.4	2	0.0672
H. r. rustica × *H. r. transitiva*	–	–	–	–	125[d]	2.4	1	0.121	134[d]	1.7	1	0.192	45[d]	0	1	0
H. r. erythrogaster × *H. r. gutturalis*	67[a]	0.8	1	0.371	64[a]	1.5	1	0.221	73[a]	1.5	1	0.221	61[a]	1.7	1	0.192
H. r. erythrogaster × *H. r. transitiva*	66[d]	0.3	1	0.584	59[d]	0.2	1	0.655	70[d]	0.3	1	0.584	40[d]	0	1	0
Males																
H. r. rustica × *H. r. erythrogaster*	126[a]	8.0	1	0.0047*	105[a]	8.9	1	0.0029*	126[a]	6.8	1	0.0091*	124[a]	6.7	1	0.0096*
H. r. rustica × *H. r. gutturalis*	115[c]	10.6	2	0.0050*	90[a]	7.4	1	0.0065*	117[c]	9.6	2	0.0082*	84[c]	5.6	2	0.0608
H. r. erythrogaster × *H. r. gutturalis*	58[a]	0.5	1	0.480	53[a]	1.5	1	0.221	62[a]	1.3	1	0.254	51[a]	1.5	1	0.221
H. r. erythrogaster × *H. r. transitiva*	54[d]	0.8	1	0.371	45[d]	1.4	1	0.237	35[d]	1.5	1	0.221	35[d]	1.5	1	0.221

[a]Tail length, throat colour.

[b]Tail length, tail asymmetry, ventral colour, throat colour.

[c]Tail length, size of white spots on tail, throat colour.

[d]Tail length, ventral colour.

