Sexual Selection Strategy of Northeastern Chinese Barn Swallows (*Hirundo rustica*)

Thesis by

Yu Liu

Submitted in partial fulfillment of the requirements of the Degree of

Doctor of Philosophy

Queen Mary, University of London

2016

(Submitted September 1, 2016)

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Details of collaboration and publications:

Morphological data of Chinese barn swallows were collected with Elizabeth Scordato, Caroline Glidden and Rachel Lock from the laboratory of Rebecca Safran in University of Colorado, Colorado, US. This work has not been published by the submission date of the thesis.

Acknowledgements

I would like to express the deepest appreciation to my supervisor, Prof. Matthew Evans, and advisory panel members Prof. Stephen Rossiter and Prof. Richard Nichols for their guidance on my PhD project.

I would like to thank Dr. Rebecca Safran and Dr. Elizabeth Scordato, who organized the sampling and data collection of barn swallows in China and helped me with the morphological data analysis of Chinese barn swallows.

I am grateful to Prof. Zhang Zhengwang and colleagues of the avian research group in Beijing Normal University. With their support I accomplished the paternity test and part of the spectrophotometer test of my project.

In addition, a thank you to Jiao Qiao, Liang Tianjiao and Jiao Yuelang for their assistance during my field work. Also to Caroline Glidden and Rachel Lock, who had the sampling trip with me in China.

I wish to express my sincere thanks to my family, especially to my parents and Ma Tianran, and my friends, Li Nana and Liu Zetian, for their unceasing encouragement and support during my research.

Financial support was provided by the China Scholarship Council and Queen Mary, University of London.

Abstract

Sexual selection can be defined as the difference in reproductive success among individuals that is related to their ability to acquire mates and fertilizations. Mathematical models have shown that combined with natural selection, sexual selection can be an important driver for speciation. There have also been a few case studies showing that sexual selection alone can lead to speciation in nature. Over the past 30 years, the barn swallow has become a classic model animal for sexual selection. The barn swallow has at least two sexually selected traits in different subspecies: the length of tail streamers in European barn swallows, *H. r. rustica* and ventral plumage colour in north American barn swallows, *H. r. erythrogaster*. Meanwhile molecular research on barn swallows shows that these barn swallow subspecies are recently derived and thus the barn swallow becomes an ideal model animal to test the theory that sexual selection drives speciation. The Barn Swallow species complex is comprised of six closely related subspecies distributed throughout the Holarctic. Whereas experimental studies in Europe, the Middle East and North America have been conducted, little is known about populations distributed across Asia.

During my PhD study I collected barn swallow samples from more than 20 locations across China and compared the difference in morphology of Chinese barn swallow populations. The results show that in the northeastern part of China the barn swallow is intermediate in phenotype between subspecies in North America (*H. r. erythrogaster*) and subspecies in Europe (*H. r. rustica*), and is characterized by rusty ventral plumage colour and medium length tail streamers. Using morphological and colour traits, northeastern Chinese swallow populations can be separated from other Chinese populations, and this pattern of phenotypic variation may form under both natural and sexual selection. I also conducted an observational and experimental study on one of these populations, aiming to determine the extent to which variation in plumage color

and tail streamers is underlain by sexual selection. The observational study reveals that for male barn swallows in my study population, clutch initiation date, the number of both social and genetic offspring and the body condition of nestlings can be predicted by the colouration of their ventral plumage, while the experiment shows that the reproductive success of male barn swallows tended to increase with experimental enhancement on their plumage colouration. My research supports that the ventral plumage colour is the sexually selected trait in northeastern Chinese barn swallows, and further experiments are needed to clarify the effect of male ventral plumage colour manipulation on their breeding success with the limitation of small sample size in my study.

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General Introduction

Species and speciation

A species can be considered to be a population of organisms that has the following characteristics: 1. Monophyly: The individuals of one species have a common ancestor; 2. Cohesion: Within one species, all organisms must be genetically and ecologically cohesive, which means that they have the potential to interbreed (genetically) and share the same ecology environment (ecologically); 3. Separation: Different species are separated from each other morphologically, behaviourally or genetically; 4. Distinguishability: Every organism of one species can be distinguished from other species with single or multiple traits or from their DNA sequence (Stearns & Hoekstra 2000). Based on these criteria, the concept of species can be defined from different angles, like the biological species concept (BSC) in which species are defined as groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups (Mayr et al. 1963), or the phylogenetic species concept (PSC) in which species are defined as a monophyletic group composed of the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent (Cracraft 1983). Both these concepts of species have their shortcomings: the BSC is problematic when considering inter-species hybrids, e.g. the carrion crow (*Corvus corone*) and the hooded crow (*Corvus cornix*) are phenotypically distinct but have stable zones of hybridization, which confused their taxonomic status (de Knijff 2014); the PSC is restricted by the resolution of the sequencing methods, resulting in established species being split into more groups when the analysis is conducted at a high resolution and vice versa. Therefore, general species criteria should also be considered when we try to identify a group of organisms as one species, no matter which concept of species is being used.

A basic concept in evolution, speciation refers to the process by which reproductively isolated populations evolve to become distinct species. In the genic view, cumulative genetic differentiations eventually produce speciation: firstly, due to differences in selection a few genes might differentiate between populations that still have the ability to interbreed. Then after separation more genes start to differentiate, during which populations still have the chance to fuse. When populations diverge beyond the point at which they can fuse, they will have only limited hybridization and will share only a small portion of their genes via gene flow. Finally reproductive isolation is complete and different species stop sharing alleles at any part of their genomes through breeding (Wu 2001). It is commonly recognized that genes are the basic units of species differentiation and the process described above outlines the conceptualized stages of species differentiation and defines species on the basis of reproductive isolation at the genic level.

Allopatric, sympatric and parapatric speciation

At least three different modes of speciation are known: allopatric speciation, sympatric speciation and parapatric speciation. Allopatric speciation, speciation of populations isolated in space, develops with independent accumulation of genetic differences. In the allopatric model of speciation firstly populations are separated geographically, e.g. populations separated by water on distant islands. Exposed to divergent selection, populations evolve independently while genetic differences accumulate. Speciation occurs when reproductive isolation completes, after which even if these populations have the chance of secondary contact they cannot interbreed or their hybrids are sterile (Mayr et al. 1963). One iconic example of allopatric speciation is the finches on the Galápagos Islands, which inspired Darwin, the present species are descendants from a single ancestor species. On the Galápagos Islands about 14 different finch species are recognized, which presumably share one ancestor group of birds from South or Central America. After the population colonized at least one of the islands, ancestor birds dispersed onto other islands, on which ecological environments varied. Under the different selective forces on the Galápagos Islands, these birds differentiated into various species, despite interbreeding still being possible during the process (Grant 1999; Palmer & Kronforst 2015). Benefiting from the accelerated advance in genome sequencing technology nowadays, insights into the evolutionary genetics of Darwin's finches help to elucidate the molecular basis of speciation, e.g. whole-genome re-sequencing of 120 individuals representing all of the Darwin's finch species and two close relatives revealed the role of introgressive hybridization - the incorporation of alleles from one species into the gene pool of a second, divergent species (Harrison & Larson 2014) - throughout the radiation in rising species of mixed ancestry. Also introgression influenced the beak shape, one key phenotypic trait with huge diversity across different Darwin's finch species. The research also revealed that the *ALX1* gene, which affecting craniofacial development, contributes to the evolution process of beak shape diversity (Lamichhaney et al. 2015).

Different from allopatric speciation, in which populations are isolated geographically at the start of the speciation process, sympatric speciation occurs within a single population. In sympatric speciation, the population differentiates despite the existence of gene flow between the diverging groups. The sympatric speciation model assumes that some degree of genetic separation exists within a single population, then complete isolation between the diverging groups is formed by further differentiation and genetic separation. Though it has long been controversial whether sympatric speciation is likely and common, some iconic examples show that sympatric speciation does exist. One example includes the cichlids living in Lake Ejagham, Cameroon. In this small lake with a surface area of about 0.49 km^2, five forms of cichlids of the genus *Tilapia* are identified. This species flock is very young and originated from a common ancestor that came from an adjacent river approximately 10,000 years ago. Two forms of these cichlids seem to be in the process of speciation, with differences in body size, habitat depth and eye size and similarities in other morphological traits. Though their utilization of different food resources makes them differentiate in habitat depth, inshore and pelagic respectively, their breeding overlaps both in time and space. Highly restricted gene flow was found in the analysis on the microsatellite polymorphisms between these two forms and this reproductive isolation appeared to be achieved by size assortative mating (Schliewen et al. 2001). Recent studies showed that for *Tilapia* in Lake Ejagham, strong assortative mating by diet, colour, size and morphology occur, however the speciation progress is quite limited (Martin 2013). Also with next-generation sequencing (NGS), genotyping single nucleotide polymorphisms (SNPs) of crater lake cichlid fishes in Cameroon showed that gene flow from allopatric species may also facilitate sympatric speciation (Martin et al. 2015).

Allopatric speciation is more general than sympatric speciation, e.g. studies on putative sister species showed that in 309 speciation events 9.4% of them resulted in sister species with over 90% contemporary range overlap, which may be candidates for sympatric speciation (Bolnick & Fitzpatrick 2007). While allopatric speciation happens at a higher frequency, even treated as a null model in research, sympatric speciation should not be neglected despite difficulty in demonstrating it.

Parapatric speciation refers to the speciation occurring in adjacent populations sharing a border, and unlike allopatric speciation in this scenario gene flow may exist between populations. As an intermediate type of speciation between allopatric speciation and sympatric speciation, parapatric speciation can start in either way: parapatric speciation can be basically allopatric, in which daughter population accumulates genetic changes geographically isolated from parental population and then expands until it meets with the parental population; or parapatric speciation can form with range expansion in the shape of a ring, during which populations go under differential selection and suffer from reproductive isolation when crossed, though across the whole range contiguous populations can interbreed. For instance, greenish warbler (*Phylloscopus trochiloides*) has two geographically adjacent but reproductive isolated subspecies, *viridanus* and *plumbeitarsus*. These two Siberian subspecies are connected by a long chain of intergrading populations circling around the Tibetan Plateau, while they do not recognize each other's songs, which was inferred as the result of parallel selection pressure on the song complexity and structure (Irwin, Bensch & Price 2001).

Sexual selection is an important driver for speciation

In the book *The Descent of Man and Selection in Relation to Sex*, Charles Darwin proposed the idea of sexual selection as competition over mates that can cause differences in reproductive success (Darwin 1871). Sexual selection is defined as "the differences in reproduction that arise from variation among individuals in traits that affect success in competition over mates and fertilizations" (Andersson 1994).

In organisms in which females invest more in reproduction than males, sexual selection can operate via two types of processes: male-male competition and female choice. In male-male competition, intrasexual selection, stronger males that have an advantage in combating other males gain greater reproductive success. One case study of male-male competition is the red deer *Cervus elaphus*, in which the reproductive success of males largely depends on their mating success (Clutton-Brock 1988). Stags holding larger antlers or holding antlers longer have stronger fighting ability and higher dominance rank within the population, obtaining larger size harems and obtaining harems longer thus they gain greater reproductive success than other stags (Clutton-Brock et al. 1979; Gibson & Guinness 1980).

Sexual selection also occurs between the two sexes, because females are choosy over their mates, selecting them on the basis of direct and/or indirect benefits. In the female choice process, females evaluate males through specific sexually selected traits and adjust their investment in reproduction according to the quality of their mates (Andersson 1994; Davies, Krebs & West 2012). One possible reason that females choose males as their mates based on particular traits is that these traits can reflect the quality of males ('honest signals'), which may due to the handicap brought to males by these traits ('Zahavi's handicap principle') (Zahavi 1975, 1977). These traits are reliable to females as they are costly for males either to produce or to maintain, particularly so for males of low quality. Thus, males of high quality are more able to afford the cost of these traits and benefit from acquiring greater reproductive fitness than males of low quality (Grafen 1990a, b). Driven by female choice, sexually select traits are typically exaggerated in males, for example, the tail of male long-tailed widowbirds *Euplectes progne* with a remarkable length or the elaborate train with numerous eye-spots of peacocks *Pavo cristatus* (Andersson 1982; Petrie, Tim & Carolyn 1991). Through choice, female animals may gain direct benefits from their mates like food resources or parental care on their offspring, as in North American bullfrogs *Rana catesbeiana* where the highest quality breeding territories are defended by the strongest males. Thus females prefer these males with the highest quality territories and benefit from good laying sites which are optimal for embryo development (Howard 1978a, b). In red-winged blackbirds *Agelaius phoeniceus*, experienced males which take better care of their young are preferred by females and have more offspring and offspring in better condition (Muldal, Moffatt & Robertson 1986; Yasukawa 1981). Besides direct benefits females may also achieve indirect benefits with choice, like giving birth to sexually attractive offspring or offspring of high genetic quality, especially in species that females get nothing from males but sperm to fertilize their eggs. One case study is from natural populations of *Colias* butterflies, where older and more discriminating females prefer males with certain phosphoglucose isomerase (PGI) enzyme genotype, which confers an advantage in diverse fitness-related properties like flight capacity, survivorship and mating success (Watt, Carter & Donohue 1986).

Fisher-Lande Coevolution

The Fisherian runaway sexual selection process assumes that females prefer a particular male trait that indicates male quality and is genetically heritable. Thus a positive feedback loop is created between the sexually selected trait and female preference for that trait, producing extravagant exaggeration in the trait until survival costs halt further exaggeration (Fisher 1930). This basic model, sketched by Fisher and specified by Lande, describes the coevolution between a sexually selected male trait and female preference for it (Lande 1981). Besides the unstable outcome (known as the runaway process), Fisher-Lande process (FLP) can also result in a stable (walk-toward) outcome which requires fewer extreme genetic assumptions and has a better chance to happen than the runaway process. Meanwhile, the restrictive conditions under which the runaway process may happen have been clarified (Hall, Kirkpatrick & West 2000). Operating along with other forms of selection, the Fisher-Lande process has the potential to facilitate sympatric speciation and likely allopatric speciation, though the latter has not been modeled explicitly. Examples representing the Fisher-Lande process leading to speciation could be the long feathers of particular birds, e.g. the birds of paradise. The ornaments of birds of paradise, family Paradisaeidae, are considered to be a prominent example of sexual selection and they show a complex biogeographical distribution. These birds develop and advertise remarkable elongated and elaborate tail feathers of great variety, with which males display complex courtships and often showing hidden features of their plumage. Phylogenetic research on birds of paradise revealed that the extraordinary diversification in male plumage and courtship displays is the result of evolution over about 26 million years, which does not support the view that sexually selected traits evolve particularly rapidly (Irestedt et al. 2009). Comparison between the patterns observed in birds of paradise and predictions of basic FLP model of sexual selection supported that the sexual radiation of birds of paradise can be explained with the FLP model under restrictive conditions (Arnold & Houck 2016).

Reminiscent of Fisher's runaway process, the chase-away process is the result of sexual conflict between the two sexes may also facilitate speciation. Sexual conflict, "a conflict between the evolutionary interests of individuals of the two sexes (Parker 1979)", results in females perpetually evolving to resist male traits and decrease the mating rate while males evolve to increase their mating rate. Under these assumptions, if different resistance strategies form in females, males may chase each of them and sexual isolation may be facilitated between populations (Gavrilets & Waxman 2002).

Assortative Mating

In sympatric speciation or during the reinforcement process after allopatric divergence, assortative mating may become the force to drive speciation along with disruptive selection. Assortative mating refers to female choice of male phenotypes that match themselves or type of specific traits that are preferred by females. Depending on the distribution of phenotypes, traits or preferences in the population, e.g. if the distribution is skewed in different directions by ecological niche divergence, assortative mating can produce selection patterns that act in different directions in diverging populations (Servedio 2016). On the contrary, in a model that females prefer a trait that indicates the quality of males and ecological disruptive selection exists, no divergence of mating preferences is needed as natural and sexual selection work in concert to achieve local adaption and reproductive isolation (van Doorn, Edelaar & Weissing 2009). One flagship case study about assortative mating facilitating speciation is the research on African cichlids (genus *Haplochromis*) especially cichlids from Lake Victoria in central Africa. Lake Victoria was completely dry less than 13000 years ago, after it refilled 500-1000 cichlids species have evolved in the lake. For sympatric species, male always differ in colour, and colour is always the sexually selected trait, while females do not have bright colours. As many closely related species are distinguished by colour, sexual selection is considered as the main driving force behind this. Phylogenetic analyses on the cichlid colour patterns against a wider assemblage of cichlids revealed that male nuptial colour is most likely driven by sexual selection while the evolution of bar patterns is associated with cichlid ecological habitat (Burress 2015; Van Alphen 1999). Sequencing genomes and transcriptomes of five lineages of African cichlids provided the evidence that neutral processes seem to be crucial to amassing genomic variation, while adaptive processes may be important in facilitating subsequent evolutionary diversification (Brawand et al. 2014).

The barn swallow: a classic and ideal animal for speciation research

The barn swallow *Hirundo rustica* is a small passerine that feeds on insects in the air. This Holarctic bird has a wide distribution on all continents except Antarctica and is commonly recognized by people all over the world (Scordato & Safran 2014; Turner 2006). Except for a few non-migratory populations, every year in the spring barn swallows migrate from southern wintering sites to their northern breeding sites, and for adults they usually return to the site they used the previous year (Turner 2006). Males always arrive at the breeding sites earlier than females and after arrival they search for suitable nesting sites, usually artificial structures such as cowsheds, corridors or even stairwells ((Turner 2006), also see General Methods). After finding their nesting sites, males will defend them while attracting the attention of females with courtship singing and display. The barn swallow is monogamous and after a male successfully acquires a mate they will build a new nest or refine an old nest together with little pieces of mud (Turner 2006). After fertilization, female barn swallows usually lay one egg each day and lay three to six eggs in total (Turner 2006). Whether males help to incubate eggs differs among different populations and after about two weeks of incubation nestlings hatch out. Nestlings are fed by both parents for about three weeks before fledglings leave their nests, and after leaving their nests fledglings may still be fed by parents for a few days (Turner 2006). Barn swallows may have one to three clutches every year, after which they will migrate back to their southern wintering sites (Pagani-Núñez et al. 2016; Turner 2006).

Along with its wide distribution, the barn swallow varies in morphology among different populations, particularly in body size, streamer length, ventral plumage colour and chest bands (Table 1). Six subspecies are usually recognized in the barn swallow: the European subspecies *H. r. rustica*, the Egyptian subspecies *H. r. savignii*, the Middle East subspecies *H. r. transitiva*, the northern Asian subspecies *H. r. tytleri*, the southern Asian subspecies *H. r. gutturalis* and the North American subspecies *H. r. erythrogaster*. The nominate *H. r. rustica* Linnaeus, 1758 breeds in Europe, North Africa, the central Himalaya and western China. *H. r. rustica* is large in size with long streamers, pale ventral plumage and a full breast band. *H. r. savignii* and *H. r. transitiva* are two sedentary subspecies and they have relatively large body size, long streamers, dark ventral plumage and full breast bands, though *H. r. savignii* is darker in plumage colour than *H. r. transitiva*. *H. r. gutturalis* occurs from the eastern Himalaya, southern, central and eastern China to Korea and Japan and has the smallest body size and shortest tail streamers among all subspecies, along with pale ventral plumage and narrow breast band. *H. r. tytleri*, the northern Asian subspecies, typically breeds in the Baikal region and has intermediate body size, intermediate streamer length, dark plumage colour and narrow breast band. *H. r. erythrogaster*, the North American subspecies, has streamers intermediate in length to other subspecies, dark ventral plumage and discontinued breast band. A phylogenetic hypothesis for the six barn swallow subspecies based on mitochondrial DNA sequencing supports that different subspecies share a common African ancestor to which *H. r. savignii* and *H. r. transitiva* are closest relatives. During the expansion throughout Eurasia in barn swallows, *H. r. rustica* and *H. r. gutturalis* diverged via geographic isolation. About 100,000 years ago barn swallows from the Asian populations crossed the Bering Strait and established the North American populations, giving rise to the *H. r. erythrogaster* subspecies. Similarly, about 27,000 years ago North American barn swallows re-crossed the Bering Strait and colonized the Baikal region, giving rise to the *H. r. tytleri* subspecies (Dor et al. 2010; Zink et al. 2006). At least three subspecies occur in China: *H. r. rustica* breeds in the western part of China, particularly in Xinjiang Province and Xizang Province; *H. r. tytleri* is encountered during migratory in northeastern part of China, particularly in Heilongjiang Province; and *H. r. gutturalis* breeds in other parts of China with the widest range. Meanwhile a potential fourth subspecies is also recorded as *H. r. mandschurica*, especially in Heilongjiang Province (Zheng 1987). Recently *H. r. mandschurica* is considered as an intergrading form of *H. r. tytleri* and *H. r. gutturalis* based on restriction-site associated DNA sequencing (RADSeq) analysis (Safran & Scordato, unpublished data), and in this thesis I use the term *H. r. mandschurica* to stand for these *tytleri-gutturalis* intergrade populations in the northeastern part of China.

Table 1 Morphological differences among six barn swallow subspecies. Differences in body size, streamer length, ventral plumage colour and chest band shape are listed (Møller 1994c; Scordato & Safran 2014; Turner 2006).