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Arai *et al.* (2015)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	throat colour	correlation	laying date	1	laying date 1 brood	31	0.50878	1
Balbontín *et al.* (2007)	Spain	Badajoz	1994–2006	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	322	0.25184	1
Bańbura (1986)	Poland	Goślub	1978–1982	*rustica*	M	adult	tail length	correlation	laying date	1	laying date 1 brood	32	0.57634	0
Bańbura (1986)	Poland	Goślub	1978–1983	*rustica*	F	adult	tail length	correlation	laying date	1	laying date 1 brood	33	0.10741	0
Bańbura (1986)	Poland	Goślub	1978–1981	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	139	0.40592	1
Bańbura (1986)	Poland	Goślub	1978–1981	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	149	0.20169	1
Bradley *et al.* (2014)	USA	Colorado	2008–2011	*erythrogaster*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	35	0.06957	1
Bradley *et al.* (2014)	USA	Colorado	2008–2011	*erythrogaster*	F	unspecified	throat colour	correlation	overall reproductive success		overall reproductive success	37	0.19228	1
Brown&Brown (1999)	USA	Nebraska	1996	*erythrogaster*	M	unspecified	tail asymmetry	correlation	survival		survival	16	0.19230	1
Brown&Brown (1999)	USA	Nebraska	1996	*erythrogaster*	F	unspecified	tail asymmetry	correlation	survival		survival	29	0.31000	1
Brown&Brown (1999)	USA	Nebraska	1996	*erythrogaster*	M	unspecified	tail length	correlation	survival		survival	16	0.14270	1
Brown&Brown (1999)	USA	Nebraska	1996	*erythrogaster*	F	unspecified	tail length	correlation	survival		survival	29	0.47030	1
Cadée (2000)	Denmark	Brønderslev	1996	*rustica*	F	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	21	0.25623	1
Cadée (2000)	Denmark	Brønderslev	1996	*rustica*	F	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	19	-0.00942	1
Cadée (2000)	Denmark	Brønderslev	1997	*rustica*	F	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	38	0.11595	1
Cadée (2000)	Denmark	Brønderslev	1997	*rustica*	F	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	35	0.18962	1
Cadée (2000)	Denmark	Brønderslev	1998	*rustica*	F	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	28	-0.09205	1
Cadée (2000)	Denmark	Brønderslev	1998	*rustica*	F	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	28	-0.02573	1
Cadée (2000)	Denmark	Brønderslev	1996	*rustica*	M	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	20	-0.05973	1
Cadée (2000)	Denmark	Brønderslev	1997	*rustica*	M	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	31	0.09333	1
Cadée (2000)	Denmark	Brønderslev	1997	*rustica*	M	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	28	0.20885	1
Cadée (2000)	Denmark	Brønderslev	1998	*rustica*	M	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	23	0.04951	1
Cadée (2000)	Denmark	Brønderslev	1998	*rustica*	M	unspecified	tail asymmetry	correlation	offspring physiology	1	offspring quality 1 brood	23	-0.13296	1
Cadée (2000)	Denmark	Brønderslev	1996	*rustica*	M	unspecified	tail asymmetry	correlation	offspring size	1	offspring quality 1 brood	21	-0.16082	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	breeding success	1	breeding success 1 brood	29	0.04223	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	breeding success	2	breeding success 2 brood	22	0.08440	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	29	0.15216	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	female care provisioning	1	care provisioning 1 brood	29	0.01560	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	male care provisioning	1	care provisioning 1 brood	29	0.04790	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	male care provisioning	2	care provisioning 2 brood	22	0.45207	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	experiment	female care provisioning	2	care provisioning 2 brood	22	0.36317	1
Cuervo&Møller (2006)	Spain	Sevilla	1997	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	31	0.45383	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	67	0.43743	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	36	-0.09892	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	36	0.04242	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	experiment	female care provisioning	1	care provisioning 1 brood	36	0.00444	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	46	0.28090	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	46	-0.19435	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	experiment	laying date	1	laying date 1 brood	48	-0.04300	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	46	0.19969	1
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	46	-0.03816	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Cuervo *et al.* (1996)	Spain	Badajoz	1994	*rustica*	F	unspecified	tail length	experiment	overall reproductive success		overall reproductive success	46	0.06700	1
Cuervo *et al.* (2003)	Spain	Badajoz	1994	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	25	-0.05501	1
Cuervo *et al.* (2003)	Spain	Badajoz	1995	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	25	0.00550	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	breeding success	1	breeding success 1 brood	36	0.66590	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	breeding success	2	breeding success 2 brood	30	0.32107	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	female care provisioning	1	care provisioning 1 brood	18	0.45550	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	laying date	1	laying date 1 brood	36	0.18210	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	laying date	2	laying date 2 brood	30	-0.26860	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	offspring size	1	offspring quality 1 brood	36	-0.13920	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	offspring size	2	offspring quality 2 brood	30	0.09440	1
de Lope&Møller (1993)	Spain	Badajoz		*rustica*	M	unspecified	tail length	experiment	overall reproductive success		overall reproductive success	55	0.58270	1
Eikenaar *et al.* (2011*a*)	USA	Virginia	2010	*erythrogaster*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	65	0.15101	1
Eikenaar *et al.* (2011*a*)	USA	Virginia	2010	*erythrogaster*	M	unspecified	ventral colour	correlation	male care provisioning	1	care provisioning 1 brood	65	0.21292	1
Eikenaar *et al.* (2011*b*)	USA	Virginia	2009–2010	*erythrogaster*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	52	0.08755	1
Eikenaar *et al.* (2011*b*)	USA	Virginia	2009–2010	*erythrogaster*	M	unspecified	ventral colour	correlation	overall reproductive success		overall reproductive success	52	0.07351	1
Eikenaar *et al.* (2011*b*)	USA	Virginia	2009–2010	*erythrogaster*	M	unspecified	tail length	correlation	paternity	1, 2	paternity	92	0.17711	1
Eikenaar *et al.* (2011*b*)	USA	Virginia	2009–2010	*erythrogaster*	M	unspecified	tail length	correlation	paternity	1	paternity	50	0.20377	1
Eikenaar *et al.* (2011*b*)	USA	Virginia	2009–2010	*erythrogaster*	M	unspecified	ventral colour	correlation	paternity	1, 2	paternity	92	0.25448	1
Galván&Møller (2013)	Denmark	Kraghede	1996	*rustica*	M+F	unspecified	throat colour	correlation	survival		survival	117	0.30905	1
Galván *et al.* (2014)	Denmark	Vendsyssel	1993–1994	*rustica*	M	unspecified	tail length	correlation	survival		survival	55	0.22349	1
Galván *et al.* (2014)	Denmark	Vendsyssel	1993–1994	*rustica*	F	unspecified	tail length	correlation	survival		survival	37	0.33547	1
Galván *et al.* (2014)	Denmark	Vendsyssel	1993–1994	*rustica*	M+F	unspecified	white spots on tail	correlation	survival		survival	92	-0.11258	1
Galván *et al.* (2014)	Denmark	Vendsyssel	1993–1994	*rustica*	M	unspecified	white spots on tail	correlation	survival		survival	55	-0.16007	1
Galván *et al.* (2014)	Denmark	Vendsyssel	1993–1994	*rustica*	F	unspecified	white spots on tail	correlation	survival		survival	37	-0.15746	1
Garamszegi *et al.* (2005)	Spain	Badajoz	2000–2001	*rustica*	M	unspecified	tail length	correlation	survival		survival	55	0.37981	1
Garamszegi *et al.* (2006)	Spain	Badajoz	2000–2001	*rustica*	M	unspecified	tail length	correlation	mating success		mating success	171	-0.33947	1
Garamszegi *et al.* (2006)	Spain	Badajoz	2000–2001	*rustica*	M	unspecified	tail length	correlation	survival		survival	171	-0.16036	1
Grüebler&Naef–Daenzer (2008b)	Switzerland	Lucerne	2000–2004	*rustica*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	88	0.21764	1
Grüebler&Naef–Daenzer (2010)	Switzerland	Lucerne	2000–2004	*rustica*	M	unspecified	tail length	correlation	breeding success	2	breeding success 2 brood	60	0.15421	1
Grüebler&Naef–Daenzer (2010)	Switzerland	Lucerne	2000–2004	*rustica*	F	unspecified	tail length	correlation	breeding success	2	breeding success 2 brood	60	0.19192	1
Hasegawa&Arai (2013)	Japan	Kyushu, Hokkaio, Tohoku	2005–2006	*gutturalis*	M	adult	tail length	correlation	overall reproductive success		overall reproductive success	20	0.04475	1
Hasegawa&Arai (2013)	Japan	Kyushu, Hokkaio, Tohoku	2005–2008	*gutturalis*	M	adult	throat colour	correlation	overall reproductive success		overall reproductive success	20	0.50473	1
Hasegawa&Arai (2013)	Japan	Kyushu, Hokkaio, Tohoku	2005–2009	*gutturalis*	M	adult	throat patch size	correlation	overall reproductive success		overall reproductive success	20	0.46644	1
Hasegawa&Arai (2013)	Japan	Kyushu, Hokkaio, Tohoku	2005–2007	*gutturalis*	M	adult	white spots on tail	correlation	overall reproductive success		overall reproductive success	20	0.09515	1
Hasegawa&Arai (2015)	Japan	Joetsu	2007–2009	*gutturalis*	M	unspecified	throat patch size	experiment	male care provisioning	1	care provisioning 1 brood	22	0.54410	1
Hasegawa&Arai (2015)	Japan	Joetsu	2007–2009	*gutturalis*	M	unspecified	throat patch size	experiment	female care provisioning	1	care provisioning 1 brood	22	-0.22950	1
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	adult	tail length	correlation	laying date	1	laying date 1 brood	21	-0.32300	0
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	125	0.14122	1
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	adult	throat colour	correlation	laying date	1	laying date 1 brood	21	0.40800	0

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	unspecified	throat colour	correlation	laying date	1	laying date 1 brood	125	0.19141	1
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	adult	throat patch size	correlation	laying date	1	laying date 1 brood	21	0.19700	0
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	unspecified	throat patch size	correlation	laying date	1	laying date 1 brood	125	-0.06333	1
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	adult	white spots on tail	correlation	laying date	1	laying date 1 brood	21	0.43800	0
Hasegawa *et al.* (2010*b*)	Japan	Joetsu	2005–2006	*gutturalis*	M	unspecified	white spots on tail	correlation	laying date	1	laying date 1 brood	125	0.13619	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	tail length	correlation	laying date	1	laying date 1 brood	17	0.20273	0
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	31	0.56260	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	throat colour	correlation	laying date	1	laying date 1 brood	17	-0.07762	0
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	unspecified	throat colour	correlation	laying date	1	laying date 1 brood	31	0.20273	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	throat patch size	correlation	laying date	1	laying date 1 brood	17	-0.22745	0
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	unspecified	throat patch size	correlation	laying date	1	laying date 1 brood	31	0.14238	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	white spots on tail	correlation	laying date	1	laying date 1 brood	17	0.25003	0
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	unspecified	white spots on tail	correlation	laying date	1	laying date 1 brood	31	0.42365	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	tail length	correlation	mating date		mating date	15	-0.25003	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	throat colour	correlation	mating date		mating date	15	0.17497	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	throat patch size	correlation	mating date		mating date	15	-0.07762	1
Hasegawa *et al.* (2012*a*)	Japan	Joetsu	2007	*gutturalis*	M	adult	white spots on tail	correlation	mating date		mating date	15	0.20273	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	tail length	correlation	female incubation	1	incubation 1 brood	23	0.10367	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	tail length	correlation	male incubation	1	incubation 1 brood	23	-0.03411	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	throat colour	correlation	female incubation	1	incubation 1 brood	23	-0.39761	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	throat colour	correlation	male incubation	1	incubation 1 brood	23	0.30974	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	throat patch size	correlation	female incubation	1	incubation 1 brood	23	0.00520	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	throat patch size	correlation	male incubation	1	incubation 1 brood	23	-0.04473	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	white spots on tail	correlation	female incubation	1	incubation 1 brood	23	1.13050	1
Hasegawa *et al.* (2012*b*)	Japan	Joetsu	2006	*gutturalis*	M	unspecified	white spots on tail	correlation	male incubation	1	incubation 1 brood	23	0.25573	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	29	-0.34153	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	F	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	29	0.28411	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	M	unspecified	throat colour	correlation	male care provisioning	1	care provisioning 1 brood	29	0.43351	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	F	unspecified	throat colour	correlation	female care provisioning	1	care provisioning 1 brood	29	-0.09116	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	M	unspecified	throat patch size	correlation	male care provisioning	1	care provisioning 1 brood	29	-0.02886	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	F	unspecified	throat patch size	correlation	female care provisioning	1	care provisioning 1 brood	29	-0.21719	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	M	unspecified	white spots on tail	correlation	male care provisioning	1	care provisioning 1 brood	29	0.08156	1
Hasegawa *et al.* (2014*a*)	Japan	Joetsu	2006–2009	*gutturalis*	F	unspecified	white spots on tail	correlation	female care provisioning	1	care provisioning 1 brood	29	-0.22539	1
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	adult	tail length	correlation	survival		survival	20	0.40759	0
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	unspecified	tail length	correlation	survival		survival	113	0.17460	1
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	adult	throat colour	correlation	survival		survival	20	0.45804	0
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	unspecified	throat colour	correlation	survival		survival	113	0.09341	1
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	adult	throat patch size	correlation	survival		survival	20	0.54551	0
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	unspecified	throat patch size	correlation	survival		survival	113	0.23074	1
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	adult	white spots on tail	correlation	survival		survival	20	-0.05483	0
Hasegawa *et al.* (2014*b*)	Japan	Joetsu	2005–2007	*gutturalis*	M	unspecified	white spots on tail	correlation	survival		survival	113	0.08591	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Kleven *et al.* (2006)	Canada	Ontario	2003	*erythrogaster*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	86	0.36667	1
Kleven *et al.* (2006)	Canada	Ontario	2003	*erythrogaster*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	86	0.44769	1
Kleven *et al.* (2006)	Canada	Ontario	2003	*erythrogaster*	M	unspecified	tail length	correlation	paternity	1	paternity	86	0.29907	1
Kojima *et al.* (2009)	Japan	Kato, Shikoma	2006–2007	*gutturalis*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	28	0.01924	1
Kojima *et al.* (2009)	Japan	Kato, Shikoma	2006–2007	*gutturalis*	M	unspecified	tail length	correlation	paternity	1, 2	paternity	38	0.06082	1
Kose&Møller (1999)	Estonia	Haademeeste	1995–1997	*rustica*	M	unspecified	white spots on tail	experiment	breeding success	1	breeding success 1 brood	54	-0.07745	1
Kose&Møller (1999)	Estonia	Haademeeste	1995–1997	*rustica*	M	unspecified	white spots on tail	experiment	laying date	1	laying date 1 brood	54	0.24297	1
Kose&Møller (1999)	Estonia	Haademeeste	1995–1997	*rustica*	M	unspecified	white spots on tail	experiment	overall reproductive success		overall reproductive success	54	0.33990	1
Kose *et al.* (1999)	Estonia	Haademeeste	1995–1998	*rustica*	M	unspecified	white spots on tail	experiment	breeding success	1	breeding success 1 brood	51	0.28837	1
Kose *et al.* (1999)	Estonia	Haademeeste	1995–1998	*rustica*	M	unspecified	white spots on tail	experiment	laying date	1	laying date 1 brood	51	0.24256	1
Kose *et al.* (1999)	Estonia	Haademeeste	1995–1998	*rustica*	M	unspecified	white spots on tail	experiment	overall reproductive success		overall reproductive success	51	0.53943	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	adult	tail length	correlation	overall reproductive success		overall reproductive success	38	0.35409	0
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	56	0.27825	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	yearling	tail length	correlation	overall reproductive success		overall reproductive success	18	-0.01000	0
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	adult	ventral colour	correlation	overall reproductive success		overall reproductive success	38	0.01000	0
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	unspecified	ventral colour	correlation	overall reproductive success		overall reproductive success	56	0.16139	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	yearling	ventral colour	correlation	overall reproductive success		overall reproductive success	18	-0.14093	0
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	adult	tail length	correlation	paternity	1	paternity	38	0.31615	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	yearling	tail length	correlation	paternity	1	paternity	18	0.03527	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	adult	ventral colour	correlation	paternity	1	paternity	38	0.09737	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	yearling	ventral colour	correlation	paternity	1	paternity	18	0.07089	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	F	unspecified	tail length	correlation	survival		survival	54	0.08506	1
Lifjeld *et al.* (2011)	Canada	Ontario	2003–2004	*erythrogaster*	M	unspecified	tail length	correlation	survival		survival	56	0.04370	1
Maguire&Safran (2010)	USA	New Jersey	2007	*erythrogaster*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	36	-0.05472	1
Maguire&Safran (2010)	USA	New Jersey	2007	*erythrogaster*	M	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	36	-0.15397	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	22	-0.15054	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	F	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	22	-0.20214	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	F	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	22	-0.09983	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	M	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	22	0.21783	1
Maguire&Safran (2010)	USA	New Jersey	2007	*erythrogaster*	M	unspecified	throat colour	correlation	male care provisioning	1	care provisioning 1 brood	36	0.10933	1
Maguire&Safran (2010)	USA	New Jersey	2007	*erythrogaster*	M	unspecified	throat colour	correlation	female care provisioning	1	care provisioning 1 brood	36	0.27618	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	M	unspecified	throat colour	correlation	male care provisioning	1	care provisioning 1 brood	22	0.15746	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	M	unspecified	throat colour	correlation	female care provisioning	1	care provisioning 1 brood	22	0.59773	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	F	unspecified	ventral colour	correlation	female care provisioning	1	care provisioning 1 brood	22	0.09566	1
Maguire&Safran (2010)	USA	New York	2002	*erythrogaster*	F	unspecified	ventral colour	correlation	male care provisioning	1	care provisioning 1 brood	22	-0.02306	1
Matyjasiak (2013)	Poland	Warsaw	2007–2008	*rustica*	M+F	unspecified	tail length	correlation	arrival date		arrival date	124	0.17248	1
Møller&de Lope (1994)	Denmark, Spain	Kraghede, Badajoz	1987–1991	*rustica*	M	unspecified	tail length	correlation	survival		survival	336	0.10297	1
Møller&Nielsen (1997)	Denmark	Kraghede	1993–1994	*rustica*	M	unspecified	tail asymmetry	correlation	survival		survival	804	0.61698	1
Møller&Nielsen (1997)	Denmark	Kraghede	1993–1994	*rustica*	F	unspecified	tail asymmetry	correlation	survival		survival	785	-0.12600	1
Møller&Nielsen (1997)	Denmark	Kraghede	1993–1994	*rustica*	M	unspecified	tail length	correlation	survival		survival	804	0.65497	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Møller&Nielsen (1997)	Denmark	Kraghede	1993–1994	*rustica*	F	unspecified	tail length	correlation	survival		survival	785	0.23783	1
Møller&Tegelström (1997)	Denmark	Kraghede	1988–1989	*rustica*	M	unspecified	tail length	correlation	paternity	1	paternity	47	0.63639	1
Møller (1988)	Denmark	Kraghede	1970–1987	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	74	0.67767	1
Møller (1988)	Denmark	Kraghede	1970–1987	*rustica*	M	unspecified	tail length	experiment	mating success		mating success	42	0.42070	1
Møller (1988)	Denmark	Kraghede	1970–1987	*rustica*	M	unspecified	tail length	experiment	overall reproductive success		overall reproductive success	44	0.45525	1
Møller (1988)	Denmark	Kraghede	1970–1987	*rustica*	M	unspecified	tail length	experiment	paternity	1	paternity	44	0.47700	1
Møller (1989)	Denmark	Kraghede	1988	*rustica*	M	unspecified	tail length	correlation	survival		survival	20	0.97129	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	74	0.36544	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	74	-0.11045	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	63	0.18198	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	63	0.33680	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	M	unspecified	tail length	correlation	mating success		mating success	74	0.25541	1
Møller (1990*b*)	Denmark	Kraghede	1984–1986	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	63	0.44120	1
Møller (1991*a*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	396	0.67427	1
Møller (1991*a*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	396	0.25402	1
Møller (1991*a*)	Denmark	Kraghede	1984–1990	*rustica*	F	unspecified	tail length	correlation	survival		survival	396	-0.02985	1
Møller (1991*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	adult	tail length	correlation	survival		survival	159	0.18590	0
Møller (1991*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	survival		survival	380	0.22820	1
Møller (1991*b*)	Denmark	Kraghede	1984–1990	*rustica*	F	unspecified	tail length	correlation	survival		survival	395	-0.01640	1
Møller (1991*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	yearling	tail length	correlation	survival		survival	221	0.19290	0
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	breeding success	1	breeding success 1 brood	96	0.01560	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	breeding success	1	breeding success 1 brood	96	0.21278	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	breeding success	2	breeding success 2 brood	96	0.02629	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	breeding success	2	breeding success 2 brood	96	0.23113	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	laying date	1	laying date 1 brood	96	0.42020	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	laying date	1	laying date 1 brood	96	1.63048	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	mating success		mating success	96	0.47498	1
Møller (1992*a*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	mating success		mating success	96	0.91436	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	261	0.13816	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	breeding success	2	breeding success 2 brood	120	0.16364	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	129	0.28768	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	female care provisioning	2	care provisioning 2 brood	120	0.21317	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	261	0.19126	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	laying date	2	laying date 2 brood	120	-0.13686	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	mating success		mating success	485	0.20926	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	yearling	tail length	correlation	mating success		mating success	306	0.21738	0
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	offspring size	1	offspring quality 1 brood	261	0.03467	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	offspring size	2	offspring quality 2 brood	120	0.02478	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	396	0.33238	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	paternity	1	paternity	161	0.47366	1
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	yearling	tail length	correlation	paternity	1	paternity	71	0.33328	0