	Body size	Streamer length	Ventral colour	Chest band
H. r. rustica	large	long	pale	Thick, connected

H. r. savignii	relatively large	long	dark	Thick, connected
H. r. transitiva	relatively large	long	dark	Thick, connected
H. r. gutturalis	small	short	pale	Narrow, broken
H. r. tytleri	intermediate	intermediate	dark	Narrow, broken
H. r. erythrogaster	intermediate	intermediate	dark	Narrow, broken

European barn swallows: tail streamer as the sexually selected trait

Inspired by the sexual dimorphism in the length of tail streamers, many studies have shown that the tail streamer is the sexually selected trait in European barn swallows *H. r. rustica* (Møller 1994c). A series of tests on the flight performance of barn swallows with streamer length manipulation have suggested that natural selection also acted on streamer morphology: in both sexes sexual selection has extended streamer length by around 10 mm beyond its optimum length for flight, which should be the result of natural selection (Buchanan & Evans 2000; Evans 1998; Evans 1999; Evans & Thomas 1997; Hedenström & Møller 1999). There is a strongly positive relationship between the tail length of male barn swallows and that of their male offspring, suggesting that the length of tail streamers shows a statistically significant heritability (Møller 1991a). First reported in 1988 by Møller, it was shown that males with experimentally elongated tail streamers can obtain mates earlier than males with shorter tails, thus they are more likely to have second clutches and have a greater reproductive output during the whole breeding season, meanwhile these males tend to have a higher chance of extra-pair copulations (Møller 1988). Empirical studies showed that male swallows with naturally longer streamers arrive at breeding sites earlier than males with shorter tails, they are more likely to acquire their mates and find their mates more quickly, and benefit from an earlier start to breeding, which allows raising more nestlings and having second clutches within a single season (Møller 1990b). Early arrival indicates that long tailed swallows have high body condition, which have good migratory performance and can survive the potential costs of early arrival such as poor environment conditions in early spring, thus tail streamers in male barn swallows are an “honest signal” chosen by females (Møller 1994b). This is consistent with the finding that viability is positively related to the streamer length in male barn swallows, particularly among unmated and yearling male swallows (Møller 1991b).

Females prefer long tailed males and invest more in breeding with these males, in addition to the advantage in seasonal number of clutches, males with elongated streamers also have more nestlings in single broods compared with males with shortened streamers. Along with a larger number of offspring in each breeding season, long tailed male swallows also produce offspring of high quality, e.g. with partial cross-fostering experiments it was shown that offspring of male swallows with long tail ornaments have smaller loads of parasitic mites (Møller 1990a). Also, there is a strong positive relationship between offspring longevity and tail length of their fathers while there is a negative relationship between relative male provisioning and male tail length, further supporting the view that females gain an indirect fitness advantage in offspring quality through mate choice (Møller 1994a). Females mated to males with elongated tails also have significantly higher feeding rates than those mated to males with shortened tails, which is considered as a compensation to the reduced foraging ability by males with long tails on (De Lope & Møller 1993). As a monogamous bird, female barn swallows also prefer males with long tails as extra-pair mates (Møller 1988), and paternity analysis using multi-locus DNA fingerprinting showed that the proportion of extra-pair offspring is negatively correlated with the tail length of the male swallow attending the nest, indicating that long tailed males are more sexually attractive to females (Møller & Tegelström 1997).

American barn swallows: ventral plumage colour as the sexually selected trait

Unlike European barn swallows *H. r. rustica,* which have long tail streamers and pale ventral plumage colour, North American barn swallows *H. r. erythrogaster* have short tail streamers and dark ventral plumage colouration. Barn swallows colour their ventral plumage brown with melanin pigments which can be synthesized from amino acids by animals themselves instead of acquiring them from diet like carotenoids, the other main pigments in animal colouration (McGraw et al. 2004). However, the expression of this melanin-based visual signal can be costly in other ways, e.g. in natural conditions darker *H. r. erythrogaster* males have higher androgen concentrations than other males and colour manipulation experiments show that enhancement in ventral plumage colouration can increase the concentrations of circulating androgens and lighten the body mass of barn swallows (Saino et al. 2013a), and male swallows with darker ventral plumage colour have lower next-year viability compared with those with paler plumage, supporting the view that the response of this visual signal expression can be costly through bidirectional feedbacks between sexual signal and physiological condition (Safran et al. 2008). The concentration of two melanin forms, pheomelanin and eumelanin, can predict the colouration of ventral plumage, which is highly heritable in both *H. r. rustica* and *H. r. erythrogaster* subspecies (Hubbard, Jenkins & Safran 2015; Saino et al. 2013b). Along with diverging ventral plumage colour phenotypes, pigmentation strategies vary geographically among different subspecies, e.g. for *H. r. erythrogaster* males the concentration of pheomelanin is not significantly positively correlated with the concentration of eumelanin (McGraw, Safran & Wakamatsu 2005), and this pattern is different from that of *H. r. rustica* (Saino et al. 2013b).

In *H. r. erythrogaster*, it has been found that plumage colouration can predict the assortative mating patterns and annual number of fledglings produced by a male, suggesting that plumage colouration is the sexually selected trait in *H. r. erythrogaster*

(Neuman, Safran & Lovette 2007; Safran & McGraw 2004), but also see (Kleven et al. 2006). Male *H. r. erythrogaster* with naturally dark ventral plumage colour have higher testosterone concentration and suffered less from cuckoldry than males with pale colour, though dark males do not have higher extra-pair or total reproductive success than pale males (Eikenaar et al. 2011). Similarly an egg removal experiment showed that males with artificially enhanced ventral plumage colouration can gain greater paternity from their social mates while males in the control group did not, indicating that ventral plumage colour, as an sexually selected trait, is under dynamic selection from female choice (Safran et al. 2005). Also in *H. r. erythrogaster* the offspring of males with dark plumage receive more provisioning from females, supporting the differential allocation theory that females give more care to offspring when mated with high quality males (Maguire & Safran 2010).

Sexually selected traits in other barn swallow subspecies

The Middle East barn swallow subspecies *H. r. transitiva*, a subspecies possessing both elongated long tail streamers and dark ventral colouration, combines tail streamer length and ventral plumage colour as their sexually selected traits - empirical studies showed that both streamer length and ventral colour can predict their annual breeding success, e.g. the total number of young raised successfully over the entire breeding season (Vortman et al. 2011). This result was confirmed with phenotype manipulation experiments, in which males with both tail elongation and colour darkening were favoured by females and suffered less from cuckoldry (Vortman et al. 2013). Both traits, ventral colouration and tail streamer length, are heritable in *H. r. transitiva*, meanwhile the relative allocation towards one or the other trait is strongly correlated between fathers and sons. This result supports that the genetic strategy for resource allocation among multiple sexual signals may be under selection during the evolutionary process

(Vortman et al. 2015).

For the southern Asian subspecies *H. r. gutturalis*, no significant positive relationship was found between male streamer length and the number of extra-pair young sired, indicating that the length of tail streamers plays no role in sexual selection in *H. r. guttrualis* (Kojima et al. 2009). Further observational analysis showed that red throat patch and the white spot on the tail may be the sexually selected traits in this subspecies as males with less saturated throat plumage and larger white spots in the tail had an advantage in breeding time over other males (Hasegawa et al. 2010b). However, it was also reported that males with more pheomelanin, which is positively correlated with feather colour saturation (McGraw et al. 2005), bred earlier than other males (Arai et al. 2015). Males with large throat patches acquired more experienced and heavier females with higher viability as their mates and again this result was in accordance with differential access hypothesis that males with more exaggerated sexually selected trait can acquire females of higher quality and increase their reproductive fitness than other males (Hasegawa & Arai 2013a). Males with more colourful throat patches also tended to occupy territories of higher quality compared with their less colourful neighbours while male feeding rate was negatively correlated with the colourfulness of male throat patch (Hasegawa et al. 2014a). Further a trait manipulation experiment showed that males with artificially reduced throat patches invested more in provisioning compared with individuals in the control group (Hasegawa & Arai 2015). Unlike tail streamer length (measured in this study as fork depth), the red throat patch and white tail spot have a clinal variation along latitude among *H. r. gutturalis* populations, which could be explained by latitudinal clines of sexually selected advantages (Hasegawa & Arai 2013b). The size and colouration of throat patches, along with tail length could predict the viability of male *H. r. gutturalis* (Hasegawa et al. 2014b).

In summary, as a classic example of sexual selection, both empirical and experimental studies have shown that the length of tail streamers is positively related to the reproductive success in male European barn swallows (Møller et al. 1998; Møller 1994c). Extensive studies have shown that tail streamer and ventral colour are both sexually selected traits which act separately in two barn swallow subspecies, *H. r. rustica* and *H. r. erythrogaster* (Scordato & Safran 2014). To demonstrate a detailed evolutionary history of the barn swallow subspecies complex, more behavioural studies are required to investigate variation in sexually selected traits in other barn swallow populations. Especially for Asian barn swallows (*H. r. gutturalis*, *H. r. tytleri*), little is known about their sexual selection strategies, which is a gap between previous sexual selection studies on European and North American populations. Also benefiting from the advantage of genome sequencing techniques, it is promising that a high resolution phylogenetic analysis on the subspecies complex can be done in the near future. If we can combine the phylogeny of the barn swallow subspecies complex with the behaviour studies, it will help to reveal how natural selection and sexual selection act together on the formation of the barn swallow subspecies complex.

In this thesis, I aim to test the sexual selection strategy of northeastern Chinese barn swallows *H. r. mandschurica*. Firstly, barn swallow samples were collected from more than 20 sites across China and the morphological variation of Chinese barn swallows was tested, especially the difference between *H. r. mandschurica* and other populations (Chapter I). Then I tested if there was a correlational relationship between the reproductive success of barn swallows and their potential sexual signals in *H. r. mandschurica* with an observational study, both considering their social offspring (Chapter II) and genetic offspring (Chapter III). I predicted that ventral plumage colour, not tail streamers, would be the sexually selected trait in this population and that the ventral colour of male barn swallows would predict their reproductive fitness.

Following the observational study, a trait manipulation experiment was conducted on male ventral colour to determine the mechanism of sexual selection in *H. r. mandschurcia*. If the ventral colour is the sexual signal in this population, males are expected to gain greater reproductive success, e.g. more offspring with an artificial enhancement in their ventral colour.

Chapter I. Phenotype variation in barn swallows across China

Abstract

Phenotype will be affected by the interaction between genotype and environment, and both natural and sexual selection may drive the divergence in phenotype between different populations of the same species. The barn swallow subspecies complex consists of about six subspecies distributed on all continents except the Antarctic, and different subspecies vary extensively in morphology, especially in the streamer length and ventral plumage colour, which are also two potential sexually selected traits in the barn swallow. In this study I tested the morphological variation between different barn swallow populations in China, including two confirmed subspecies, *H. r. rustica* and *H. r. gutturalis*, and one potential subspecies *H. r. mandschurica*. I found that different populations can be separated using morphological data, and the formation of the phenotype variation could be due to both natural selection and sexual selection.

Introduction

Natural and sexual selection are two forces that can drive speciation, including morphological divergence between sister species (Ritchie 2007). For instance, Darwin's finches vary in beak size and beak shape among different species, which is considered the result of local adaptation to food sources, while the varying elaborate and elongated feathers of birds of paradise (Paradisaeidae) are inferred to be the result of sexual selection (Frith & Beehler 1998; Grant 1999). During the process of speciation, while disruptive selection can increase phenotype variation and assortative mating can reinforce this process, gene flow between populations can weaken the phenotype variation by reproducing individuals of intermediate phenotypes anew every generation (Rueffler et al. 2006; van Doorn et al. 2009). However, the relative significance of natural selection and sexual selection on speciation remains largely unknown with few case studies. Meanwhile phenotype is considered as the result of genotype-environment interaction (Via & Lande 1985). Due to genetic variation and geographic differences continuous phenotypic variation may exist, e.g. the shells of snails show pronounced clinal phenotypic variation along a latitudinal gradient (Trussell 2000). Similarly, it remains unknown how different sexual selection pressures work on the formation of continuous variation in phenotypes.

The barn swallow (*Hirundo rustica*) is a small passerine with a wide distribution on all continents except Antarctica (Turner 2006). Among different populations phenotypes vary extensively, mainly in traits like streamer length, ventral plumage colouration, body size, chest band, etc. For instance, European barn swallows (*H. r. rustica*) have long tail streamers and pale ventral plumage colour, while the streamers of North American barn swallows (*H. r. erythrogaster*) are relatively short and the plumage colour is dark and rusty. Correspondingly, different subspecies of barn swallows use different traits as sexual signals: *H. r. rustica* is treated as a classic example of sexual selection with considerable research on the relationship between the breeding success and male streamer length, however for *H. r. erythrogaster* males with darker ventral plumage colour gain greater reproductive success than males with less colourful plumage (Møller 1994c; Safran & McGraw 2004). Besides sexual selection, ecological factors like food resources or climate conditions can simultaneously contribute to the variation in expression of sexually selected traits within or among populations (Safran et al. 2013). To understand phenotype variation among different barn swallow populations, a careful quantitative analysis of various morphological features is needed. Six subspecies of barn swallow are commonly recognized (General Introduction, Table 1), of which two barn swallow subspecies breed in China: *H. r. rustica* in the west part of China, *H. r. gutturalis* in most other places, while *H. r. tytleri* is encountered during migration (Cheng 1987). Besides the six commonly recognized subspecies, the distributions of another two potential subspecies, *H. r. saturata* and *H. r. mandschurica,* are close to China - in Russia. In history *H. r. saturata* and *H. r. mandschurica* have been regarded as synonyms for *H. r. erythrogastyer* or *H. r. gutturalis* (Dickinson & Dekker 2001; Dickinson, Eck & Milensky 2002). The seventh potential subspecies, *H. r. mandschurica*, is recorded in the northeastern part of China (Zheng 1987). However, though this potential subspecies has been proposed, no comprehensive sampling has been done to investigate its status as a separate cluster among the barn swallow subspecies complex. In this chapter I use the term *H. r. mandschurica* to refer to these northeastern Chinese barn swallow populations. A comprehensive phenotype analysis is presented here from an extensive sampling transect across China. I explore patterns of sexual dimorphism within populations and the extent of trait variation among closely related populations using several quantitative measures of morphological variation. Using these data, I test the extent to which phenotypes are different among Chinese barn swallow populations especially for *H. r. mandschurica* populations, and what factors, e.g. natural selection or sexual selection pressure may play a role in the formation of phenotype divergence across a large distribution of one species.

Methods

Fieldwork methods

From April to June 2014 and May to June 2015, I collected samples from 23 cities within China (Figure 1.1). In the western part of China, I considered three populations (WLMQ, GEM, DH) to be subspecies *H. r. rustica*, and five populations (YM, JQ, ZY, GT, WW) to be hybrids between *H. r. rustica* and *H. r. gutturalis*. Two populations (SYS, QQHE) from northeastern China were predefined as *H. r. mandschurica* while all other populations were classified as *H. r. gutturalis*. Swallows were caught with mist nets near their nests, either during the daytime or at night. I took standard measurements on swallows after I banded them with numbered aluminum rings, and the time and location of the capture was recorded. I collected quantitative data on seven morphological traits including tarsus length, beak length, beak width, beak depth, wing length, streamer length and body weight. Tarsi and beaks were measured with vernier calipers to the nearest 0.05 mm, and body weight was measured with a spring balance (Pesola, Switzerland) to the nearest 0.01 g. I measured the right-wing length and the lengths of both streamers of each bird to the nearest 0.5 mm with rulers. All length measurements were taken three times (repeatability: 2014 data: $r = 0.82 – 0.99$, all $p < 0.01$ (Lessells & Boag 1987).) and results were averaged for the analysis. Blood samples (about 70 μL from each bird) were collected with capillaries by brachial venipuncture. Feather samples were plucked from throat, breast, belly and vent regions of each bird.

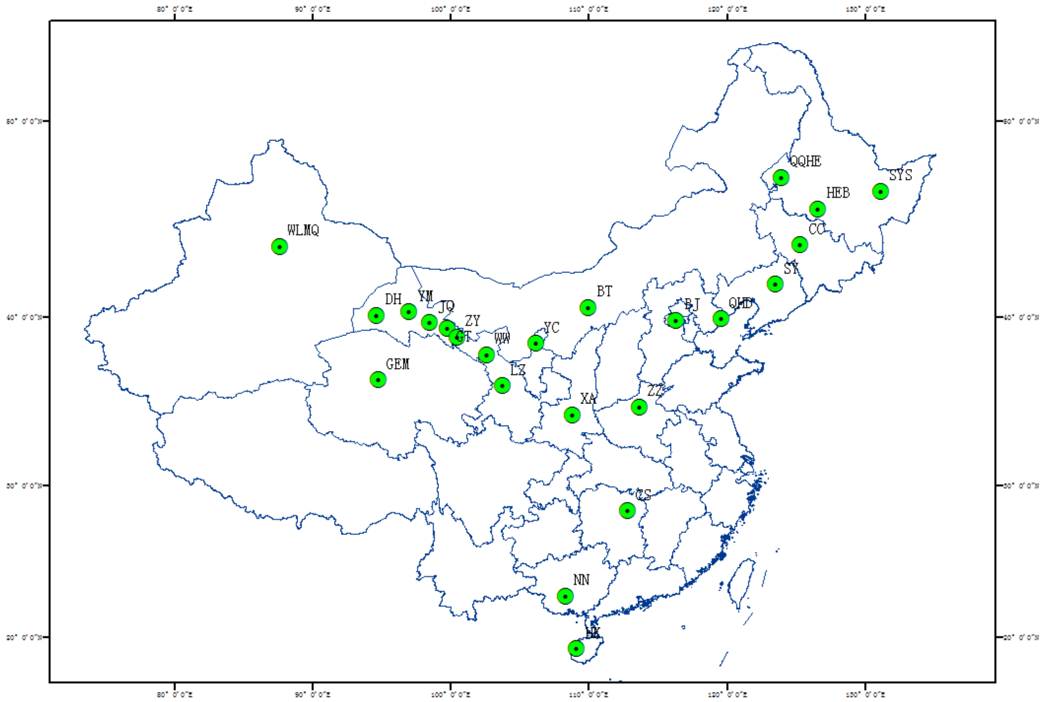

Figure 1.1 Sampling sites of barn swallows in China. Morphological data of barn swallows were collected from 23 sites across China.

Plumage colour measurement

Feather samples from different body regions were mounted on index cards separately and stored in the dark for later measurement. I measured feather samples with a reflectance spectrometer (USB4000, light source: PX-2, Ocean Optics, US). Each feather patch was measured three times and the spectra were averaged for scoring. I calculated colour variables according to three dimensions of colour vision: hue, brightness and chroma. Calculation of colour variation is based on spectra with wavelengths between 300 and 700 nm, which are the range of spectral sensitivity in birds (Cuthill et al. 2000). Hue is represented with the wavelength corresponding to the maximum reflectance; red chroma is the proportion of total reflectance occurring in the red region (600 – 700 nm) as a measure of spectral saturation; and brightness is the mean reflectance over the spectra. Though no particular dimension is used to describe reflection in the ultraviolet (UV) range, the barn swallow plumage reflects little in the UV so no colour information loses with the HSB colour space (Safran & McGraw 2004).

Statistical analyses

Firstly, the correlations among each morphological and colour trait were calculated using Pearson's correlation analysis in male and female barn swallows separately, which enabled me to choose a subset of representative morphological and colour traits. Then I applied Levene's test to examine whether the variances of traits in male and female barn swallow were equal and I tested whether these traits were sexually dimorphic using generalized linear mixed-effect models (GLMMs) with sex as a fixed factor and population as a random factor.

To assess the extent to which various morphological and colour traits vary among populations, I analysed population differences using post hoc analyses following ANOVAs. By means of Tukey's test I made multiple comparisons of all populations and divided populations into different levels by $\alpha = 0.05$. Traits in males and females were tested separately to avoid statistical error caused by sex bias. Also for potential hybrid populations between *H. r. rustica* and *H. r. gutturalis*, and between *H. r. gutturalis* and *H. r. mandschurica*, I tested whether linear variation exists among various morphological and colour traits.

Additionally, I analysed standardized trait differences between all populations using an unbiased effect size metric, ΔP (Safran et al. 2012). Using ΔP values, I applied a Mantel test to analyse whether trait divergence among populations is correlated with geographic distance.

Finally, I examined the accuracy with which the different subspecies of barn swallow within China can be separated using morphological traits, colour traits or all traits using discriminant function analysis (DFA) for male or female barn swallows separately.

Results

For morphological traits, bill length, width and depth were correlated (Pearson correlation: male: coefficient = 0.24 – 0.42, n = 213, all $p < 0.01$; female: coefficient = 0.21 – 0.23, n = 216 – 217, all $p < 0.01$). For colour traits, brightness, hue and chroma were correlated with each other respectively among breast, belly and vent regions (Pearson correlation: male: brightness: coefficient = 0.56 – 0.73, n = 213, all $p < 0.01$; hue: coefficient = 0.20 – 0.30, n = 213, all $p < 0.01$; chroma: coefficient = 0.66 – 0.78, n = 213, all $p < 0.01$; female: brightness: coefficient = 0.56 – 0.66, n = 217, all $p < 0.01$; hue: coefficient = 0.33 – 0.37, n = 217, all $p < 0.01$; chroma: coefficient = 0.66 – 0.76, n = 217, all $p < 0.01$). Therefore, I used bill length to represent bill length, bill width and bill depth and breast colour traits to present breast, belly and vent colour traits in further analysis. The colours of the different body regions did not correlate with each other (Pearson correlation: male: belly brightness – belly hue: coefficient = -0.07, n = 213, $p = 0.30$, belly hue – belly chroma: coefficient = -0.04, n = 213, $p = 0.57$; female: belly hue – belly chroma: coefficient = -0.08, n = 217, $p = 0.26$), and all three measures (hue, brightness, chroma) were tested separately in following analyses. The result of all correlation tests is shown in Table S1.