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Møller (1992*b*)	Denmark	Kraghede	1984–1990	*rustica*	M	unspecified	tail length	correlation	survival		survival	461	0.03453	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	breeding success	1	breeding success 1 brood	34	0.00227	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	breeding success	2	breeding success 2 brood	15	0.23520	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	laying date	1	laying date 1 brood	34	0.59015	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	laying date	2	laying date 2 brood	15	0.45353	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	mating success		mating success	34	0.59342	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	offspring size	1	offspring quality 1 brood	34	-0.03035	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	offspring size	2	offspring quality 2 brood	15	-0.14720	1
Møller (1993*a*)	Denmark	Kraghede	1992	*rustica*	M	unspecified	tail asymmetry	experiment	overall reproductive success		overall reproductive success	34	0.60106	1
Møller (1993*b*)	Ukraine	Chernobyl		*rustica*	M	unspecified	tail asymmetry	correlation	laying date	1	laying date 1 brood	45	0.40320	1
Møller (1993*b*)	Ukraine	Chernobyl		*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	45	0.85933	1
Møller (1993*b*)	Ukraine	Kiev, Kanev		*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	118	0.79150	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	343	0.03392	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	80	0.59834	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	breeding success	2	breeding success 2 brood	212	0.11307	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	breeding success	2	breeding success 2 brood	66	0.43252	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	female care provisioning	1	care provisioning 1 brood	130	-0.01154	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	female care provisioning	2	care provisioning 2 brood	87	0.00067	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	female incubation	1	incubation 1 brood	80	0.34283	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	female incubation	2	incubation 2 brood	66	0.26611	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	343	0.23179	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	80	0.70892	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	laying date	2	laying date 2 brood	212	0.19985	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	laying date	2	laying date 2 brood	66	0.41180	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	mating date		mating date	343	0.21013	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	mating date		mating date	80	0.60416	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	mating success		mating success	343	0.05796	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	mating success		mating success	80	0.43561	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	offspring size	1	offspring quality 1 brood	130	0.07622	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	offspring size	2	offspring quality 2 brood	87	0.08389	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	343	0.09011	1
Møller (1993*c*)	Denmark	Kraghede	1984–1991	*rustica*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	80	0.67908	1
Møller (1994*a*)	Denmark	Kraghede	1984–1991	*rustica*	M	unspecified	tail length	correlation	male care provisioning	1	care provisioning 1 brood	131	0.19234	1
Møller (1994*a*)	Denmark	Kraghede	1984–1991	*rustica*	M	unspecified	tail length	correlation	male care provisioning	2	care provisioning 2 brood	90	0.18198	1
Møller (1994*a*)	Denmark	Kraghede	1984–1991	*rustica*	M	unspecified	tail length	correlation	offspring size	1	offspring quality 1 brood	131	0.05520	1
Møller (1994*a*)	Denmark	Kraghede	1984–1991	*rustica*	M	unspecified	tail length	correlation	offspring size	2	offspring quality 2 brood	90	-0.03449	1
Møller (1994*b*)	Denmark	Kraghede	1990–1992	*rustica*	M	unspecified	tail asymmetry	correlation	arrival date		arrival date	124	-0.06287	1
Møller (1994*b*)	Denmark	Kraghede	1984–1992	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	697	0.14850	1
Møller (1994*b*)	Denmark	Kraghede	1987	*rustica*	M	unspecified	tail length	correlation	survival		survival	29	0.91018	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	F	unspecified	tail asymmetry	correlation	arrival date		arrival date	121	0.00000	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	M	unspecified	tail asymmetry	correlation	laying date	1	laying date 1 brood	215	-0.12600	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	F	unspecified	tail asymmetry	correlation	laying date	1	laying date 1 brood	231	0.15773	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	M	unspecified	tail asymmetry	correlation	mating success		mating success	367	0.17365	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	M	unspecified	tail asymmetry	correlation	overall reproductive success		overall reproductive success	239	0.03142	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	F	unspecified	tail asymmetry	correlation	overall reproductive success		overall reproductive success	252	-0.07862	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	M	unspecified	tail asymmetry	correlation	survival		survival	246	0.17365	1
Møller (1994*d*)	Denmark	Kraghede	1990–1992	*rustica*	F	unspecified	tail asymmetry	correlation	survival		survival	269	0.28659	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	female care provisioning	1	care provisioning 1 brood	69	0.41910	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	female care provisioning	1	care provisioning 1 brood	69	0.65267	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	female care provisioning	2	care provisioning 2 brood	39	0.48180	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	female care provisioning	2	care provisioning 2 brood	39	0.71640	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	offspring size	1	offspring quality 1 brood	69	0.09540	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	offspring size	1	offspring quality 1 brood	69	0.12392	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail asymmetry	experiment	offspring size	2	offspring quality 2 brood	39	0.03245	1
Møller (1994*e*)	Denmark	Kraghede	1991	*rustica*	M	unspecified	tail length	experiment	offspring size	2	offspring quality 2 brood	39	0.19720	1
Møller *et al.* (1998*b*)	Italy	Milan	1995	*rustica*	M	unspecified	tail length	correlation	paternity	1	paternity	26	0.47784	1
Møller *et al.* (2003)	Denmark	Kraghede	1994	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	86	0.18642	1
Møller *et al.* (2003)	Denmark	Kraghede	1994	*rustica*	M	unspecified	tail length	correlation	paternity	1, 2, 3	paternity	86	0.08650	1
Møller *et al.* (2004)	Spain	Badajoz	1997–1998	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	37	-0.00398	1
Møller *et al.* (2004)	Spain	Badajoz	1997–1998	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	37	0.02286	1
Møller *et al.* (2004)	Denmark	Kraghede	1986–1999	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	192	0.71550	1
Møller *et al.* (2004)	Denmark	Kraghede	1986–1999	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	159	0.40339	1
Møller *et al.* (2004)	Denmark	Kraghede	1988	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	60	0.69266	1
Møller *et al.* (2004)	Denmark	Kraghede	1988	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	55	0.52709	1
Møller *et al.* (2004)	Italy	Milan	1999	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	283	0.15390	1
Møller *et al.* (2004)	Italy	Milan	1999	*rustica*	F	unspecified	tail length	correlation	arrival date		arrival date	270	0.17016	1
Møller *et al.* (2005*a*)	Spain	Badajoz	before 2005	*rustica*	M	unspecified	tail asymmetry	correlation	survival		survival	32	0.16564	1
Møller *et al.* (2005*a*)	Spain	Badajoz	before 2005	*rustica*	F	unspecified	tail asymmetry	correlation	survival		survival	32	-0.06236	1
Møller *et al.* (2005*a*)	Denmark	Kraghede	before 2005	*rustica*	M	unspecified	tail asymmetry	correlation	survival		survival	20	0.07433	1
Møller *et al.* (2005*a*)	Denmark	Kraghede	before 2005	*rustica*	F	unspecified	tail asymmetry	correlation	survival		survival	24	-0.04998	1
Møller *et al.* (2005*a*)	Italy	Milan	before 2005	*rustica*	M	unspecified	tail asymmetry	correlation	survival		survival	34	0.24548	1
Møller *et al.* (2005*a*)	Italy	Milan	before 2005	*rustica*	F	unspecified	tail asymmetry	correlation	survival		survival	32	-0.10913	1
Møller *et al.* (2005*a*)	Spain	Badajoz	before 2005	*rustica*	M	unspecified	tail length	correlation	survival		survival	32	-0.06655	1
Møller *et al.* (2005*a*)	Spain	Badajoz	before 2005	*rustica*	F	unspecified	tail length	correlation	survival		survival	32	-0.10749	1
Møller *et al.* (2005*a*)	Denmark	Kraghede	before 2005	*rustica*	M	unspecified	tail length	correlation	survival		survival	20	-0.02255	1
Møller *et al.* (2005*a*)	Denmark	Kraghede	before 2005	*rustica*	F	unspecified	tail length	correlation	survival		survival	24	-0.14692	1
Møller *et al.* (2005*a*)	Italy	Milan	before 2005	*rustica*	M	unspecified	tail length	correlation	survival		survival	34	0.00750	1
Møller *et al.* (2005*a*)	Italy	Milan	before 2005	*rustica*	F	unspecified	tail length	correlation	survival		survival	32	0.03659	1
Neumann *et al.* (2007)	USA	New York	2002	*erythrogaster*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	53	0.15872	1
Neumann *et al.* (2007)	USA	New York		*erythrogaster*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	237	0.10727	1
Neumann *et al.* (2007)	USA	New York	2002	*erythrogaster*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	51	0.13807	1
Neumann *et al.* (2007)	USA	New York		*erythrogaster*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	232	-0.21474	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Neumann *et al.* (2007)	USA	New York		*erythrogaster*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	246	0.20666	1
Neumann *et al.* (2007)	USA	New York	2002	*erythrogaster*	M	unspecified	tail length	correlation	paternity	1	paternity	46	-0.21230	1
Ninni *et al.* (2004)	Spain	Badajoz	1999	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	103	0.31650	1
Ninni *et al.* (2004)	Spain	Badajoz	2000	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	102	0.17410	1
Ninni *et al.* (2004)	Spain	Badajoz	1999	*rustica*	M	unspecified	throat colour	correlation	arrival date		arrival date	103	-0.44436	1
Ninni *et al.* (2004)	Spain	Badajoz	2000	*rustica*	M	unspecified	throat colour	correlation	arrival date		arrival date	102	0.25832	1
Pap (2002)	Hungary	Balmazujvaros	2000	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	60	0.26759	1
Pap (2002)	Hungary	Balmazujvaros	2000	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	68	0.44235	1
Pap *et al.* (2005)	Hungary	Balmazujvaros	1999–2003	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	325	0.15102	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	29	0.04774	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	52	0.12014	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	M	unspecified	throat colour	correlation	laying date	1	laying date 1 brood	29	0.41695	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	M	unspecified	ventral colour	correlation	laying date	1	laying date 1 brood	29	0.41123	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	F	unspecified	ventral colour	correlation	laying date	1	laying date 1 brood	52	0.38342	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	29	0.44102	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	52	-0.03234	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	M	unspecified	ventral colour	correlation	overall reproductive success		overall reproductive success	28	0.40535	1
Safran&McGraw (2004)	USA	New York	2001	*erythrogaster*	F	unspecified	ventral colour	correlation	overall reproductive success		overall reproductive success	47	0.43652	1
Safran *et al.* (2005)	USA	New York		*erythrogaster*	M	unspecified	throat colour	experiment	paternity	1	paternity	27	0.37654	1
Safran *et al.* (2005)	USA	New York		*erythrogaster*	M	unspecified	ventral colour	experiment	paternity	1	paternity	27	0.37654	1
Saino *et al.* (1995)	Italy	Milan	1993	*rustica*	M	unspecified	tail length	correlation	arrival date		arrival date	249	0.23419	1
Saino *et al.* (1997*a*)	Italy	Milan	1994–1995	*rustica*	M	unspecified	tail length	correlation	survival		survival	85	0.30191	1
Saino *et al.* (1997*b*)	Italy	Milan	1994	*rustica*	M	unspecified	tail length	experiment	mating success		mating success	108	0.14422	1
Saino *et al.* (1997*b*)	Italy	Milan	1994	*rustica*	M	unspecified	tail length	correlation	paternity	1, 2	paternity	52	0.41366	1
Saino *et al.* (1997*b*)	Italy	Milan	1994	*rustica*	M	unspecified	tail length	correlation	paternity	1	paternity	52	0.47215	1
Saino *et al.* (1997*b*)	Italy	Milan	1994	*rustica*	M	unspecified	tail length	experiment	paternity	1, 2	paternity	108	0.23322	1
Saino *et al.* (1999*a*)	Italy	Milan	1996–1997	*rustica*	M	unspecified	tail length	correlation	survival		survival	100	0.23706	1
Saino *et al.* (1999*b*)	Italy	Milan	1994–1995	*rustica*	M	unspecified	tail length	experiment	paternity	1	paternity	38	0.33389	1
Saino *et al.* (2003)	Italy	Milan	1993–2000	*rustica*	M	unspecified	tail length	correlation	paternity	1	paternity	84	0.33389	1
Saino *et al.* (2004)	Italy	Milan	1993–2002	*rustica*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	409	0.15471	1
Saino *et al.* (2004)	Italy	Milan	1993–2002	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	409	0.58969	1
Saino *et al.* (2011)	Italy	Milan	2006–2010	*rustica*	M+F	unspecified	tail length	correlation	survival		survival	427	0.09998	1
Saino *et al.* (2013)	Italy	Milan	1997–2010	*rustica*	M	unspecified	ventral colour	correlation	survival		survival	216	-0.27583	1
Saino *et al.* (2013)	Italy	Milan	1997–2010	*rustica*	F	unspecified	ventral colour	correlation	survival		survival	132	-0.06011	1
Shykoff&Møller (1999)	Denmark	Kraghede	1988–1989	*rustica*	M	unspecified	tail asymmetry	correlation	overall reproductive success		overall reproductive success	40	0.52731	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	correlation	arrival date		arrival date	23	0.24477	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	F	unspecified	tail length	correlation	arrival date		arrival date	20	0.25541	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	breeding success	1	breeding success 1 brood	20	0.15772	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	17	0.02582	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	laying date	1	laying date 1 brood	20	0.44604	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	correlation	mating success		mating success	17	0.01633	1