Trait Variance and Sexual Dimorphism

The mean values of all morphological and colour traits are shown in Table 1.1.

Variances for streamer length and body mass were not equal between males and females (Levene's test: streamer length: F = 20.24, $p < 0.01$; body mass: F = 27.07, $p < 0.01$), while variances for other morphological traits were not significantly different (Levene's test: F = 0.28 – 0.94, $p = 0.33 – 0.59$). For colour traits, variances for throat brightness and breast chroma were significantly different between the two sexes (Levene's test: throat brightness: F = 4.62, $p = 0.03$; breast chroma: F = 5.38, $p = 0.02$) while others were not (Levene's test: F = 0.31 – 3.39, $p = 0.07 - 0.58$). For morphological traits, GLMM using sex as a fixed factor and population as a random factor revealed sexual dimorphism in wing length, streamer length and body mass (all $p < 0.01$) but not in tarsus length and bill length ($p = 0.15 – 0.67$). For colour traits, throat brightness, throat hue, breast brightness and breast chroma were sexually dimorphic (GLMM: all $p < 0.05$) while throat chroma and breast hue were not (GLMM: $p = 0.11 – 0.20$).

Table 1.1 Mean value of each trait and sexual dimorphism. Last two columns are coefficients and *p* values obtained from GLMMs using sex as a fixed factor and population as a random factor.

		Male			Female				
		mean value	SD	n	mean value	SD	n	coefficient	*p*
Morphological traits									
wing		119.79	4.24	213	117.88	4.72	217	1.66	<0.01
streamer		101.15	9.92	211	85.01	7.13	217	15.89	<0.01
tarsus		10.48	0.52	213	10.53	0.44	217	-0.06	0.15
bill length		5.57	0.32	213	5.60	0.40	217	-0.01	0.67
weight		16.10	1.45	211	17.43	2.25	217	-1.45	<0.01
Colour traits									
throat	brightness	8.51	2.14	212	10.49	3.42	216	-1.91	<0.01
	hue	673.76	12.82	212	670.06	14.92	216	3.86	<0.01
	chroma	0.54	0.02	212	0.52	0.03	216	-0.003	0.20
breast	brightness	44.41	10.89	213	46.33	9.90	217	-1.50	0.05
	hue	619.46	29.76	213	616.20	28.94	217	4.31	0.11
	chroma	0.36	0.04	213	0.35	0.009	217	-2.18	<0.01

Trait Differences among Populations

Morphological Traits

In general, western Chinese barn swallows were larger in body size with significantly longer wings, longer streamers, longer tarsi, longer bill and greater body mass than central or eastern Chinese barn swallows (Table S2, Figure 1.2). For wing length, western Chinese barn swallows (DH, GEM, WLMQ, JQ, ZY) were significantly longer than northeastern ones (SY, SYS, QHD, HEB, CC) and one southern population (NN) both in males and in females. For streamer length, males in two (WLMQ, DH) and females in one (WLMQ) population were significantly longer than other populations from central or eastern part of China. For body mass, males in two western populations (WLMQ, DH) were significantly heavier than central populations (YC, WW, LZ, XA, ZZ, BT) and those in these central populations were significantly heavier than northeastern populations (CC, HEB, QHD, SY) and one southern population (NN), while females in two western populations (YM, WLMQ) were significantly heavier than other populations from central or eastern China. For tarsus length, males showed no significant differences between populations, while females in one western population (WLMQ) were significantly longer than some other populations (ZZ, HEB, SYS, HK, LZ, CS, QHD, NN, CC, BJ). In neither sex did bill length show any significant differences between populations.

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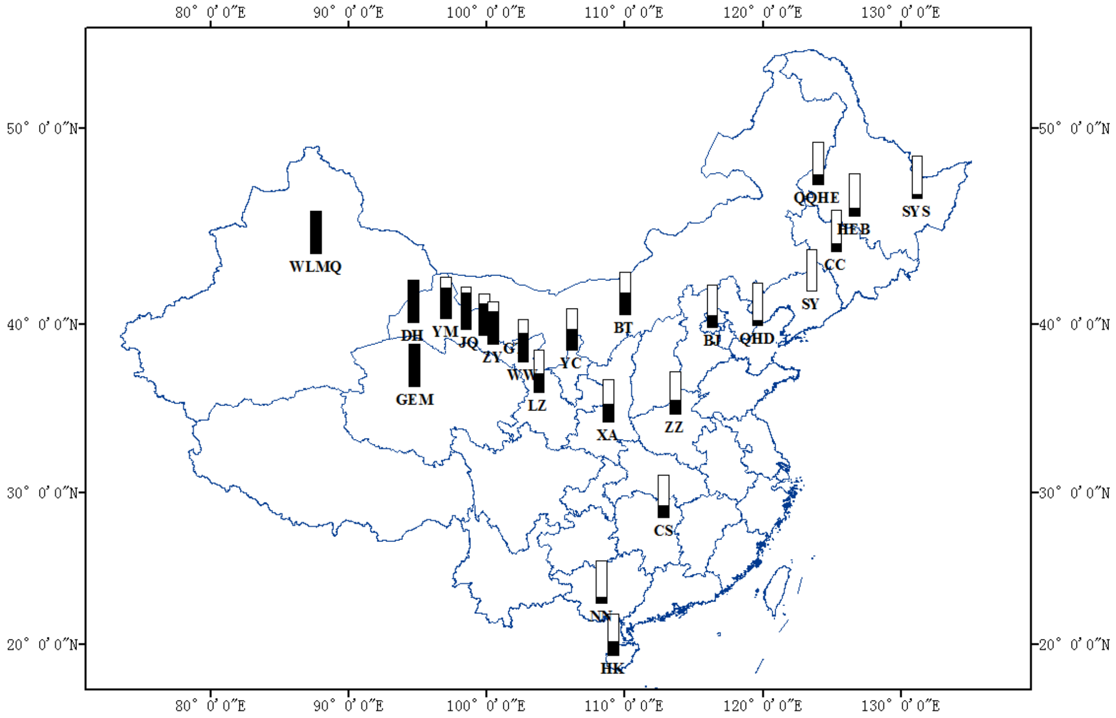
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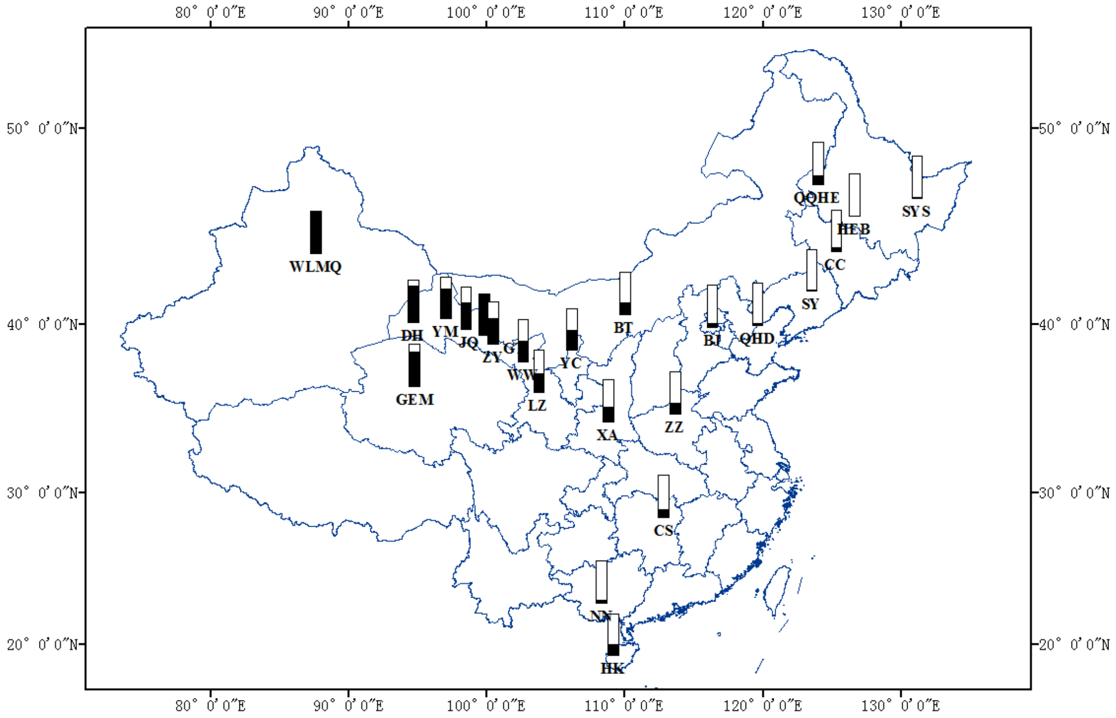
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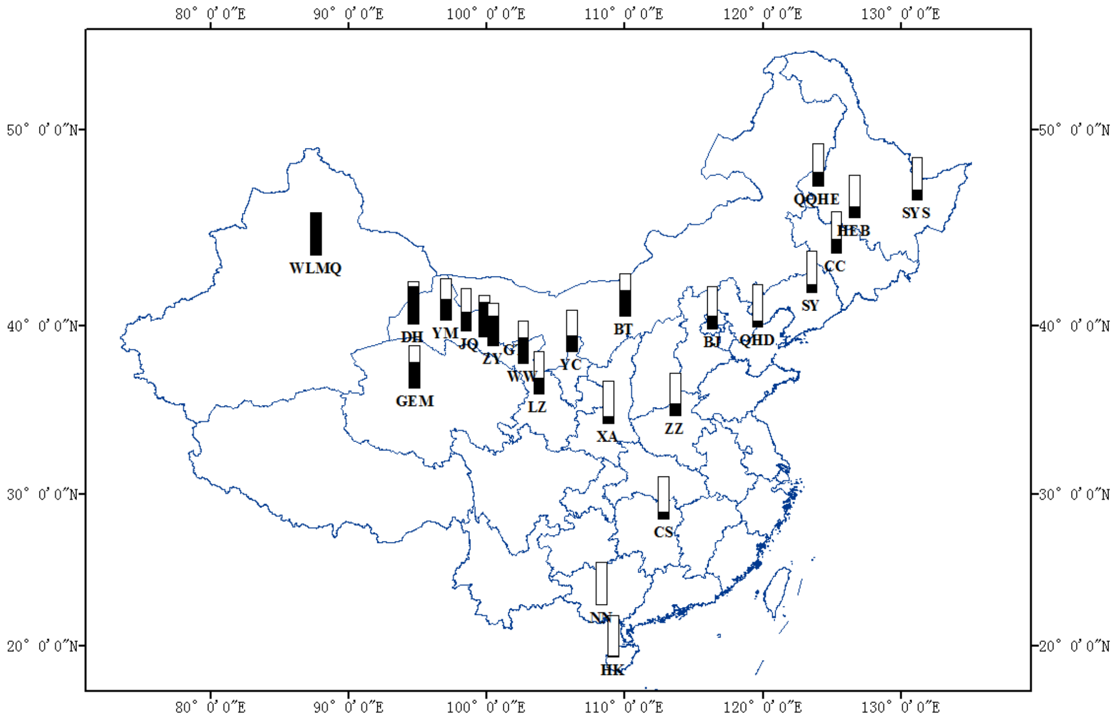
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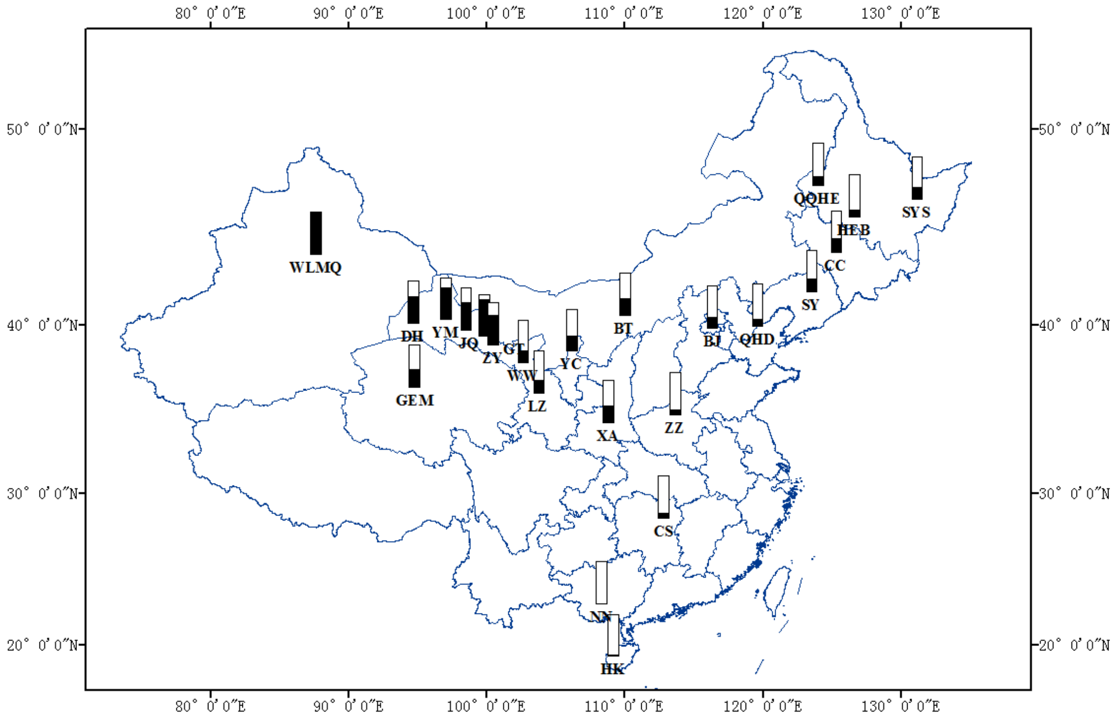
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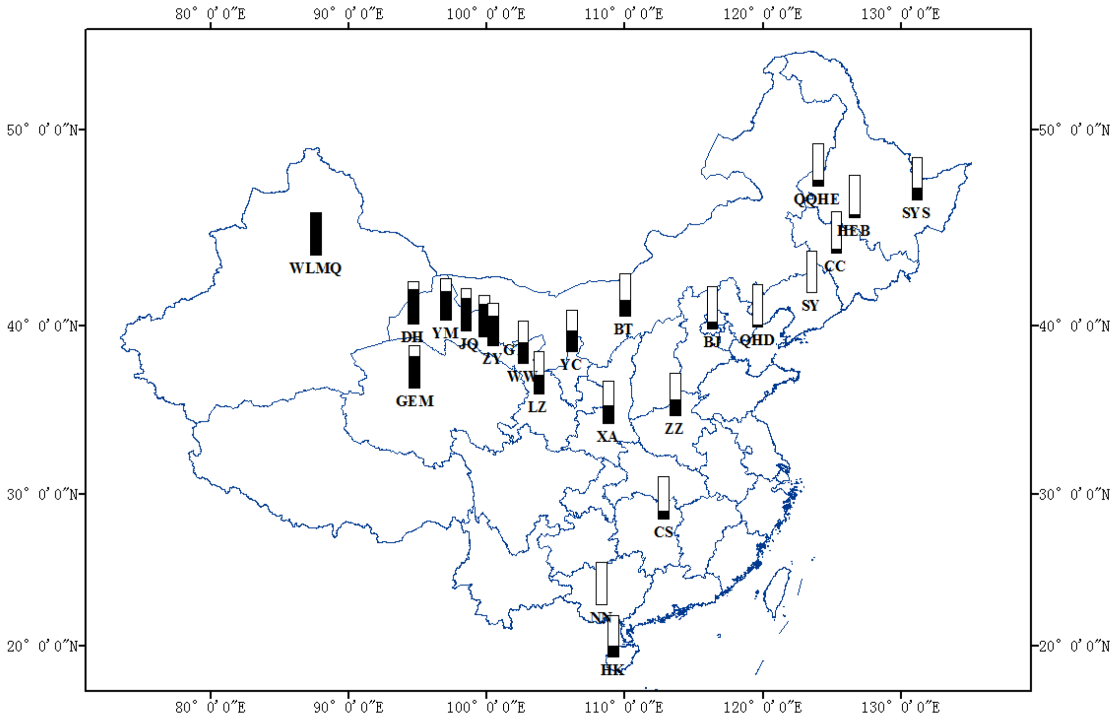
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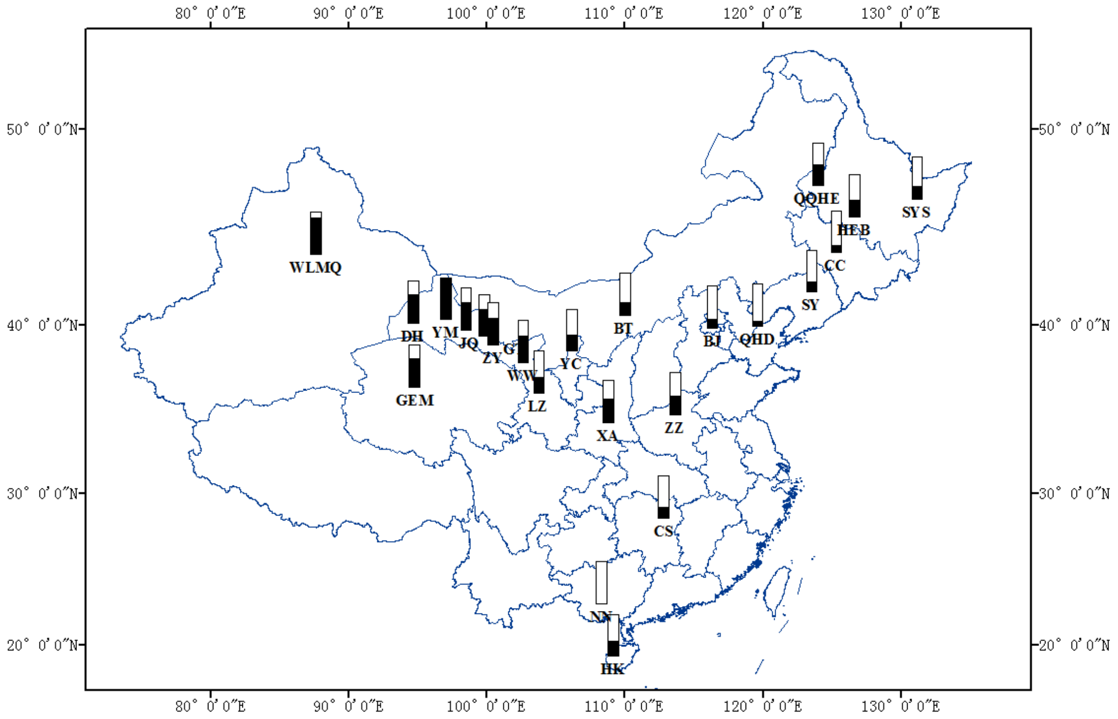
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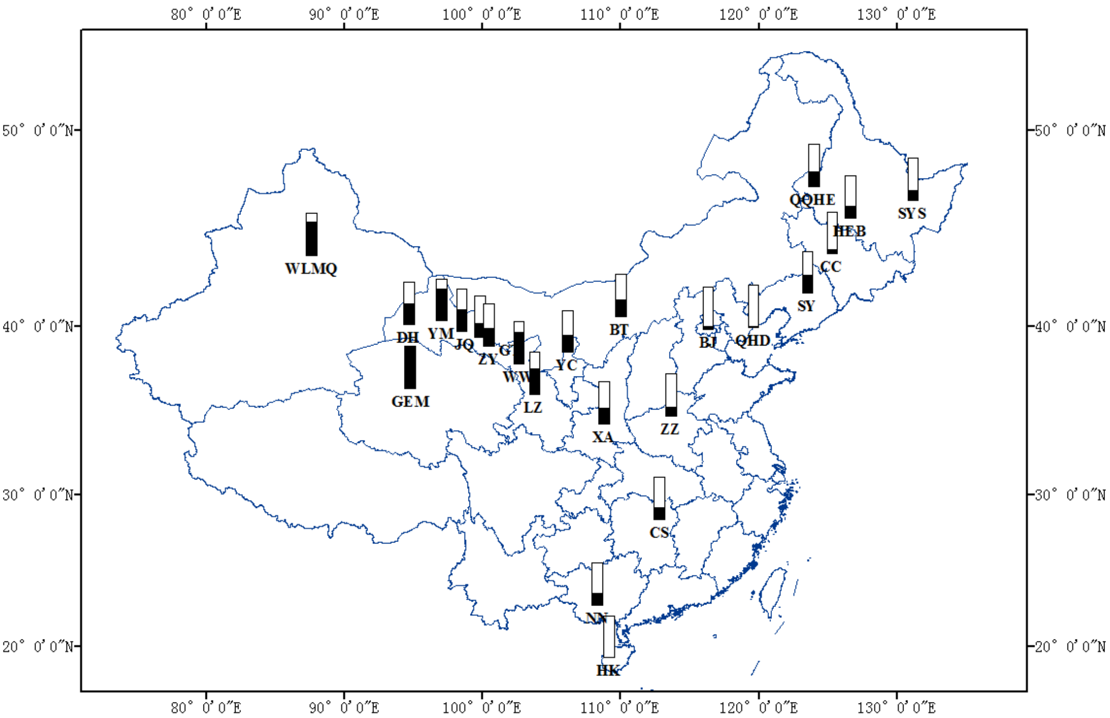
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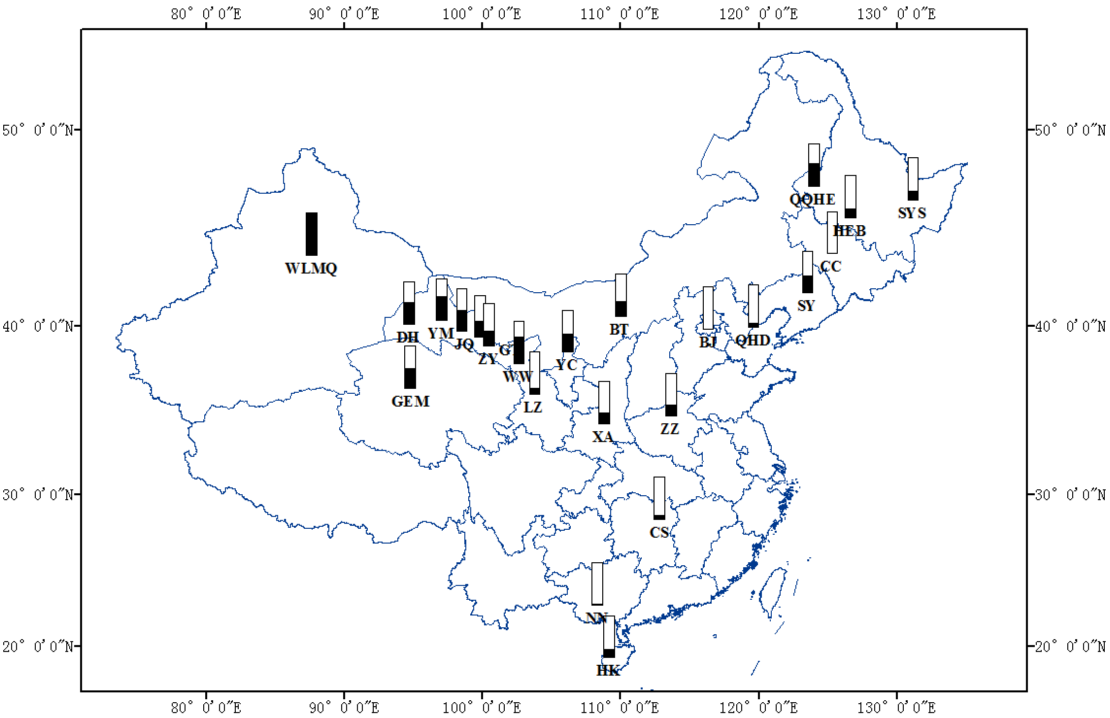
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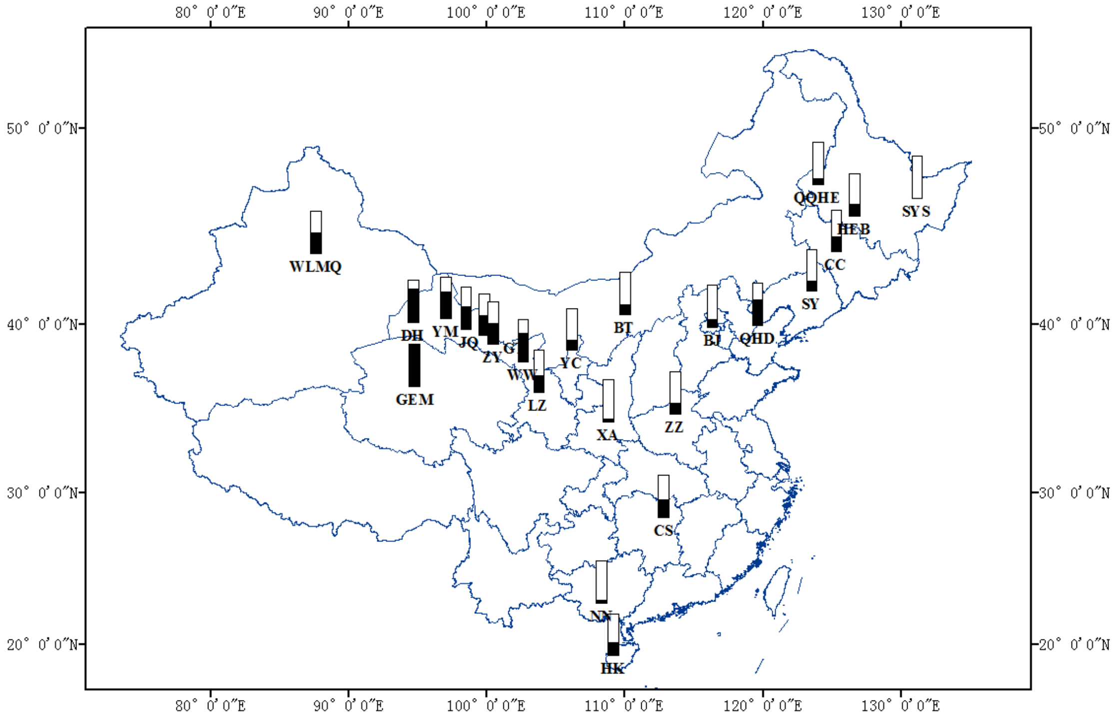
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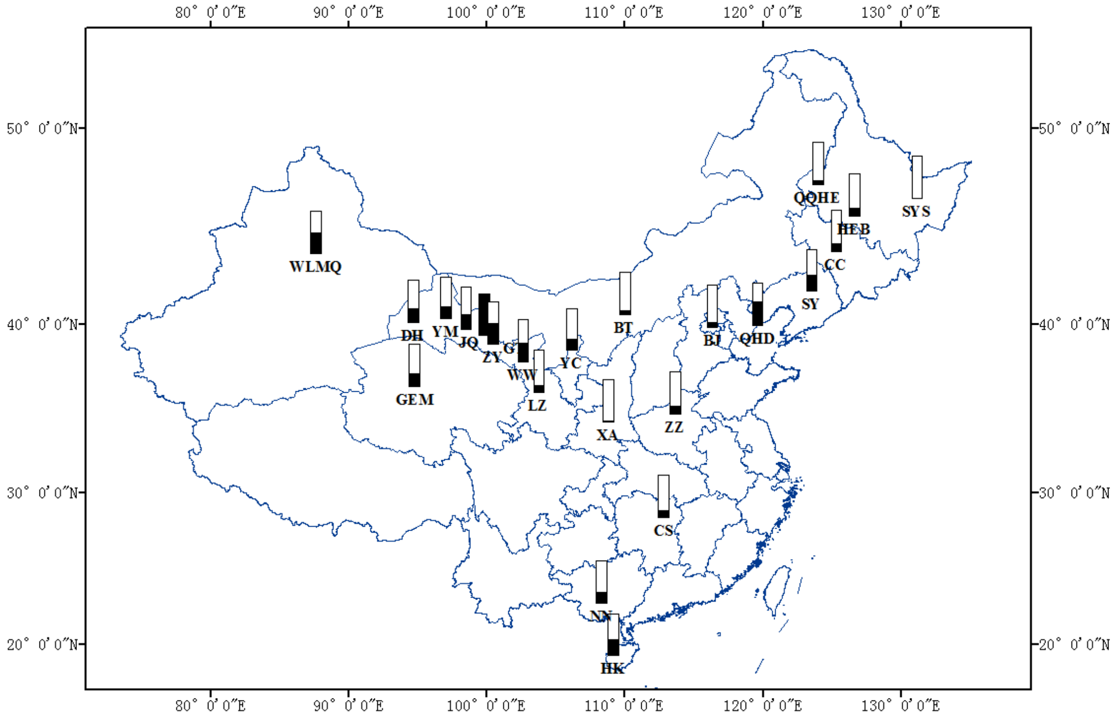
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Figure 1.2 A map with bar charts showing the difference in mean values of morphological traits among different populations. The mean value (Table S2) is represented with the height of black bar in the chart with the largest mean value represented with a full black bar chart and the smallest one represented with a blank bar chart. a) male wing length, b) female wing length, c) male streamer length, d) female streamer length, e) male body mass, f) female body mass, g) male tarsus length, h) female tarsus length, i) male bill length and j) female bill length.