Study	Country	Study Area	Data Year	Subspecies	Sex	Age	Plumage Ornament	Study Type	Fitness Proxy	Brood	Breeding Stage	*N*	*Zr*	Data Set
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	mating success		mating success	42	0.54301	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	offspring size	1	offspring quality 1 brood	20	-0.04537	1
Smith&Montgomerie (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	overall reproductive success		overall reproductive success	20	0.00085	1
Smith&Montgomerie (1992)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	correlation	male incubation	1	incubation 1 brood	16	-0.12058	1
Smith&Montgomerie (1992)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	male incubation	1	incubation 1 brood	16	-0.15615	1
Smith *et al.* (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	breeding success	1	breeding success 1 brood	11	0.24747	1
Smith *et al.* (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	correlation	paternity	1	paternity	11	0.84821	1
Smith *et al.* (1991)	Canada	Ontario	1989	*erythrogaster*	M	unspecified	tail length	experiment	paternity	1	paternity	11	-0.72875	1
Soler *et al.* (1998)	Spain	Badajoz	1997	*rustica*	F	unspecified	tail length	correlation	laying date	1	laying date 1 brood	14	0.01941	1
Soler *et al.* (1998)	Spain	Badajoz	1997	*rustica*	M	unspecified	tail length	correlation	laying date	1	laying date 1 brood	14	0.48244	1
Soler *et al.* (1998)	Spain	Badajoz	1997	*rustica*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	14	0.64229	1
Soler *et al.* (1998)	Spain	Badajoz	1997	*rustica*	F	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	14	0.18477	1
Teplitsky *et al.* (2011)	Denmark	Kraghede	1985–2009	*rustica*	M+F	unspecified	tail length	correlation	arrival date		arrival date	191	0.30075	1
Teplitsky *et al.* (2011)	Spain	Badajoz	1991–2007	*rustica*	M+F	unspecified	tail length	correlation	arrival date		arrival date	709	0.10539	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	M	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	29	0.12874	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	F	unspecified	tail length	correlation	breeding success	1	breeding success 1 brood	26	0.47024	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	M	unspecified	ventral colour	correlation	breeding success	1	breeding success 1 brood	29	0.47788	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	F	unspecified	ventral colour	correlation	breeding success	1	breeding success 1 brood	26	-0.15216	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007	*transitiva*	M	unspecified	tail length	correlation	overall reproductive success		overall reproductive success	13	0.77876	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007	*transitiva*	M	unspecified	ventral colour	correlation	overall reproductive success		overall reproductive success	12	0.83342	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007	*transitiva*	M	unspecified	tail length	correlation	paternity	1, 2	paternity	13	0.79995	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	M	unspecified	tail length	correlation	paternity	1	paternity	29	0.25402	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007	*transitiva*	M	unspecified	ventral colour	correlation	paternity	1, 2	paternity	12	0.74875	1
Vortman *et al.* (2011)	Israel	Hula Valley	2007–2008	*transitiva*	M	unspecified	ventral colour	correlation	paternity	1	paternity	29	0.59348	1
Vortman *et al.* (2013)	Israel	Hula Valley	2008–2010	*transitiva*	M	unspecified	tail length	experiment	paternity	1	paternity	6	0.40245	1
Vortman *et al.* (2013)	Israel	Hula Valley	2008–2010	*transitiva*	M	unspecified	ventral colour	experiment	paternity	1	paternity	8	0.25605	1