Colour Traits

For throat colour traits, most populations did not show significant differences between each other, while two northeastern populations were significantly different from other populations in breast brightness and breast chroma (Table S3, Figure 1.3). For throat brightness, most populations were not significantly different; only one (YM) in males and two populations in females (QHD, SYS) were significantly different from other populations. Similarly, throat hue did not show any significant differences between populations in either sex, while for throat chroma one population (CS) in males and three in females (CC, QHD, WLMQ) were significantly different from other populations.

For breast brightness, two northeastern populations (SYS, QQHE) were significantly different from western populations (JQ, ZY, YM, WLMQ) and one central population (QHD) in both sexes. Similarly, for breast chroma these two populations were significantly higher than other western or central populations in both sexes. In neither sex did breast hue show any significant difference between populations.

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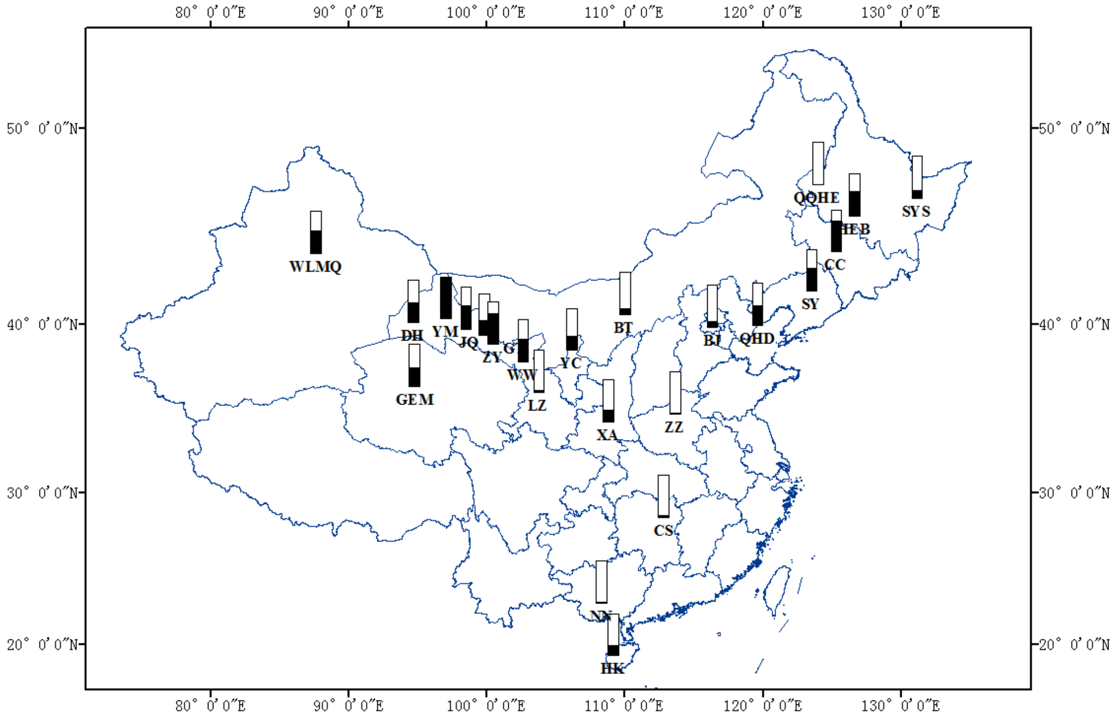
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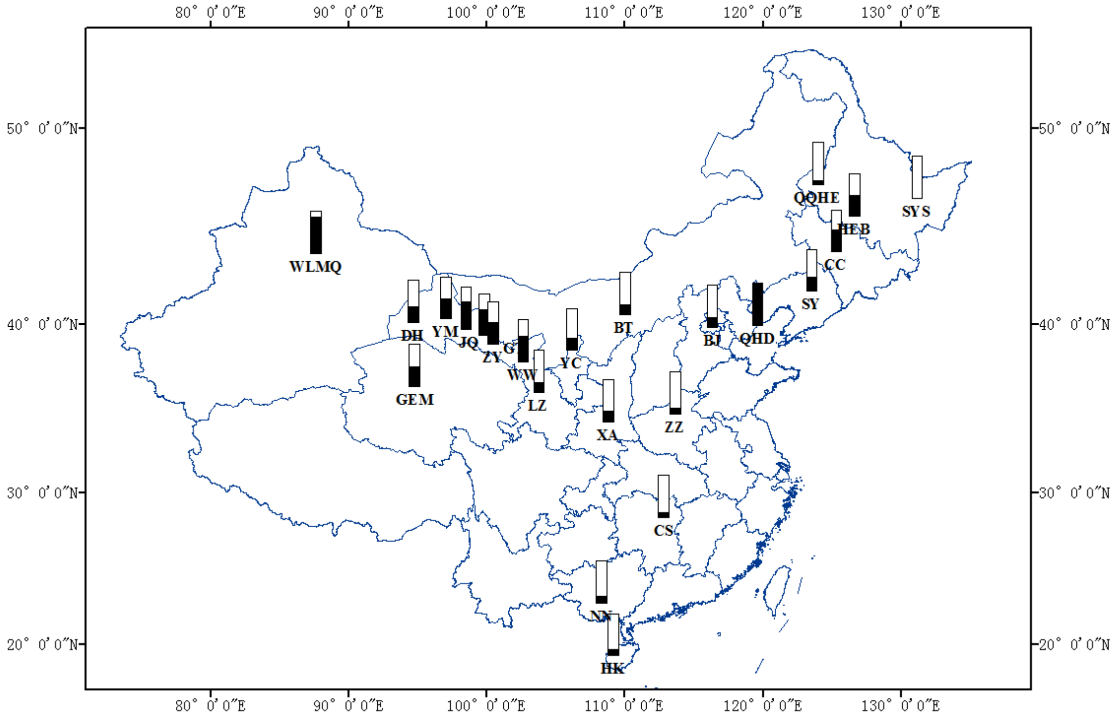
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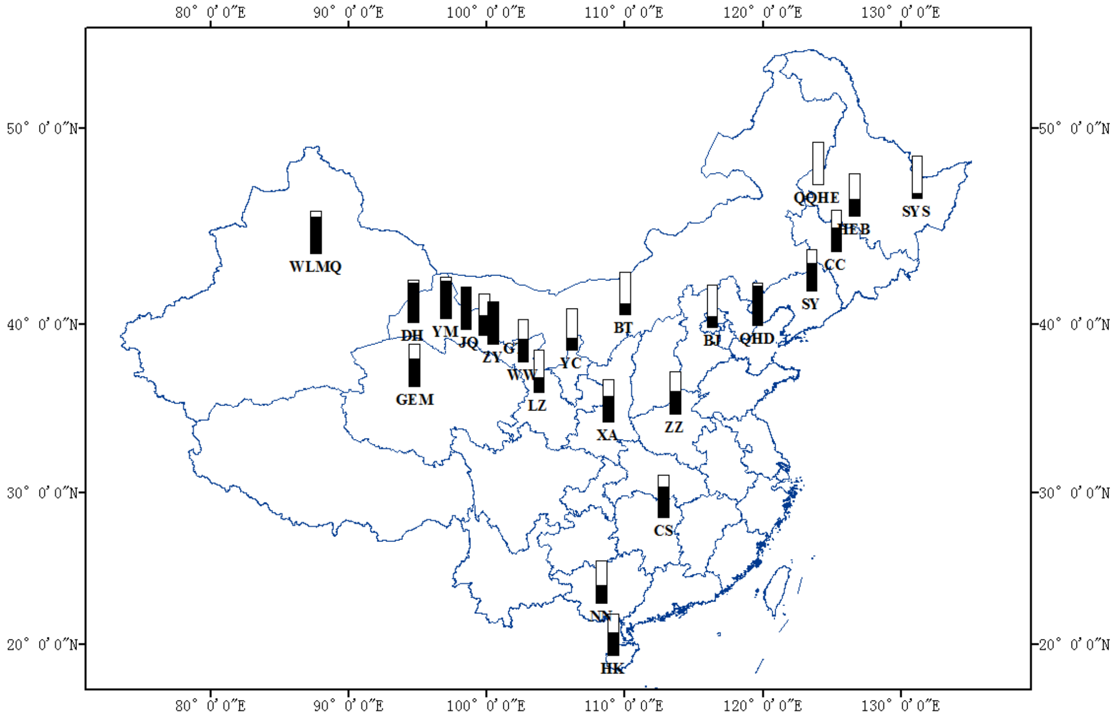
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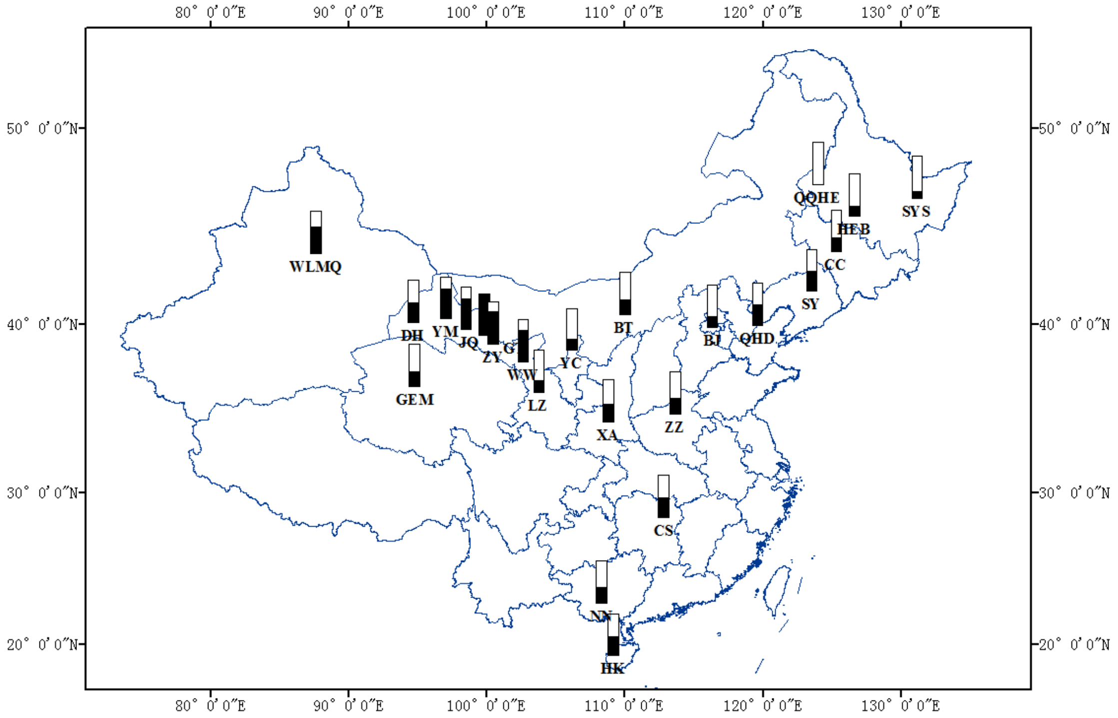
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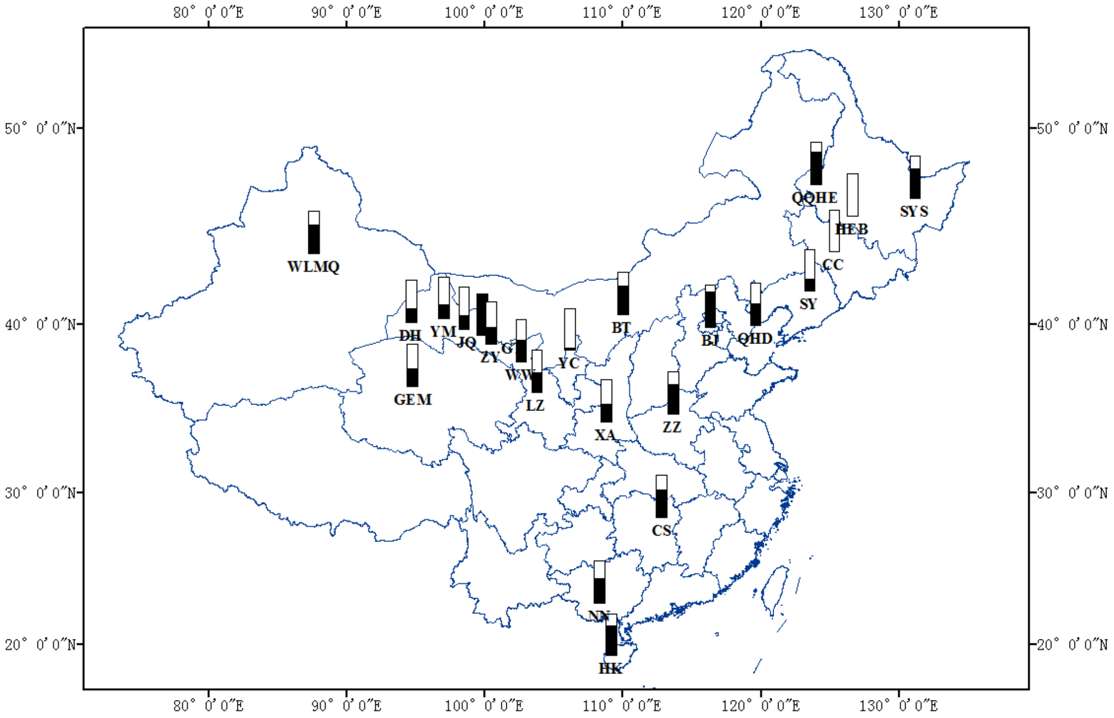
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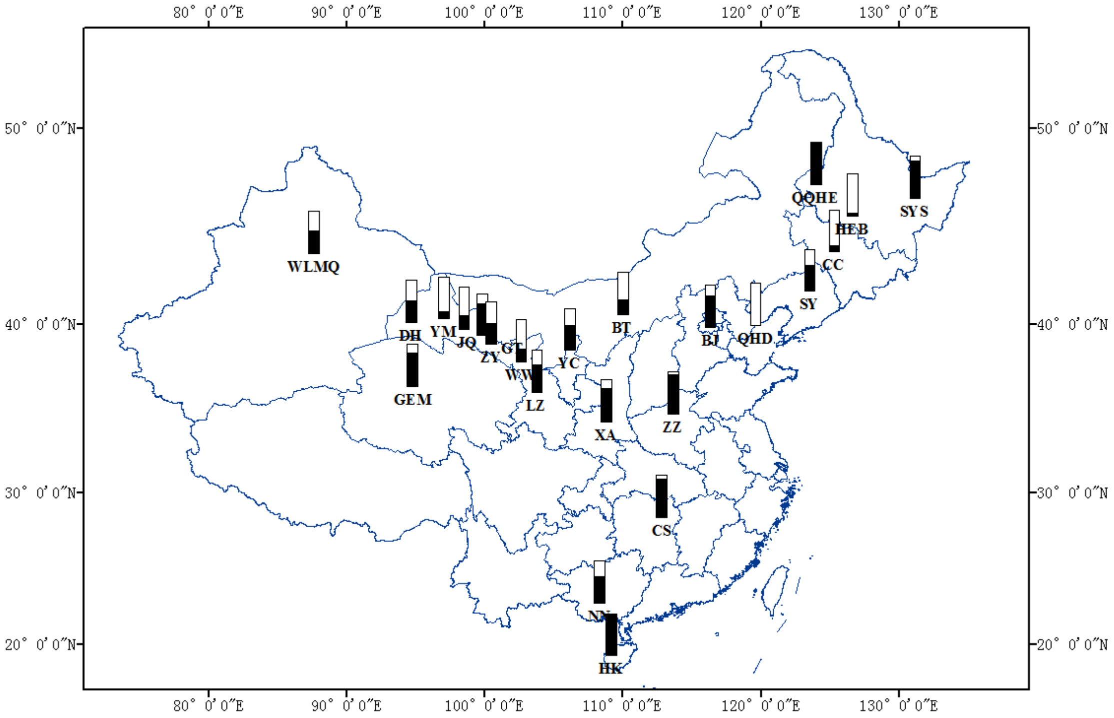
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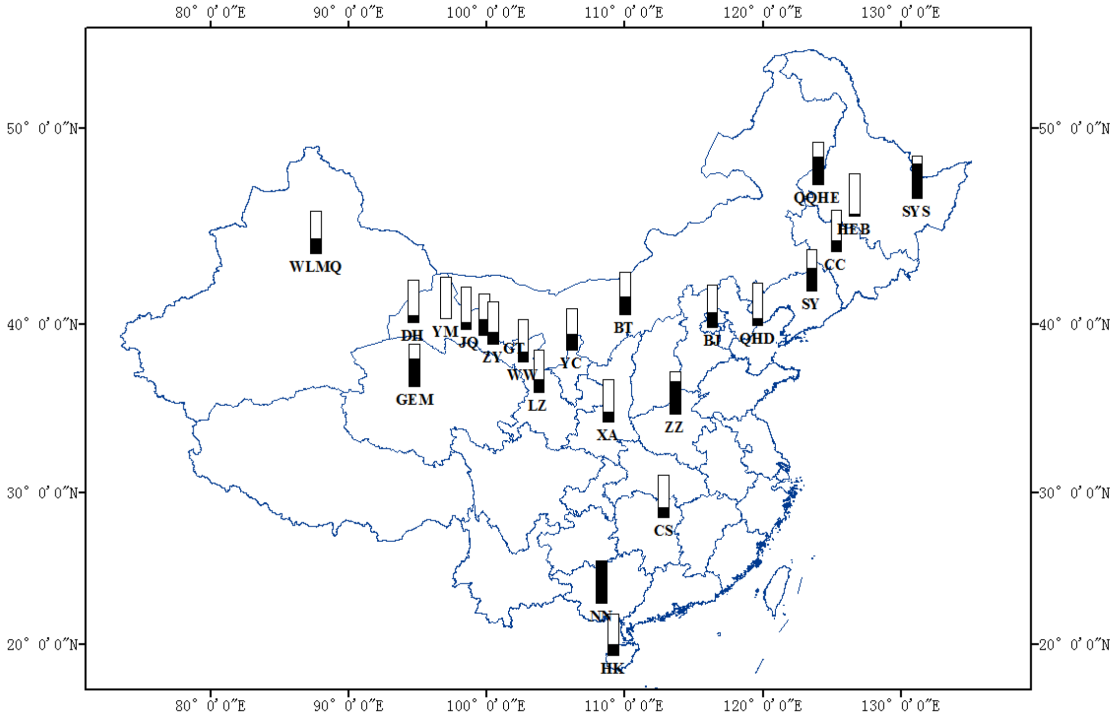
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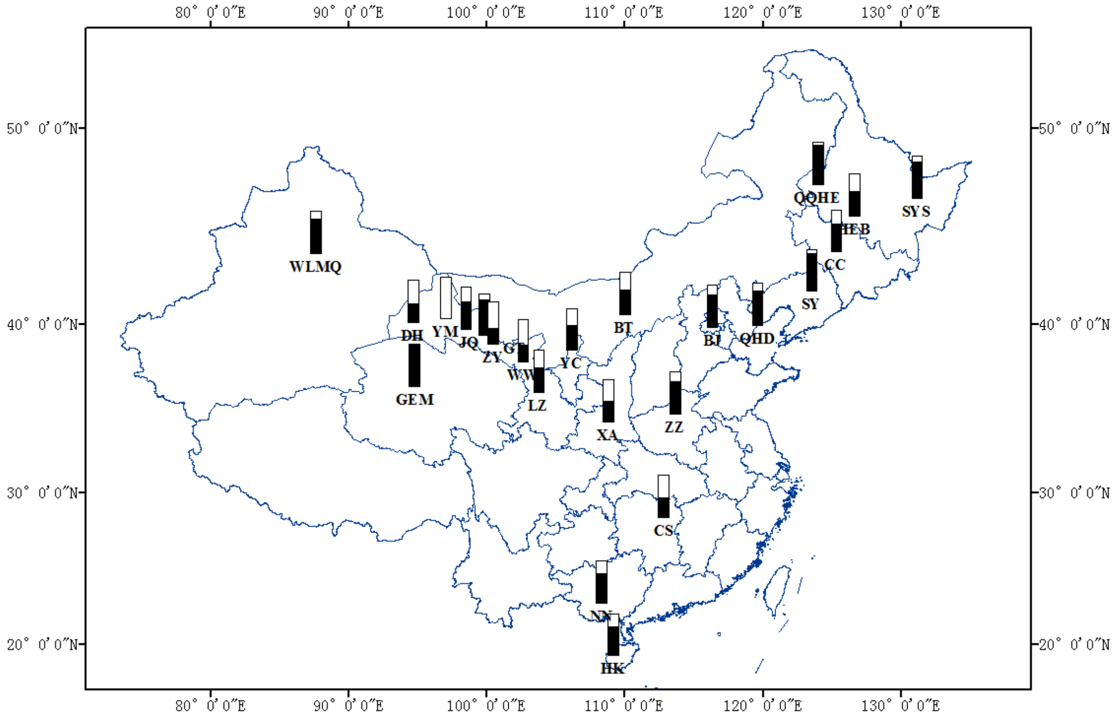
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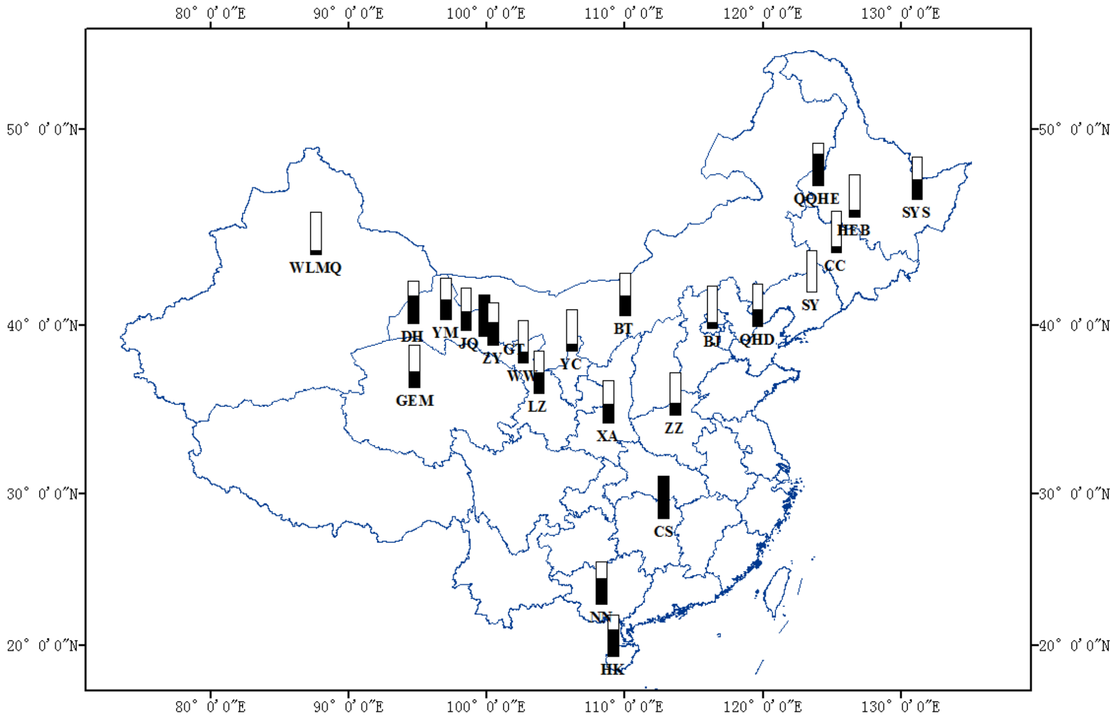
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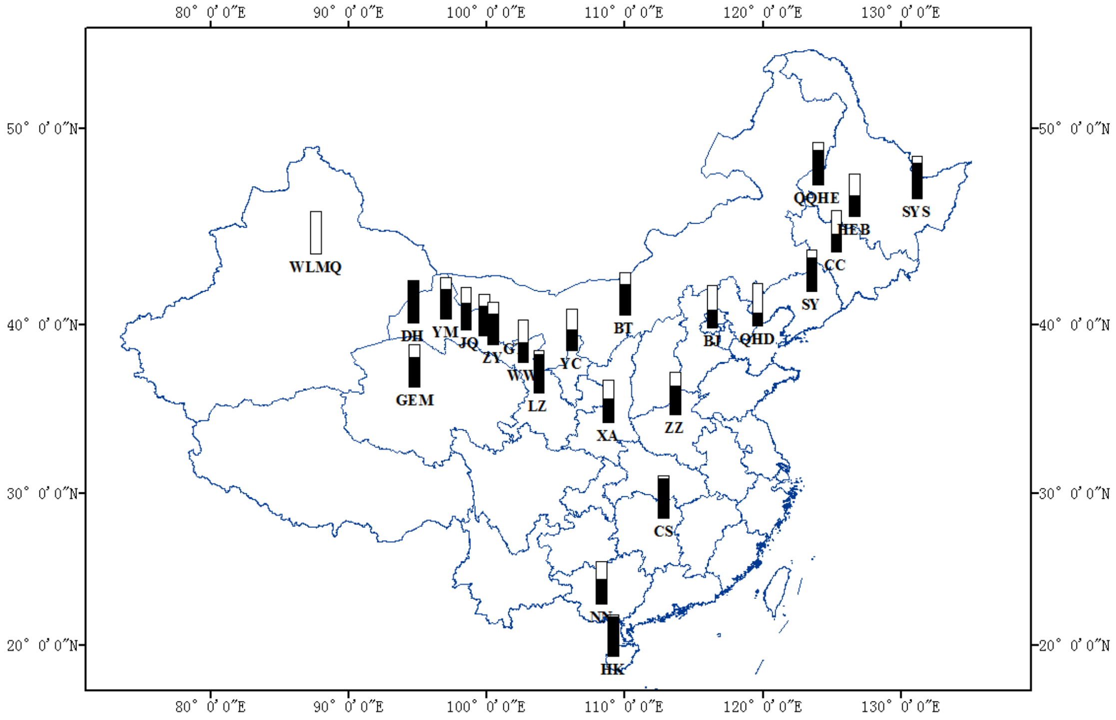
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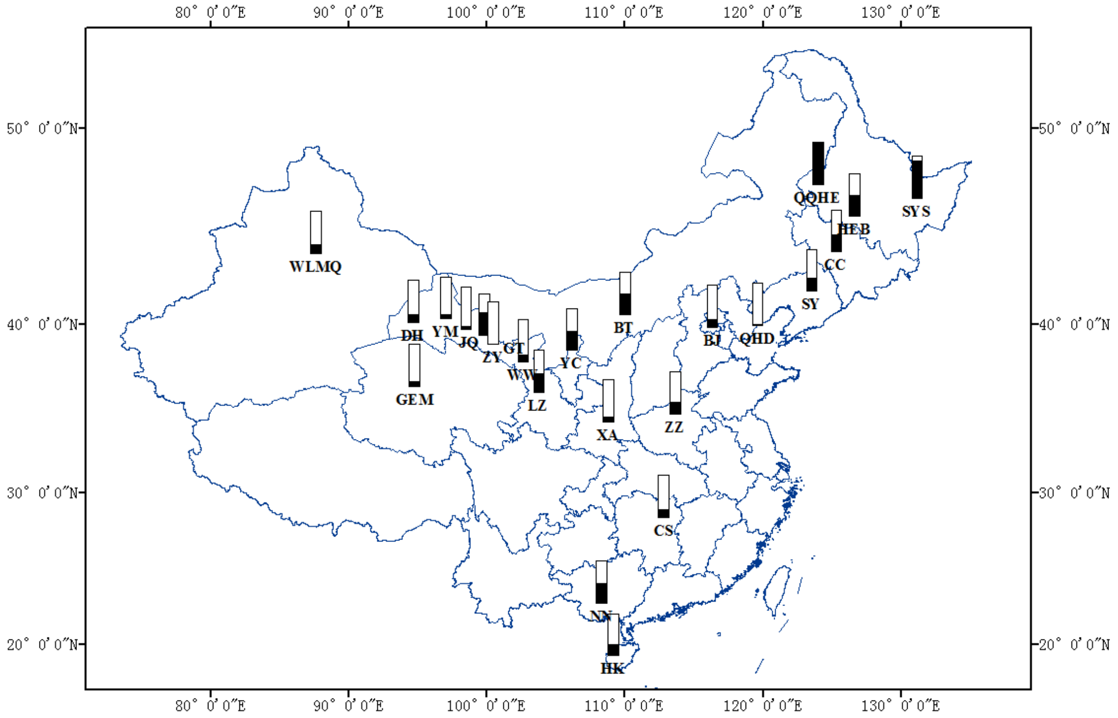
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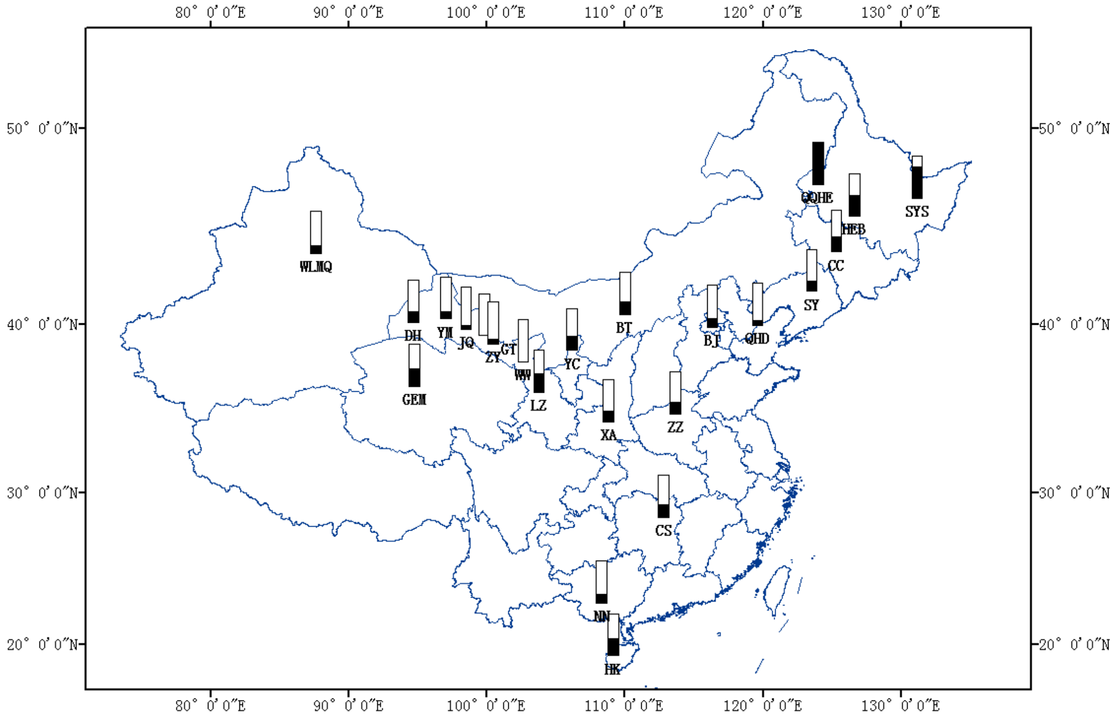
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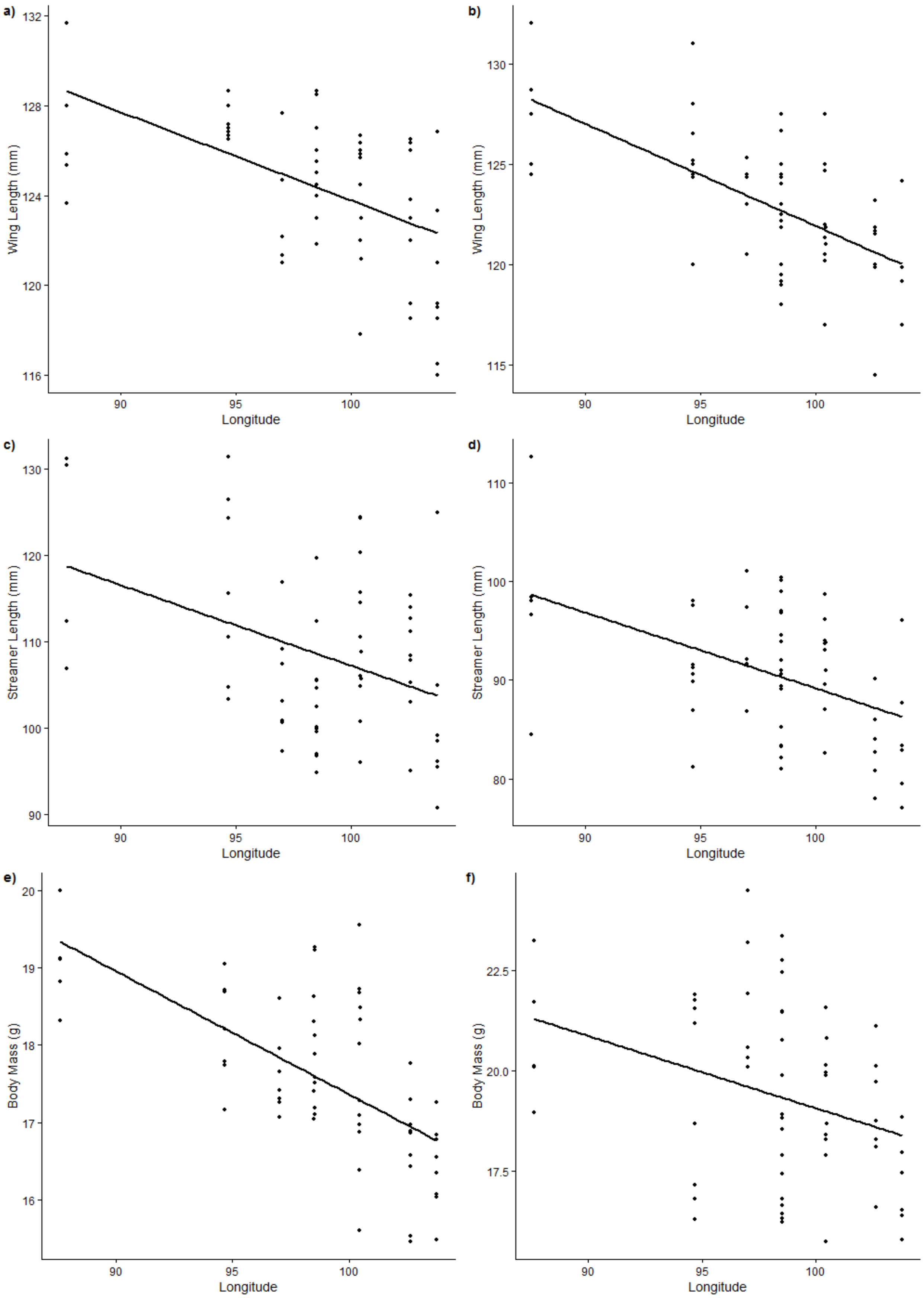
Figure 1.3 A map with bar charts showing the difference in mean values of colour traits among different populations. The mean value is represented with the height of black bar in the chart with the largest mean value represented with a full black bar chart and the smallest one represented with a blank bar chart. a) male throat brightness, b) female throat brightness, c) male breast brightness, d) female breast brightness, e) male throat hue, f) female throat hue, g) male breast hue, h) female breast hue, i) male throat chroma, j) female throat chroma, k) male breast chroma and l) female breast chroma.

Table 1.2 One-way analyses of variance of morphological and colour traits of barn swallows within and among populations.