12. PhD Student Final Report

UNIVERSITÀ DEGLI STUDI DI MILANO

PhD ESciences Environmental

Ph.D. Course in Environmental Sciences

PhD Student Final Report

PhD Student:	**COSTANZO Alessandra**
PhD Course Cycle:	XXIX
Scientific tutor:	Prof. SAINO Nicola
Scientific co-tutor:	Prof. GIANFRANCESCHI Luca
Thesis project title:	Melanin-based colouration as a signal of individual quality and its potential role in sexual selection in the Barn swallow (*Hirundo rustica*)
Project performed at:	University of Milan; Department of Biosciences; Laboratory of Behavioral and Evolutionary Ecology.

Research Period Abroad			
December 2014-March 2015	Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud, Orsay Cedex, **FRANCE**	Local Supervisor: Prof. MØLLER Anders Pape	Research Project Title: Geographical and seasonal variation in the intensity of sexual selection in the Barn swallow *Hirundo rustica*: a meta-analysis.

List of Scientific Publications

Costanzo A., Panseri S., Giorgi A., Romano A., Caprioli M., Saino N. (2016) The odour of sex: sex-related differences in volatile compound composition among barn swallow eggs carrying embryos of either sex. Plos one. e0165055.

Costanzo A., Parolini M., Bazzi G., Khoriauli L., Santagostino M., Possenti C.D., Romano A., Nergadze S.G., Rubolini D., Giulotto E., Saino N. (2016). Brood size, telomere length, and parent-offspring color signaling in barn swallows. Behavioral ecology. 28:204-211.

Romano A., Costanzo A., Rubolini D., Saino N., Møller A.P. (2016), Geographical and seasonal variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*: a meta-analysis. Biological review. 97:1582-1600.

Romano A., Bazzi G., Caprioli M., Corti M., Costanzo A., Rubolini D., Saino N. (2016). Nestling sex and plumage color predict food allocation by barn swallow parents. Behavioral ecology. 27:1198-1205.

Romano A., Costanzo A., Caprioli M., Parolini M., Ambrosini R., Rubolini D., Saino N. (2016). Better-surviving barn swallow mothers produce more and better-surviving sons. Evolution.70:1120-1128.

Saino N., Romano M., Romano A., Rubolini D., Ambrosini R., Caprioli M., Parolini M., Scandolara C., Bazzi G., Costanzo A. (2015) White tail spots in breeding Barn Swallows *Hirundo rustica* signal body condition during winter moult. Ibis. 157:722-730.

Bazzi G., Ambrosini R., Caprioli M., Costanzo A., Liechti F., Gatti E., Gianfranceschi L., Podofillini S., Romano A., Romano M., Scandolara C., Saino N., Rubolini D. (2015). Clock gene polymorphism and scheduling of migration: a geolocator study of the barn swallow *Hirundo rustica*. Scientific reports. 5:12443.

Romano A., Romano M., Caprioli M., Costanzo A., Parolini M., Rubolini D., Saino, N. (2015). Sex allocation according to multiple sexually dimorphic traits of both parents in the barn swallow (*Hirundo rustica*). Journal of evolutionary biology. 28:1234-1247

Saino N., Romano M., Rubolini D., Caprioli M., Costanzo A., Canova L., Møller, A.P. (2014). Melanic coloration differentially predicts transfer of immune factors to eggs with daughters or sons. Behavioral ecology. 25:1248-1255.

Saino N., Romano M., Rubolini D., Ambrosini R., Romano A., Caprioli M., Costanzo A., Bazzi G. (2014). A trade-off between reproduction and feather growth in the barn swallow (*Hirundo rustica*). Plos one. e96428

Saino N., Romano M., Scandolara C., Rubolini D., Ambrosini R., Caprioli M., Costanzo A., Romano A. (2014). Brownish, small and lousy barn swallows have greater natal dispersal propensity. Animal behaviour. 87:137-146

Costanzo A., Ambrosini R., Caprioli M., Gatti E., Parolini M., Canova L., Rubolini D., Romano A., Gianfranceschi L., Saino N. Lifetime reproductive success, selection on lifespan and multiple sexual ornaments in male European barn swallows. Accepted in Evolution.