Trait		MS (Population)	MS (Residual)	*F*	df	*p*
Male						
wing length		122.20	5.95	20.53	22, 190	<0.01
streamer length		435.80	59.00	7.39	22, 188	<0.01
tarsus legnth		0.61	0.23	2.62	22, 190	<0.01
bill length		0.35	0.07	4.76	22, 190	<0.01
body mass		14.30	0.69	20.82	22, 188	<0.01
Throat	brightness	14.12	3.48	4.06	22, 189	<0.01
	hue	285.50	150.30	1.90	22, 189	0.01
	chroma	0.0013	0.0005	2.56	22, 189	<0.01
Breast	brightness	587.70	64.40	9.13	22, 190	<0.01
	hue	1684.40	793.40	2.12	22, 190	<0.01
	chroma	0.0073	0.0009	8.36	22, 190	<0.01
Female						
wing length		138.60	9.10	15.23	22, 194	<0.01
streamer length		202.89	33.62	6.04	22, 194	<0.01
tarsus legnth		0.47	0.16	2.99	22, 194	<0.01
bill length		0.31	0.14	2.23	22, 194	<0.01
body mass		26.56	2.63	10.09	22, 194	<0.01
Throat	brightness	33.04	9.28	3.56	22, 193	<0.01
	hue	411.30	201.20	2.04	22, 193	0.01
	chroma	0.0029	0.0006	4.47	22, 193	<0.01
Breast	brightness	472.50	55.50	8.51	22, 194	<0.01
	hue	1604.80	750.50	2.14	22, 194	<0.01
	chroma	0.0048	0.0006	8.14	22, 194	<0.01

Linear Variation of Morphological and Colour Traits in Potential Hybrid Zones

In the potential hybrid zone between *H. r. rustica* and *H. r. gutturalis* (populations: WLMQ, DH, YM, JQ, ZY, WW, LZ), three morphological traits showed linear variation with longitude in both sexes (linear model: wing length: male: $r = -0.39$, T = -4.54, df = 58, $p < 0.01$, female: $r = -0.51$, T = -4.54, df = 57, $p < 0.01$; streamer length: male: $r = -0.93$, T = -3.08, df = 56, $p < 0.01$, female: $r = -0.77$, T = -4.01, df = 57, $p < 0.01$; body mass: male: $r = -0.16$, T = -6.49, df = 58, $p < 0.01$, female: $r = -0.18$, T = -2.76, df = 57, $p < 0.01$; Figure 1.4) while colour traits did not show significant linear variation. Meanwhile in the potential hybrid zone between *H. r. mandschurica* and *H. r. gutturalis* (populations: BJ, QHD, SY, CC, HEB, QQHE, SYS), morphological traits tarsus length, bill length and body mass showed linear variation with latitude (linear model: tarsus length: male: $r = 0.038$, T = 2.58, df = 64, $p = 0.01$, female: $r = 0.037$, T = 2.40, df = 77, $p = 0.02$; bill length: male: $r = -0.049$, T = -3.64, df = 64, $p < 0.01$, female: $r = -0.055$, T = -2.88, df = 77, $p < 0.01$; body mass: male: $r = 0.085$, T = 2.17, df = 64, $p = 0.03$, female: $r = 0.24$, T = 4.04, df = 77, $p < 0.01$), and colour traits throat brightness, throat chroma, breast brightness and breast chroma showed linear variation with latitude in both male and female barn swallows (linear model: throat brightness: male: $r = -0.20$, T = -2.23, df = 64, $p = 0.03$, female: $r = -0.58$, T = -3.57, df = 77, $p < 0.01$; throat chroma: male: $r = 0.0024$, T = 2.38, df = 64, $p =0.02$, female: $r = 0.0044$, T = 3.77, df = 77, $p < 0.01$; breast brightness: male: $r = -2.50$, T = -5.31, df = 64, $p < 0.01$, female: $r = -1.59$, T = -5.26, df = 77, $p < 0.01$; breast chroma: male: $r = 0.012$, T = 6.84, df = 64, $p < 0.01$, female: $r = 0.0091$, T = 7.70, df = 77, $p < 0.01$; Figure 1.5).


a)
132
128
124
120
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Wing Length (mm)
90
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Longitude
b)
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125
120
115
Wing Length (mm)
90
95
100
Longitude
c)
130
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100
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Streamer Length (mm)
90
95
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Longitude
d)
110
100
90
80
Streamer Length (mm)
90
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100
Longitude
e)
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17
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Body Mass (g)
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100
Longitude
f)
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17.5
Body Mass (g)
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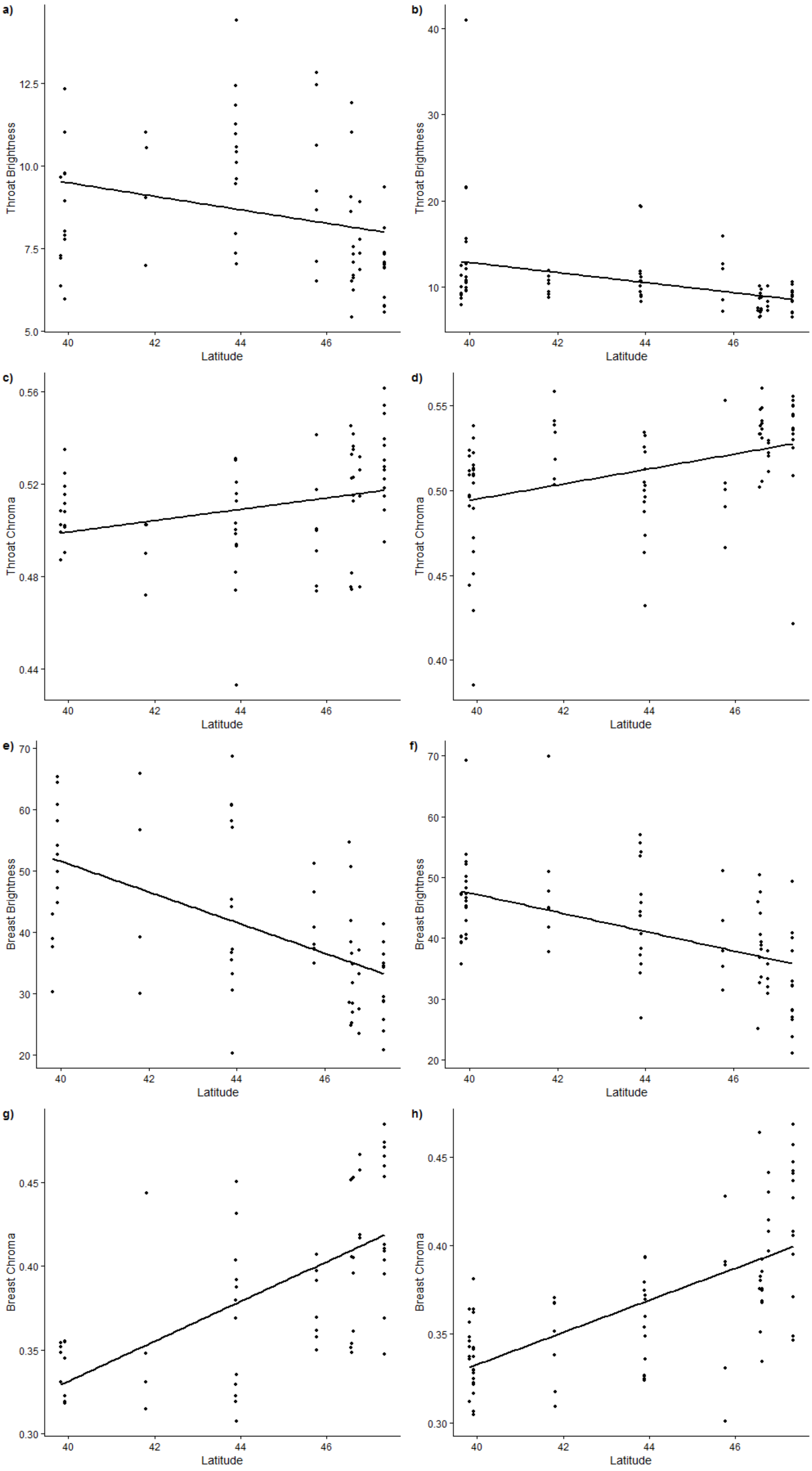
95
100
Longitude

Figure 1.4 Morphological traits showed linear variation in potential hybrid zone between *H. r. rustica* and *H. r. gutturalis*. Wing length of males (a) and females (b), streamer length of males (c) and females (d) and body mass of males (e) and females (f) were significantly negatively correlated with longitude. Regression lines from linear models are shown.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5.0
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42
44
46
Latitude
b)
Throat Brightness
40
30
20
10
40
42
44
46
Latitude
c)
Throat Chroma
0.56
0.52
0.48
0.44
40
42
44
46
Latitude
d)
Throat Chroma
0.55
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44
46
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e)
Breast Brightness
70
60
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40
30
20
40
42
44
46
Latitude
f)
Breast Brightness
70
60
50
40
30
20
40
42
44
46
Latitude
g)
Breast Chroma
0.45
0.40
0.35
0.30
40
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46
Latitude
h)
Breast Chroma
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0.40
0.35
0.30
40
42
44
46
Latitude

Figure 1.5 Colour traits showed linear variation in potential hybrid zone between *H. r. mandschurica* and *H. r. gutturalis*. Throat brightness of males (a) and females (b), breast brightness of males (e) and females (f) were significantly negatively correlated with latitude, while throat chroma of males (c) and females (d), breast chroma of males (g) and females (h) were significantly positively correlated with latitude. Regression lines from linear models are shown.

Correlation between geographic distance and trait distance

Differences in morphological and colour traits between populations were described with deltaP values. The result of the Mantel test showed that for none of the morphological or colour traits deltaP was significantly correlated with geographic distance between populations in both male and female barn swallows ($p = 0.08 – 0.99$).

Classification of Populations by Phenotype

I evaluated the accuracy of the subspecies assignation using DFA analysis. I ran DFA with morphological traits and colour traits alone and for all traits for males and females separately. For male barn swallows with only morphological traits the accuracy of subspecies assignation was 75%, while with only colour traits the accuracy was 70%. Similarly, for female barn swallows the accuracy was 75% and 73% with morphological traits and colour traits respectively. Combining morphological traits with colour traits resulted in higher prediction accuracy, 84% in male barn swallows and 83% in females (Figure 1.5). For *H. r. mandschurica*, 40 of 59 (68%) were assigned as *H. r. mandschurica* while 19 were assigned as *H. r. gutturalis*, while 15 of 296 (5%) *H. r. gutturalis* were assigned as *H. r. mandschurica*. Meanwhile *H. r. mandschurica* were totally separated from *H. r. rustica*, with no *H. r. gutturalis* assigned as *H. r. rustica* and vice versa.

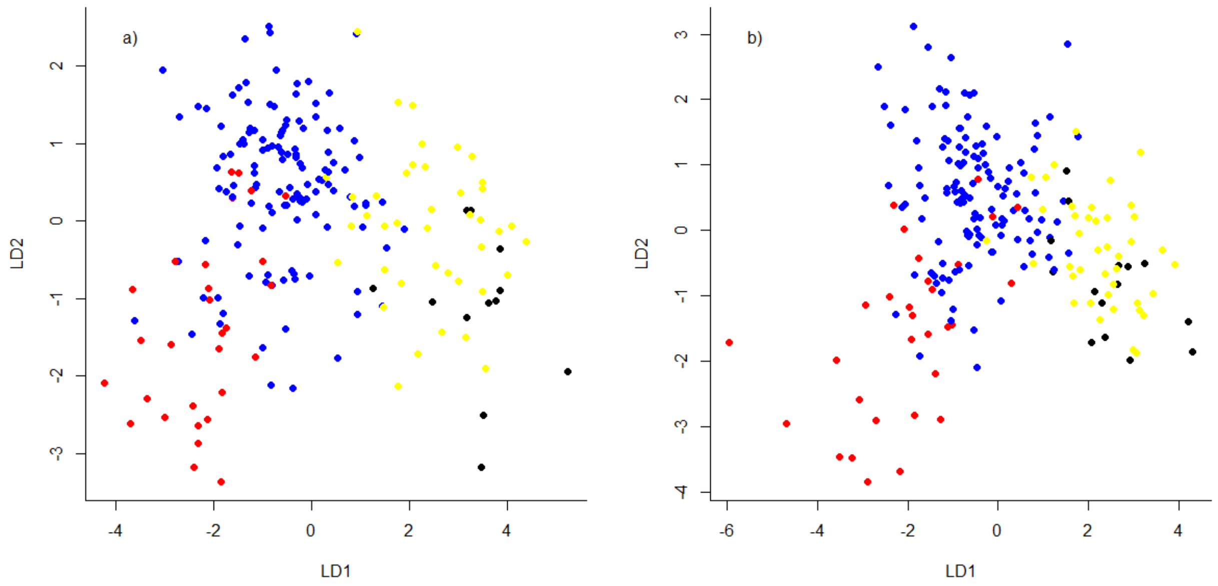


Figure 1.6 Distribution of various barn swallow subspecies along two dimensions (LD1, LD2) of DFA analysis in a) male and b) female barn swallows. Different subspecies are marked with different colours (red: *H. r. mandschurica*; blue: *H. r. gutturalis*; yellow: *gutturalis-rustica* hybrids; black: *H. r. rustica*).

Discussion

My research shows that two populations in the northeast (SYS, QQHE) of China are different from other Chinese populations in having particularly dark ventral plumage (Figure 1.3, Table S3), while three populations in the west (DH, GEM, WLMQ) have large body size (Figure 1.2, Table S2), which again separates them from the other populations. Based on these traits, our samples can be divided into three taxa with the accuracy of more than 83%. These two populations of Northeastern barn swallows can be distinguished from both *H. r. gutturalis* and *H. r. rustica* by ventral colour traits, and in this chapter I used the term *H. r. mandschurica* to stand for these two populations. With their large body size and pale ventral plumage, the two Western populations are likely to be European barn swallows, *H. r. rustica*.

In China, the size of barn swallows decreases from the west to the east, with large *H. r. rustica* in the west and small *H. r. gutturalis* and *H. r. mandschurica* in the south and east. While both *H. r. rustica* and *H. r. gutturalis* have whitish ventral colour, *H. r. mandschurica* has relatively darker plumage, which makes it different from the other two in morphology (Figure 1.3, Table S3). Between subspecies linear variation in morphological and colour traits exists, e.g. linear variation in wing length and streamer length between *H. r. rustica* and *H. r. gutturalis* and linear variation in breast brightness and breast chroma between *H. r. mandschurica* and *H. r. gutturalis*. In barn swallows several ecological factors are known to contribute to population and phenotypic divergence, like migratory distance, migratory routes and wintering grounds (Scordato & Safran 2014). While geographic variation in ornament size of European barn swallows *H. r. rustica* is considered as the result of a sexual process of reliable signaling rather than natural selection associated with migration (Møller 1995), it remains unknown whether linear variation with longitude of morphological traits in *rustica-gutturalis* hybrid zone is due to variation in migratory distance with little research on the migration of these birds. With no significant linear variation with latitude in wing length and streamer length, the variation of colour traits in the *gutturalis-mandschurica* zone is more likely to be the result of different sexual selection pressures as there is no evidence that plumage colour relates to flight ability or is under other natural selection pressure in barn swallows, while *H. r. gutturalis* and *H. r. mandschurica* both utilize plumage colour as the sexually selected trait (Hasegawa et al. 2010b).

Though there are previous studies on different barn swallow subspecies in Asia, samples from China have been lacking and morphological comparison between them was rare. Here with morphological traits I identified the existence of a *tytleri-gutturalis* intergrade, *H. r. mandschurica* (Figure 1.6), whose distribution range is surrounded by *H. r. gutturalis* in the south, *H. r. tytleri* in the west, potentially *H. r. saturata* in the east and *H. r. erythrogaster* in the east across the Bering Strait. Combining former studies and our data shows that *H. r. mandschurica* has the smallest body size compared with other barn swallow populations, while its plumage colour darkness is between *H. r. gutturalis* and *H. r. tytleri*. Previous studies have shown that the formation of subspecies *H. r. tytleri* is best explained as the result of *H. r. erythrogaster's* dispersal back into north Asia across Bering strait, which was likely to have occurred about 27,000 years ago (Dor et al. 2010; Zink et al. 2006). In this case *H. r. mandschurica* could be the intergrade between *H. r. tytleri* and *H. r. gutturalis*, which is supported by the fact that the distribution range of *H. r. gutturalis* appears to have moved towards the north along with human settlements (Smirensky & Mishchenko 1981). Also, genome sequencing using RADSeq has revealed that *H. r. mandschurica* are hybrids of *H. r. tytleri* and *H. r. gutturalis* (Safran & Scordato, unpublished data). Another possibility is that the dark colour phenotype of *H. r. mandschurica* could be formed by directional selection during the northern dispersal of barn swallows, which has been fixed in both *H. r. tytleri* and *H. r. erythrogaster* but not in *H. r. mandschurica* itself as there is likely to be gene flow from southern *H. r. gutturalis* populations. To answer this question, a higher resolution genome sequencing on the barn swallow subspecies complex is needed, as well as more detailed behaviour research on various sexual selection strategies among subspecies.

Chapter II. The correlation between potential sexually selected traits and reproductive success of northeastern Chinese barn swallows: an observational study

Abstract

The type of males preferred by females can be different even between closely related populations within a single species, and the different selection pressures can be important driving forces for speciation. For the barn swallows, at least two sexually selected traits have been identified: the length of tail streamers in European barn swallows *H. r. rustica* and ventral plumage colour in North American barn swallows *H. r. erythrogaster*. The barn swallow consists of at least six subspecies, and these two sexually selected traits vary extensively among different subspecies. In this study I tested the relationship between the reproductive success and potential sexually selected traits in a northeastern Chinese barn swallow population, on which little research has been done on sexual selection and mate choice. I found that ventral plumage colour, not the length of tail streamers, is the sexually selected trait in this population, this pattern is more similar to North American swallows than European swallows.

Introduction

First proposed by Darwin in 1871, sexual selection is considered to be an important part of natural selection (Darwin 1871; Davies et al. 2012). Sexual selection can be defined as the difference of reproductive success among individuals that is related to their ability to acquire mates and fertilizations. It can often give rise to exaggerated traits such as the antlers of red deer *Cervus elaphus* or the tail of long-tailed widowbird *Euplectes progne*, which can predict the reproductive success of male individuals (Andersson 1994). Sexual selection can operate in two ways: intrasexual selection usually male-male competition and intersexual selection usually female choice, in the latter females might choose mates to obtain either direct and/or indirect benefits (Davies et al. 2012). Females can choose their mates through specific sexually selected traits, and these traits are usually dimorphic between different sexes and correlated to seasonal reproductive success (Andersson 1994).

Barn swallows are small Holarctic-distributed passerines that mate monogamously; throughout their range barn swallows are insectivorous and feed on the wing (Turner 2006). European barn swallow populations and their long tail streamers, the outermost tail feathers, are a classic example of a sexually selected signal, extensive studies have shown that male tail streamers are a mate choice cue (Møller 1994c). Male swallows with longer tail streamers have better body condition, arrive at breeding sites and start breeding earlier, acquire higher quality mates, and have more within-pair and extra-pair offspring (Møller 1991b, 1993a, b, 1994b; Møller & Tegelström 1997). This has been confirmed by both observations of natural variation and tail elongation experiments (Møller 1988; Saino et al. 1997).

However, more recent work has shown that ventral plumage colour may play a role in mate choice in barn swallow subspecies outside Europe. To date, six different subspecies of barn swallows have been confirmed and they differ from each other in morphology, behaviour and genetics (Dor et al. 2010; Turner 2006; Turner 1994). With rust-coloured plumage in the ventral region, the fitness of populations of barn swallows in North America is predicted by their plumage colour, and artificial enhancement of this trait results in males gaining higher paternity from their social mates (Safran et al. 2005; Safran & McGraw 2004). Research on East-Mediterranean barn swallows (*H. r. transitiva*) showed that both streamer length and ventral plumage colour are related to the number of offspring in the first brood and to annual breeding success (Vortman et al. 2011). Further, manipulation experiments confirmed the multiple signaling role of the streamer and colour combination: local females prefer males with both elongated tails and darkened ventral plumage colour (Vortman et al. 2013). Similarly, research on *H. r. gutturalis* suggested both male throat plumage colour and streamer length could predict the laying date of clutches (Hasegawa et al. 2010b), but also see (Arai et al. 2015).

In the North-East of China there are barn swallow populations of what I believe to be intergrades of *H. r. tytleri* and *H. r. gutturalis*, and I refer to these swallows with the term *H. r. mandschurica* in this chapter (Chapter I). These barn swallow populations are interesting because there have been no previous studies of their life history or behaviour. In this chapter I investigated the potential role of both the tail streamer and ventral plumage colouration in sexual selection, and the relationships between these traits and swallow reproductive success were examined. I assume that the ventral plumage colour, rather than the length of tail streamers, is likely to be the sexually selected trait in this barn swallow population. It is supposed that males with more colourful (darker or redder) ventral plumage will gain greater reproductive success, e.g. starting breeding earlier or producing more offspring than other males.

Methods

Fieldwork methods

I studied a population of barn swallows in Shuangyashan City, Heilongjiang Province, China (46° 35’ N, 131° 14’ E) from May to September 2013 and June to September 2015. I searched possible nesting sites after barn swallows arrived at the breeding site in spring, especially under eaves or in corridors of single-storey buildings or in stairwells on the top storey of buildings of two or more storeys (Figure 2.1). After active nests were identified, I visited them every two days and the number of eggs in each nest was recorded. Adults were captured using mist nets during the first five days after their chicks hatched as experience suggested that there was a high risk of them abandoning their nests if they were caught earlier. In total 165 parent swallows (2013: male: n = 36, female: n = 39; 2015: male: n = 44, female: n = 46) were captured.

At capture, swallows were marked with a unique combination of coloured plastic rings on each of their legs. Different sexes were identified by checking the presence (females) or absence (males) of a brood patch. For each bird, the length of both wings was measured to the nearest 1 mm using a ruler with a pin-zero-stop, the length of left tarsus, the length of the shortest tail feather (mid-tail) and the length of both streamers were measured to the nearest 0.01 mm with digital calipers (Jazooli, UK), and the body mass of swallows was measured to the nearest 0.01g with a digital balance (ChangXie, CX-168, China). All measurements were taken by myself to avoid differences between observers. Plumage samples were taken from throat, belly and vent regions. For each region, four to ten feathers were plucked and stuck to white paper cards. These samples were stored in the dark for future spectrophotometer tests.

In one brood, it usually took one or two days for all chicks to hatch out and I define the hatching date of the earliest chick as Day 1 for that brood. For each brood, on Day 5, Day 10 and Day 15 a 1-hr video was taken at each nest between 0400 to 1600 hr, during which parent swallows were most active in feeding; and avoiding extreme weather during observation. The videos taken were later used to calculate the feeding frequencies of parent swallows. After the video was taken the body mass of each chick in the brood was measured. The same procedures were followed for both the first and second broods.

a)

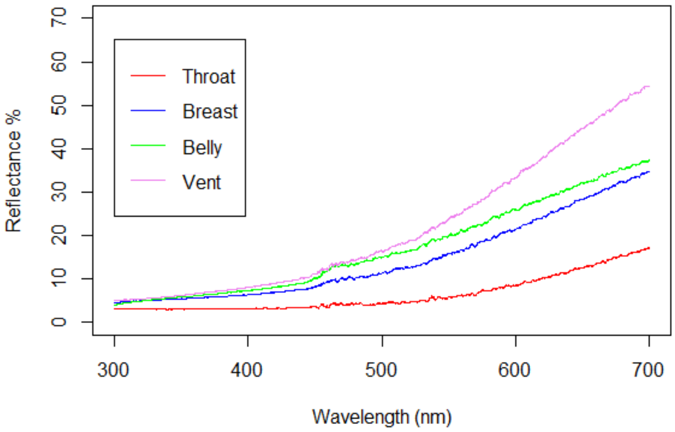
b)

Figure 2.1 Potential nesting sites of barn swallows. a) A barn swallow nesting under an eave. b) A nest built on the top of a pipe in the corner of a stairwell on the top storey of a building of seven storeys.

Plumage colour measurement

I measured the plumage samples with a spectrophotometer (USB4000, light source: PX-2 / DH-2000-BAL, Ocean Optics, US) and saved the reflectance spectra of each sample. Spectra range included ultraviolet (300 – 400 nm) and visible light (400 – 700 nm). Each sample was measured three times and three spectra from each sample were averaged for analysis. The spectra were transformed into a tetrahedral colour space 'TetraColorSpace' using the R package 'pavo', in which the colour of each sample was defined as one point in the space and described with three spherical coordinates (2) (Goldsmith 1990; Stoddard & Prum 2008). The TetraColorSpace estimates the stimulation of both visible light and ultraviolet light on the four types of retinal cones of bird eyes (ultraviolet-, short-wavelength-, medium-wavelength- and long-wavelength-sensitive cones) (Cuthill et al. 2000). In TetraColorSpace, each vertex of the tetrahedron stands for one type of cone colour channel, and the position of a colour in the space is described by spherical coordinates *theta, phi* and *r*, which are the horizontal angle, the vertical angle and the distance to the origin point (Stoddard & Prum 2008). Thus, *theta* roughly represents the hue of visible light colour, *phi* represents the ultraviolet hue and *r* describes the chroma (saturation) of the colour. The brightness of the plumage was also calculated from the spectra, which is the mean reflectance over the spectra.

a)



b)

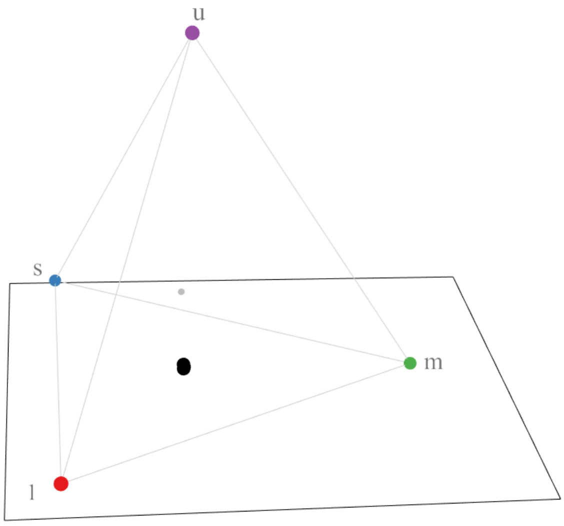


Figure 2.2 An example of the ventral plumage colour in four different body regions (throat, breast, belly and vent) of one male barn swallow shown as a) reflectance spectra or b) in Tetracolorspace. In Tetracolorspace, the four vertexes of the tetrahedron correspond to four different cone colour channels: ultraviolet (u), blue (s), green (m) and red (l).

Statistical analyses

Levene’s tests were performed to test the equality of variances for potential sexually selected traits (the length of tail streamers and ventral plumage colour) for the two sexes, and Welch two sample t-tests were performed to test the sexual dimorphism of these traits in barn swallow. Pearson's product-moment correlations were performed to explore the assortative mating patterns of potential sexually selected traits in barn swallow breeding pairs.

PCA on morphological and colour traits

As morphological traits were intercorrelated, I used principal components analysis (PCA) to collapse morphological traits (body mass, tarsus length, wing length and central tail feather length) into a smaller number of uncorrelated principal components. The PCA was performed using the function 'prcomp' in the R package 'stats', and variables were scaled to have unit variance before the analysis took place. The first three principal components (PC1, PC2 and PC3) explained more than 80% of the variation in these morphological traits (Table 2.1). Thus, these three PC scores were used in the statistical analyses.

Similarly, PCA was used to reduce the dimensionality of plumage colour traits. For the colour traits (*theta, phi, r* and brightness), the first two PC values explained more than 80% of their variation and these were used for analysis (Table 2.2).

Reproductive success

For each pair of barn swallows, reproductive success was assessed by recording the following: chick hatching date (Day 1), the number of eggs and fledglings produced during the entire breeding season. For the number of fledglings, I used the number present on Day 15, just before nestlings departed from their nest.

Generalized linear mixed models (GLMM) were used to analyse the relationship between reproductive success indexes and different traits. In each GLMM a maximal model was created using each of the measures of reproductive success as the dependent variable with using as independent variables the morphological traits (morphological PC1, PC2 and PC3 of both members of the pair) and potential sexually selected traits (streamer length of both sexes, colour PC1 and PC2 of throat, belly and vent regions of both sexes) as fixed factors and the year (2013 and 2015) as a random factor. Models were simplified from this maximal model through stepwise dropping of factors insignificantly correlated with the dependent variable until all factors were significant with $\alpha = 0.05$, and analyses of variance (ANOVA) were performed on the minimal models to calculate the proportion of explained variance by each factor. Analyses were conducted with the R software (R Foundation for Statistical Computing, Vienna, Austria). The results are presented from the minimal models, the output from the maximal models are shown in the appendices (Table S4).

Results

Morphological and colour traits of barn swallows

The first principal component of morphological traits (morphological PC1) was strongly positively correlated with two variables, mid-tail length and wing length so higher morphological PC1 represented swallows with longer mid tails and wings (Table 2.1). The second principal component (morphological PC2) was positively correlated with tarsus length and the third (morphological PC3) was negatively correlated with body mass, meaning that swallows with higher PC2 or PC3 values have longer tarsus or lighter body mass respectively (Table 2.1).

Table 2.1 The loadings and the importance of principal components for morphological traits. Correlation values between principal components and morphological traits are shown, as well as the cumulative proportion of variance.

	PC1	PC2	PC3	PC4
Tarsus length	-0.09	0.89	0.08	0.43
Mid-tail length	0.72	-0.22	-0.14	0.64
Wing length	0.68	0.36	0.15	-0.62
Body mass	-0.01	0.16	-0.98	-0.15
Standard deviation	1.12	1.04	1.00	0.81
Cumulative proportion of variance	0.31	0.58	0.83	1.00

For throat regions, the first principal component (throat colour PC1) was positively correlated with *theta* and *r* and negatively correlated with *phi*, while throat colour PC2 was negatively correlated with brightness (Table 2.2). PCA on belly and vent colour shows a similar pattern, with PC1 correlated with *theta*, *phi* and brightness and PC2 correlated with *r* (Table 2.2).

Table 2.2 The loadings and the importance of principal components for colour traits of different body regions: a) throat, b) belly, c) vent. Correlation values between principal components and morphological traits and the cumulative proportion of variance are shown.

a)

	PC1	PC2	PC3	PC4
theta	0.53	-0.45	0.40	-0.60
phi	-0.62	-0.10	-0.35	-0.70
r	0.58	0.24	-0.76	-0.17
brightness	-0.05	-0.85	-0.38	0.35
Standard deviation	1.50	1.13	0.52	0.44

Cumulative proportion of variance	0.57	0.88	0.95	1.00

b)

	PC1	PC2	PC3	PC4
theta	0.54	-0.45	0.40	-0.59
phi	-0.63	0.21	-0.04	-0.75
r	-0.20	-0.77	-0.61	-0.02
brightness	0.53	0.41	-0.68	-0.29
Standard deviation	1.51	1.18	0.50	0.28
Cumulative proportion of variance	0.57	0.92	0.98	1.00

c)

	PC1	PC2	PC3	PC4
theta	0.61	-0.06	0.45	-0.66
phi	-0.59	0.28	-0.23	-0.73
r	-0.01	-0.89	-0.40	-0.20
brightness	0.54	0.35	-0.76	-0.05
Standard deviation	1.59	1.10	0.45	0.23
Cumulative proportion of variance	0.63	0.94	0.99	1.00

Sexual dimorphism and assortative mating in potential sexually selected traits

In this population, the streamer length of male barn swallows was significantly longer than that of females (male: n = 80, mean ± SD = 100.99 ± 7.72 mm; female: n = 85, mean ± SD = 84.25 ± 5.22 mm; Welch two sample t-test: T = 16.23, df = 137.74, p < 0.01) and more variable (Levene’s test: F = 16.31, $p < 0.01$). Between males and females in each breeding pair there was no assortative mating in streamer length (Pearson's product-moment correlation: coefficient = 0.08, df = 75, $p = 0.46$).

Throat colour PC1 and PC2 values were not significantly more variable in males than females (Levene’s test: both F < 0.93, $p > 0.16$) but were significantly different in mean value from those of female swallows (Welch two sample t-test: throat PC1: T = -3.73, df = 163.92, p < 0.01; throat PC2: T = 5.89, df = 161.05, p < 0.01). Male belly colour PC values were not significantly different from those of females (Welch two sample t-test: belly PC1: T = -0.98, df = 159.98, p = 0.33; belly PC2: T = -0.83, df = 163, p = 0.41), while belly PC2 values differed significantly in variability between males and females (Levene's test: F = 8.54, $p < 0.01$) but belly PC1 values did not (Levene's test: F = 8.54, $p < 0.01$). Both vent PC1 and PC2 values differed significantly in variability between males and females (Levene's test: vent PC1 and PC2: both F > 4.56, $p < 0.05$) while only vent PC1 values differ significantly between both sexes (Welch two sample t-test: vent PC1: T = -3.05, df = 161.79, p < 0.01; belly PC2: T = -0.87, df = 163.86, p = 0.39). The ventral plumage colouration of paired barn swallows was correlated in throat and vent regions (Pearson's product-moment correlation: throat/vent colour PC1/PC2: all coefficient = 0.27 – 0.85, df = 76, $p < 0.02$), but not in both belly colour traits (Pearson's product-moment correlation: belly colour PC1: coefficient = 0.14, df = 75, $p = 0.23$; PC2: coefficient = 0.86, df = 75, $p < 0.01$).

Reproductive success and potential sexually selected traits

Hatching date

Hatching date was positively correlated with both male and female morphological PC3, suggesting that heavier male and female swallows started breeding earlier (Table 2.3). Also, hatching date was negatively correlated with female streamer length, so female swallows with longer streamers started breeding earlier than females with short streamers (Figure 2.3, Table 2.3). There was a negative correlation with male throat colour PC2 values, suggesting that male swallows with darker throat plumage tended to start breeding earlier (Figure 2.3, Table 2.3). In addition hatching date was positively correlated with male morphological PC2 and female PC1, and this indicates that males with longer tarsi or females with longer mid-tails and wings tended to breed later than other individuals (Table 2.3). The variance explained suggests that male morphological

PC3, female morphological PC3, female streamer length and male throat colour PC2 explained substantial amounts of variance (43%, Table 2.3).

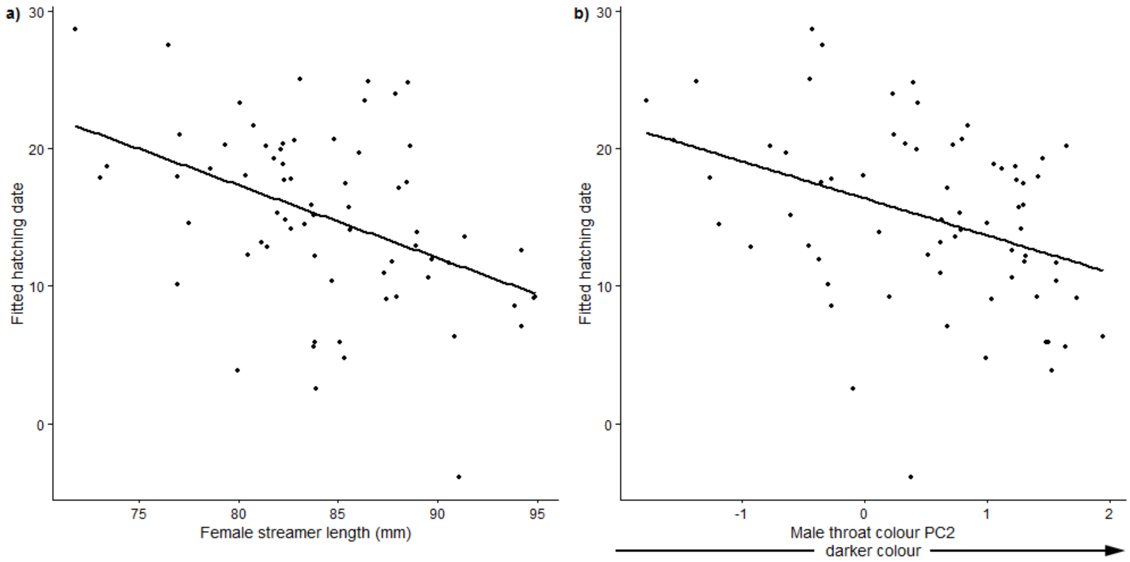


Figure 2.3 Hatching date was negatively correlated with (a) female streamer length and (b) male throat colour PC2 in the minimal GLMM model. Fitted values of the hatching date from the minimal GLMM model and simple regression lines are shown.

Number of eggs and fledglings

Variation in the number of eggs and fledglings was influenced by similar parental traits: female morphological PC3, female streamer length and male throat colour PC2 or male belly PC1 (Table 2.3). This result suggested that heavier and long-streamered female swallows had more eggs and fledglings each year, as did females mated with male swallows with darker and browner ventral plumage colour (Figure 2.4). The variance explained by GLMM models with the number of eggs and the number of fledglings as dependent variables suggested that the influence on both these variables was mainly from female body mass, female streamer length and male ventral plumage colour (Table 2.3). Other factors that may influence the number of eggs or fledglings include male morphological PC2 and female belly colour PC1 (Table 2.3).

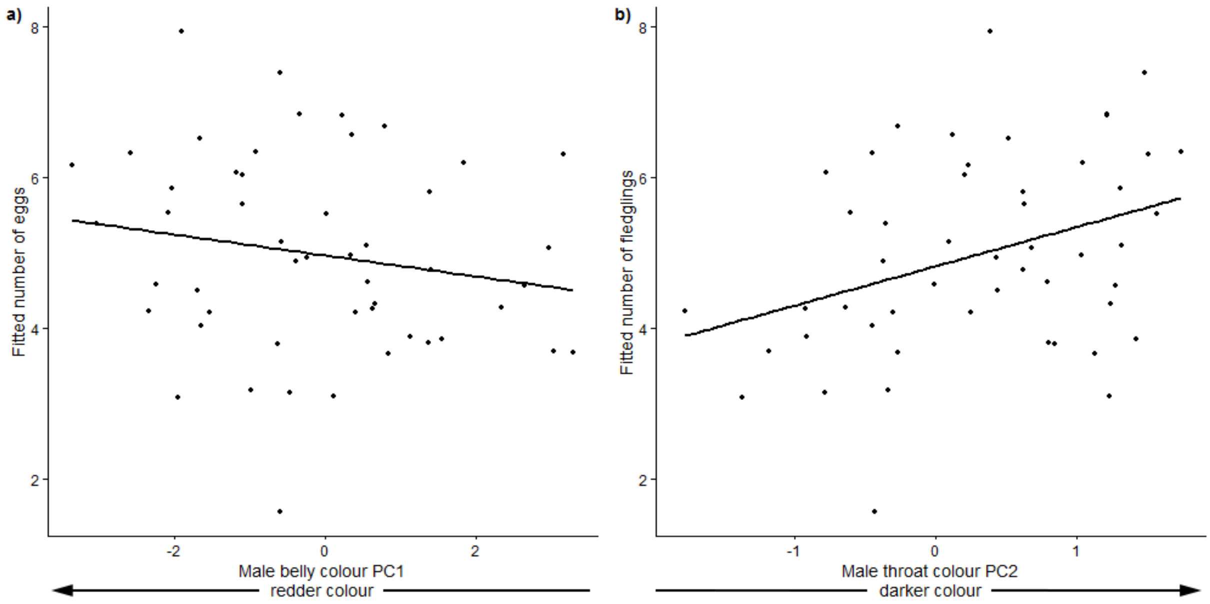


Figure 2.4 Male ventral plumage colour predicted the number of offspring in the breeding season. a) the number of eggs in relation to male belly colour PC1, b) the number of fledglings in relation to male throat colour PC2. Fitted values of the number of eggs or fledglings from minimal GLMM models and simple regression lines are shown.

Table 2.3 Factors predicting reproductive success in barn swallow. Reported values of significant factors ($p < 0.05$, in bold) are from the final model, whereas values of non-significant terms are from the model prior to the elimination of that factor. Variance explained by each fixed significant factor is shown. Sample size: hatching date: n = 68; number of eggs: n = 51; number of fledglings: n = 50.

	Hatching date				Number of eggs				Number of fledglings			
	coef.	T	*p*	PctExp	coef.	T	*p*	PctExp	coef.	T	*p*	PctExp
Morphological Traits												
male												
PC1	1.06	1.25	0.22	-	0.04	0.13	0.90	-	-0.17	-0.68	0.50	-
PC2	**2.42**	**2.79**	**<0.01**	**5.82%**	**-0.70**	**-2.59**	**0.01**	**8.65%**	**-0.64**	**-2.75**	**0.01**	**8.60%**
PC3	**4.61**	**3.39**	**<0.01**	**8.58%**	0.20	0.42	0.68	-	0.22	0.48	0.64	-
female												
PC1	**2.28**	**2.71**	**<0.01**	**5.49%**	-0.17	-0.63	0.53	-	0.00	0.00	1.00	-
PC2	1.35	1.89	0.06	-	-0.30	-1.31	0.20	-	-0.18	-0.86	0.39	-
PC3	**2.53**	**3.61**	**<0.01**	**9.73%**	**-0.59**	**-3.27**	**<0.01**	**13.75%**	**-0.39**	**-2.44**	**0.01**	**6.78%**
Streamer Length												
male	-0.12	-1.15	0.25	-	0.02	0.45	0.65	-	0.03	1.02	0.32	-
female	**-0.57**	**-3.23**	**<0.01**	**7.81%**	**0.10**	**2.37**	**0.02**	**7.21%**	**0.13**	**3.25**	**<0.01**	**12.00%**
Colour Traits												
male												
throat PC1	-1.44	-2.02	0.22	-	0.22	0.75	0.46	-	0.08	0.26	0.80	-
throat PC2	**-4.60**	**-4.75**	**<0.01**	**16.90%**	0.92	3.36	0.28	-	**0.73**	**3.08**	**<0.01**	**10.80%**
belly PC1	-0.13	-0.18	0.85	-	**-0.44**	**-2.93**	**0.01**	**11.08%**	**-0.35**	**-2.66**	**0.01**	**8.07%**
belly PC2	-0.73	-0.42	0.67	-	-0.29	-0.52	0.61	-	-0.39	-0.89	0.38	-
vent PC1	-0.99	-1.85	0.07	-	-0.01	-0.05	0.96	-	0.00	0.01	1.00	-
vent PC2	0.75	0.34	0.73	-	0.04	0.06	0.95	-	0.02	0.03	0.98	-
female												
throat PC1	0.65	0.70	0.49	-	0.05	0.11	0.91	-	-0.02	-0.06	0.95	-
throat PC2	1.12	0.89	0.38	-	0.23	0.88	0.38	-	0.11	0.36	0.72	-
belly PC1	-0.71	-1.27	0.21	-	0.24	1.42	0.16	-	**0.32**	**2.05**	**0.04**	**4.79%**
belly PC2	-0.17	-0.10	0.92	-	-0.30	-1.00	0.32	-	-0.31	-0.64	0.52	-
vent PC1	1.29	1.80	0.08	-	-0.16	-0.67	0.51	-	-0.14	-0.63	0.54	-
vent PC2	1.22	0.61	0.55	-	0.40	0.77	0.44	-	0.17	0.56	0.58	-
R-squared				0.49				0.50				0.46

Discussion

Sexual selection in barn swallows has been studied for about 25 years and it has been shown that tail streamer length is the sexually selected trait in European barn swallows (Møller 1994c) while plumage colouration fulfills this function in North American populations (Safran & McGraw 2004). Compared with other subspecies, northeastern Chinese barn swallows have intermediate streamer length and intermediate dark ventral plumage colour (Turner 2006). Molecular evidence suggested that Asian barn swallows (*H. r. tytleri*) and American barn swallows (*H. r. erythrogaster*) are part of the same clade in the phylogeny (Dor et al. 2010), and this sister relationship in evolutionary history may indicate their consistency in morphology and ethology. In my study the result shows that hatching date, the number of eggs and fledglings can be predicted by the plumage colour of male adult swallows, but not the length of tail streamers. These results are more comparable to North American barn swallows than to European swallows.