Costanzo A., Ambrosini R., Caprioli M., Gatti E., Parolini M., Romano A., Rubolini D., Gianfranceschi L., Saino N. Extra-pair fertilizations vary with female traits and pair composition, besides male attractiveness in barn swallows. Accepted in Animal behaviour.

Corti M., Bazzi G., Costanzo A., Podofillini S., Saino N., Rubolini D., Romano A. Behavioural stress response and melanin-based plumage colouration in barn swallow nestlings. Accepted in Behaviour.

Parolini M., Romano A., Costanzo A., Khoriauli L., Santagostino M., Nergadze S.G., Canova L., Rubolini D., Giulotto E., Saino N. Telomere length is reflected by plumage coloration and predicts seasonal reproductive success in the barn swallow. Submitted to Molecular ecology.

Khorialuli L., Romano A., Caprioli M., Santagostino M., Nergadze S.G., Costanzo A., Rubolini D., Giulotto E., Saino N., Parolini M. Assortative mating for telomere length and antioxidant capacity in the barn swallow (*Hirundo rustica*). Submitted to Behavioral ecology and sociobiology.

Saino N., Rubolini D., Ambrosini R., Romano A., Parolini M., Canova L., Corti M., Costanzo A. Sex- and age-dependent morphology and selection on wing shape in the barn swallow (*Hirundo rustica*). Submitted to Journal of avian biology.

Al-Murayati H., Al Rubaiee Z., Petrie M., Costanzo A., Møller A.P. Do peacock signals reveal abundance and diversity of microorganisms? In prep.

Al-Murayati H., Al Rubaiee Z., Petrie M., Costanzo A., Møller A.P. Feather bacteria may influence daily growth increments of peacock ocelli feathers. In prep.

List of attended Meetings and Congresses

1° Congresso Nazionale Congiunto S.It.E. (Società Italiana di Ecologia) -UZI (Unione Zoologica Italiana) -SIB (Società Italiana di Biogeografia). Università degli Studi di Milano-Bicocca. Milano, 30 agosto -2 settembre 2016.

XI Incontro dei Dottorandi in Ecologia e Scienze dei Sistemi Acquatici. Sapienza-Università di Roma, Dipartimento di Biologia Ambientale. Roma, 17-19 settembre 2015.

Meeting and Congress Contributions

Costanzo A., Parolini M., Romano A., Giulotto E., Saino N. Brood size, telomere length and parentoffspring color signaling in barn swallows. 1° Congresso Nazionale Congiunto S.It.E. (Società Italiana di Ecologia) - UZI (Unione Zoologica Italiana) - SIB (Società Italiana di Biogeografia): Milano, 30 agosto-2 settembre 2016.

Corti M., Romano A., Bazzi G., Caprioli M., Costanzo A., Rubolini D., Saino N. Nestling sex and plumage color predict food allocation by barn swallow parents. 1° Congresso Nazionale Congiunto S.It.E. (Società Italiana di Ecologia) - UZI (Unione Zoologica Italiana) - SIB (Società Italiana di Biogeografia): Milano, 30 agosto-2 settembre 2016.

Costanzo A., Romano A., Romano M., Caprioli M., Parolini M., Rubolini D., Saino N. Sex allocation according to multiple sexually dimorphic traits in the barn swallow (Hirundo rustica). XI Incontro dei Dottorandi in Ecologia e Scienze dei Sistemi Acquatici: Roma, 17-19 settembre 2015.

Romano A., Costanzo A., Rubolini D., Saino N., Møller A.P. Geographical and seasonal variation in the intensity of sexual selection in the barn swallow Hirundo rustica: a meta-analysis. XI Incontro dei Dottorandi in Ecologia e Scienze dei Sistemi Acquatici: Roma, 17-19 settembre 2015.

List of attended Seminars

9th April 2014. Ecotoxicology of emerging aquatic pollutants. Speaker: Marco Parolini.

24th September 2014. Valutazione dell'esposizione e marcatori biologici di dose interna. Speaker: Silvia Fustinoni.

24th September 2014. Plant reproduction: from cells to shelves. Speaker: Simona Masiero.

24th September 2014. Genes timing avian phenology in a changing climate: insights from long-distance migratory birds. Speaker: Diego Rubolini.

24th September 2014. Meccanismi di neuroinfiammazione e rilevanza nella tossicità di contaminanti ambientali. Speaker: Barbara Viviani.

10th November 2014. Primo corso pratico all'utilizzo di ImageJ. Speaker: Maurizio Abbate

11th December 2014. Ecology and Evolution at Chernobyl and Fukushima. Speaker: A. P. Møller.

12th December 2014. How to write a scientific paper. Speaker: A. P. Møller.

4th February 2015. Gendercide symbionts...e altre storie di sesso, simbiosi e sopravvivenza Speaker: Claudio Bandi

5th March 2015. Simbiosi, parassitismo e malattie infettive: un approccio evoluzionistico. Speaker: Claudio Bandi.

5th March 2015. Variabilità e cambiamenti climatici in Italia negli ultimi due secoli. Speaker: Mauro Maugeri.

5th March 2015. L'epigenetica ambientale come interfaccia tra ambiente ed espressione genica. Speaker: Valentina Bollati.

11th March 2015. Unraveling climate change effects on migration birds: a comparative approach. Speaker: D. Rubolini.

9th November 2016. Environmental DNA to understand biodiversity changes Speaker: Francesco Ficetola.

List of attended PhD Courses

March-May 2014. English course organized by the PhD course in Environmental Sciences (Prof. Carson).

June-July 2014. Statistic course organized by the PhD course in Environmental Sciences (Prof. Ambrosini).

1st-2nd October 2015. Genetica e conservazione delle popolazioni (Prof. Ettore Randi).

May-June 2016. Corso di matematica: ottimizzazione in più variabili (Dr. Paola Morando) 21st-29th October 2015. Course on molecular methods applied to environmental research. (Dr. Diego Fontaneto).

13th-15th December 2016. Environmental disasters and their ecological consequences. a short course on how to study them. Lecturer: Prof. Andrea Bonisoli Alquati.