Clutch initiation date is considered to be an important index for a swallow's breeding success, as early breeding onset leaves more time for the second clutch. In classic studies of European barn swallows, male streamer length, the mate choice cue, also indicates male quality, and males with longer streamers arrive at breeding sites earlier and gain greater reproductive success (Møller 1994b). In contrast, I found that the clutch initiation date of northeast Chinese barn swallows is explained by male plumage colour (Figure 2.2). This pattern is the same as has been found in North American barn swallows, in which male streamer length does not predict reproductive success, while the ventral plumage colour does (Neuman et al. 2007; Safran & McGraw 2004). In terms of number of fledglings, which perhaps is the most direct reflection of reproductive success, the number of fledglings was greater for male swallows with darker throat colour than for males with pale throat colour (Figure 2.3), variation in male streamer length did not correlate with the number of fledged young. Based on these results, I thus infer that plumage colour should be regarded as the sexually selected trait in northeastern Chinese barn swallow populations.

In my study population, the breeding success of barn swallows was also explained by female streamer length. For *H. r. mandschurica*, female swallows with longer streamers started breeding earlier, laid more eggs and had more offspring than female with shorter tail streamers. This result is consistent with previous research on European barn swallows, *H. r. rustica*, in which the streamer length of female barn swallows is considered as the reflection of their reproductive potential. For *H. r. rustica*, unlike streamers of male swallows that are selected by females, streamers of female swallows are not considered to be under mate choice and tail length manipulation experiments caused no significant change in their reproductive success (Cuervo, de Lope & Møller 1996a; Cuervo et al. 1996b). However, as longer-tailed females have greater seasonal reproductive success, e.g. number of fledglings, female streamer length is considered to be an honest signal of female body condition (Møller 1993b). Also, artificial elongation of female streamers is detrimental to the long-term reproduction of female barn swallows, suggesting that the streamer length of female swallows is limited to natural selection (Cuervo, Møller & de Lope 2003). Meanwhile I did not find any significant correlation between any measure of breeding success and male streamer length, nor did I find assortative mating in streamer length, again suggesting that male streamer length is not the cue for mate choice in our population. In summary I found that the streamer length of female barn swallows is probably under natural selection rather than sexual selection and is a reliable predictor of their reproductive potential, as it predicts the breeding success of female barn swallow rather than the streamer length of their mates.

In conclusion, our research showed that for northeastern Chinese barn swallows, male plumage colour is likely to be the sexually selected trait; I found little evidence for sexual selection on male streamer length. Male swallows with darker or redder plumage started breeding earlier and had more offspring than males with pale ventral colouration. This result made our population more similar to *H. r. erythrogaster* than to *H. r. rustica*, and confirmed a pattern of differentiation in sexual signals among closely related populations. Further experiments are required to investigate the intrinsic sexual selection mechanism of northeastern Chinese barn swallows.

Chapter III. Extra-pair offspring and breeding success in northeastern Chinese barn swallows

Abstract

In sexual selection, males increase their reproductive fitness by fathering more or higher quality offspring. Thus, males may seek extra-pair copulation (EPC) to sire more extra-pair offspring (EPO), while extra-pair females may also benefit from receiving EPC through improvement in the genetic quality of her offspring. Meanwhile, males may decrease their investment on parental care if their social mate has EPCs with other males, so females need to balance the costs and benefits of producing EPO. In northeastern Chinese barn swallows, I tested whether the potential sexually selected traits, the length of tail streamers and ventral plumage colouration, are related to the reproductive success of males. Also, I tested what factors, e.g. parental provisioning or the genetic quality of parents, predict the quality of offspring. The analysis shows that males with darker plumage colour fathered more and higher quality offspring than other males, which supports that ventral plumage colour is the sexually selected trait in this population.

Introduction

In sexual selection, male animals usually compete with each other directly through combat or indirectly through displaying specific sexually selected traits, aiming to get a mate(s) and reproduce (Davies et al. 2012). For instance, in northern elephant seals (*Mirounga angustirostris*) only a few males which are the winners in intrasexual competition and have high social ranks are responsible for the majority of copulations (Le Boeuf 1974), while in blue peafowls (*Pavo cristatus*) peahens prefer peacocks with more elaborate tail ornaments and males with more eyespots on their trains are more likely to have greater mating success (Petrie et al. 1991). Males with more exaggerated sexually selected traits might gain an advantage in sexual selection by copulating with more females, either their social mates or extra-pair mates, to father more offspring (Andersson 1982; Kempenaers, Verheyen & Dhondi 1997). Thus, for monogamous animals, the male winners of male-male competition or female choice gain greater fitness by producing more genetic offspring (including extra-pair offspring, EPO) than other males.

On the other hand, in sexual selection females are choosy about their mates and are typically more concerned with having offspring of higher genetic quality (Fisher et al. 2006). In this case females would likely accept mates with better body condition, which are usually males with more exaggerated sexual ornaments e.g. the three-spined stickleback *Gasterosteus aculeatus* (Milinski & Bakker 1990). When the access to better quality mates is limited, females would seek or accept extra-pair copulation to increase the genetic quality of her offspring. However having extra-pair copulations may impact on the relationship between individuals in a social pair, e.g. for species that require parental care from both parents, males are known to invest less in feeding offspring when their mates copulate with other males (Sheldon, Räsänen & Dias 1997). Decreased parental care from males may affect the body condition of the offspring, so females need to balance the potential benefits of extra-pair copulation and the potential cost of losing parental care from her social mate.

The barn swallow is a small monogamous bird, whose nestlings are usually taken care of by both parents (Turner 2006). Previous studies have shown that extra-pair offspring are common in barn swallows, with the ratio of extra-pair offspring to all offspring ranging from 18% to 31% in different populations (Turner 2006). As female swallows can easily reject males attempting to copulate with them by flying away, extra-pair offspring in barn swallows are usually treated as the result of females accepting extra-pair copulation. Under these assumptions, attractive males may have more offspring by fertilizing the eggs of extra-pair females or receiving greater paternity in their own nests. This hypothesis has been supported with both empirical and experimental studies, e.g. in European populations males with longer streamers than other males can gain more offspring during the breeding season (Møller & Tegelström 1997), while for American barn swallows males with experimentally enhanced ventral plumage colour were less cuckolded by their social mates(Safran et al. 2005).

In this study, I aim to test whether there is a relationship between barn swallows' potential sexually selected traits and their breeding success in the northeastern Chinese populations (*H. r. mandschurica*), considering both within-pair and extra-pair offspring. Further, I explore the main factor affecting the body condition of barn swallow nestlings, e.g. the body condition of their parents, parental feeding investment on nestlings or foraging competition pressure from the same nest. If the quality of nestlings can be predicted by the quality of their fathers, females are more likely to seek extra-pair copulations to acquire high quality offspring rather than to have more parental care from their social mates.

Methods

Fieldwork methods

I studied a population of barn swallows in Shuangyashan City, Heilongjiang Province, China (46° 35' N, 131° 14' E) from June to September in 2015. After active nests were identified, they were visited every two days and the number of eggs in each nest was recorded. Parent swallows were caught using mist nets during the first five days after their chicks hatched as experience suggested that there was a high risk of them abandoning their nests if they were caught earlier. Swallows were marked with a unique combination of coloured plastic rings on each of their legs. Different sexes were identified by checking for the presence (females) or absence (males) of a brood patch.

For each bird, the length of both wings was measured to the nearest 1 mm using a ruler with a pin-zero-stop, the length of left tarsus, the length of the shortest tail feather (mid-tail) and the length of both streamers were measured to the nearest 0.01mm with digital calipers (Jazooli, UK), and the body mass of swallows was measured to the nearest 0.01 g with a digital balance (ChangXie, CX-168, China). Plumage samples were taken from throat, belly and vent regions. For each plumage region, four to ten feathers were plucked and stuck to white paper cards. These samples were stored in the dark for future spectrophotometer tests. Blood samples (about 70 µL from each bird) were collected from the brachial vein; whole blood was stored in lysis buffer for later DNA extraction. Unhatched eggs and dead nestlings were also collected and stored in ethanol, -18℃. In total 43 pairs of barn swallows were caught and their breeding success was assessed. In the analysis of adult barn swallow morphological and colour traits, data of all swallows caught from this population were included (in total: male: n = 81, female: n = 85). With the hatching date of the earliest chick defined as Day 1, on Day 5, Day 10 and Day 15 a 1-hr video was taken at each nest between 0400 to 1600 hr, during which parent swallows were active in feeding and avoiding extreme weather during observation. A camcorder (SONY, HDR- CX220E, Japan) was set up about 5 m from the nests to take the videos, during the process I hid away from the nests to avoid disturbing the adult barn swallows. The videos taken on Day 10 and Day 15 were later used to calculate the feeding frequencies of parent swallows. After the video was taken I measured the body mass of each chick in the brood and on Day 15 blood samples of chicks were collected. The same procedures were followed for both the first and second broods (first broods: n = 42, second broods: n = 13).

Plumage colour measurement

I measured the plumage samples with a spectrophotometer (USB4000, light source: PX-

2 / DH-2000-BAL, Ocean Optics, US) and the reflectance spectra of each sample were saved. Each sample was measured three times and the three spectra from each sample were averaged for analysis. The spectra were transformed into the TetraColorSpace and the brightness of the plumage colour was calculated from the spectra. The detailed method of plumage colour measurement has been described in Chapter II.

Paternity analysis

DNA was extracted from both parent and nestling barn swallow blood samples, unhatched eggs and dead nestling muscle tissue using TIANamp genomic DNA Kit (TIANGEN Biotech, Beijing, China). Single or multiple polymerase chain reaction (PCR) was used to amplify 7 microsatellite loci: Escu6 (Hanotte et al. 1994), Ltr6 (McDonald & Potts 1994), POCC6 (Bensch, Price & Kohn 1997), Hir6, Hir11, Hir17 and Hir20 (Tsyusko et al. 2007). Escu6, Ltr6, Hir6 and Hir20 were combined into one multiplex mix (mix M) while POCC6, Hir11 and Hir17 were amplified individually. Multi PCR Kit (TIANGEN Biotech, Beijing, China) was used for PCR amplification of mix M and 2X Taq PCR MasterMix (TIANGEN Biotech, Beijing, China) was used for that of POCC6, Hir11 and Hir17. PCR thermal cycles were as following: initial denaturation at 95℃ for 15 min (mix M) or 94℃ for 1 min 40 s (POCC6, Hir11, Hir17); 10 cycles of denaturation at 94℃ for 30 s, annealing at 55℃ (mix M, Hir11, Hir17) or 60℃ (POCC6) for 30 s, and extension at 72℃ for 45 s; 25 cycles of denaturation at 87℃ for 30 s, annealing at 55℃ (mix M, Hir11, Hir17) or 60℃ (POCC6) for 30 s, and extension at 72℃ for 45 s; and a final extension at 72℃ for 5 min. PCR products were genotyped on a 3730 DNA Analyzer with GeneScan 500 LIZ as size standard (Thermo Fisher, MA, USA). Allele sizes were estimated with Genemapper V4.0 (Thermo Fisher, MA, USA) and rounded to the nearest whole number manually. Paternity relationship was assigned using Cervus 3.0.7 based on the confidence level of the most likely candidate parent pair (Field Genetics, London, UK).

Statistical analysis

PCA on morphological and colour traits

As morphological traits were intercorrelated, I used principal components analysis (PCA) to collapse morphological traits (body mass, tarsus length, wing length and central tail feather length) into a smaller number of uncorrelated principal components. The PCA was performed using the function 'prcomp' in the R package 'stats', and variables were scaled to have unit variance before the analysis took place. The first three principal components (PC1, PC2 and PC3) explained more than 80% of the variation in these morphological traits (Table 2.1). Thus, these three PC scores were used in the statistical analyses.

Similarly, PCA was used to reduce the dimensionality of plumage colour traits. For the colour traits (*theta, phi, r* and brightness), the first two PC values explained more than 80% of their variation and these were used for analysis (Table 2.2). See Chapter II for the result of all PCA analyses.

Reproductive success

Analyses were conducted with the R software (R Foundation for Statistical Computing, Vienna, Austria.). Generalized linear models (GLM) were used to test the relationships between male morphological traits (morphological PC1, PC2 and PC3), potential sexually selected traits (streamer length, PC1 and PC2 of throat, belly and vent colour) and the number of their genetic offspring. In GLM a maximal model was created using the number of genetic offspring as the dependent variable with the male morphological traits and potential sexually selected traits as independent variables. Models were simplified from this maximal model through stepwise dropping of factors insignificantly correlated with the dependent variable until all factors were significant with α = 0.05, and analyses of variance (ANOVA) were performed on the minimal models to calculate the percentage of explained variance by each factor. The results are presented from the minimal models, the output from the maximal models are shown in the appendices (Table S5). Using a similar method, the relationship between offspring body mass on Day 15 and parent morphological traits (morphological PC1, PC2 and PC3), potential sexually selected traits (streamer length, PC1 and PC2 of throat, belly and vent colour), number of nestlings in the brood and parent feeding frequency was tested (see Table S6 for the maximal model).

Results

Paternity analysis

For the paternity analysis, all unhatched eggs, dead nestlings and blood samples from alive nestlings were included (unhatched eggs: n = 8, dead nestlings: n =4, blood samples: n = 229). The combined exclusion probability of seven loci for the first parent was 0.995, for the second parent 0.9998 and for the parent pair 0.9999993. Overall nine of 42 first broods (21.4%) and two of 13 second broods (15.4%) hosted at least one extra-pair offspring. Within first broods 19 of 191 offspring (9.9%) were extra-pair offspring, while within second broods nine of 50 offspring (18.0%) were extra-pair offspring. Among all extra-pair offspring, 46.4% (13 out of 28) can be assigned for a father.

The number of genetic offspring of males

In first broods, the number of genetic offspring of males was negatively correlated with male morphological PC3 and positively correlated with throat colour PC1 and PC2 (Table 3.1). ANOVA analysis showed that morphological PC3 and throat colour PC2 combined to explain 28.38% of the variance (Table 3.1). This result suggests that male swallows with heavier body mass or darker throat colour tended to have more genetic offspring (Figure 3.1).

Table 3.1 Male traits influencing the number of their genetic offspring in northeastern Chinese barn swallows. Results of the minimal GLM model and the variance explained by each factor are shown. Reported values of significant factors ($p <$ 0.05, in bold) are from the final model (df = 41), whereas values of non-significant terms are from the model prior to the elimination of that factor. Variance explained by each significant factor is shown.

	Number of genetic offspring				
	coefficient	SE	T	*p*	PctExp
Male Traits					
morphological					
PC1	-0.34	0.24	-1.41	0.17	-
PC2	0.27	0.27	1.00	0.32	-
PC3	**-1.03**	**0.39**	**-2.67**	**0.01**	**11.66%**
streamer length	0.01	0.03	0.33	0.74	-
colour					
throat PC1	**1.15**	**0.48**	**2.38**	**0.02**	**9.29%**
throat PC2	**1.90**	**0.59**	**3.19**	**<0.01**	**16.72%**
belly PC1	0.31	0.17	1.82	0.08	-
belly PC2	0.35	0.54	0.66	0.52	-
vent PC1	-0.16	0.19	-0.84	0.40	-
vent PC2	-0.34	0.71	-0.48	0.63	-
R-squared					0.28

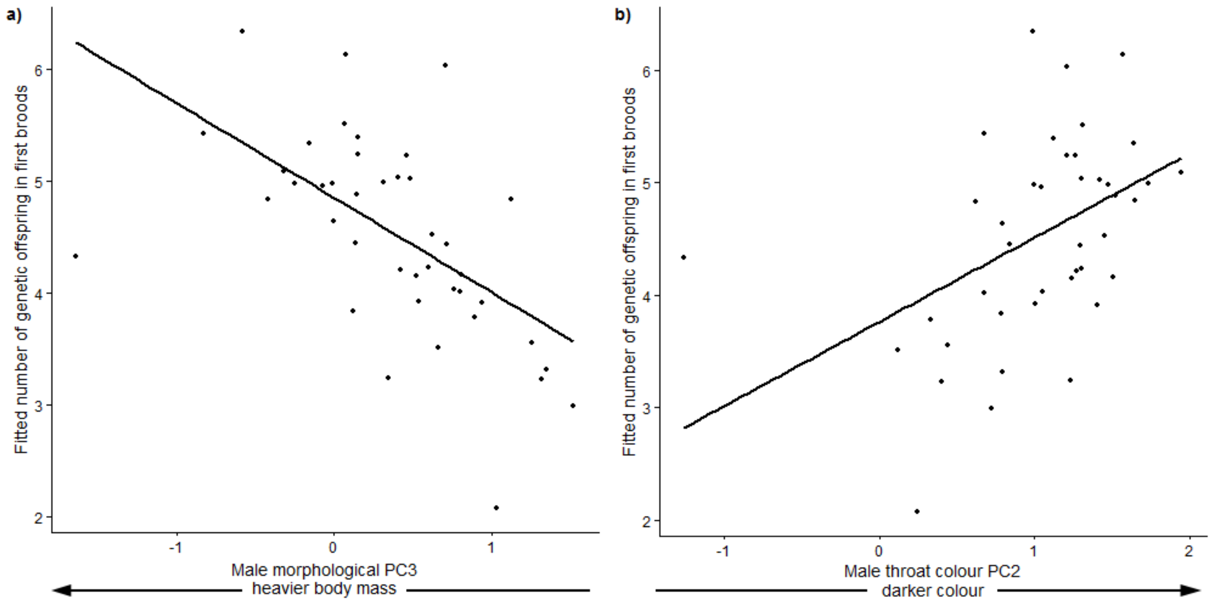


Figure 3.1 The number of genetic offspring of male barn swallows in first broods was negatively correlated with a) male morphological PC3 and positively correlated with b) male throat colour PC2 in the minimal GLM model. Fitted values of the number of genetic offspring from the model and simple regression lines are shown.

Offspring body mass

Variation in the body mass of offspring was influenced by the following traits: male streamer length, male morphological PC1 and PC2, female morphological PC2 and PC3, male belly colour PC1, male throat colour PC1 and PC2, male vent colour PC1, female throat colour PC1 and the number of nestlings in each brood (Table 3.2). Examination of the variance explained suggests that the greatest influence on offspring body mass was from male morphological PC1 (9.96%), male streamer length (9.46%), male vent colour PC1 (8.57%) and number of nestlings in the brood (3.09%) (Table 3.2). The negative correlation between offspring body mass and male streamer length suggests that male barn swallows with longer tail streamers have offspring with lighter body mass than those of other males (Figure 3.2). Offspring body mass was positively correlated with male morphological PC1, suggesting that offspring of males with longer mid-tail lengths and wings were heavier, while negatively correlated with male vent colour PC1 suggesting that so were offspring of males with ‘redder’ vent plumage than other males (Figure 3.2). Also, there was a positive correlation with offspring body mass and number of nestlings in the brood, which meant that with more nestlings in the same nest these nestlings also grew faster than those in smaller broods (Table 3.2).

Table 3.2 Parent traits influencing the body mass of offspring. Reported values of significant factors ($p < 0.05$) are from the final model (df = 210), whereas values of non-significant terms are from the model prior to the elimination of that factor. Variance explained by each significant factor is shown and factors explaining more than 3% of the variance are shown in bold.

	Nestling body mass				
	coefficient	SE	T	*p*	PctExp
Morphological Traits					
male					
PC1	**0.66**	**0.11**	**6.14**	**<0.01**	**9.96%**
PC2	0.36	0.11	3.33	<0.01	2.92%
PC3	-0.17	0.17	-1.02	0.31	-
female					
PC1	-0.11	0.09	-1.17	0.25	-
PC2	0.24	0.09	2.72	<0.01	1.96%
PC3	-0.47	0.14	-3.35	<0.01	2.96%
Streamer Length					
male	**-0.08**	**0.01**	**-5.99**	**<0.01**	**9.46%**
female	0.04	0.02	1.79	0.07	-
Colour Traits					
male					
throat PC1	0.49	0.19	2.51	0.01	1.66%
throat PC2	0.68	0.23	2.99	<0.01	2.37%
belly PC1	0.25	0.10	2.59	0.01	1.77%
belly PC2	-0.03	0.33	-0.10	0.92	-
vent PC1	**-0.40**	**0.07**	**-5.70**	**<0.01**	**8.57%**
vent PC2	0.06	0.53	0.11	0.91	-
female					
throat PC1	-0.37	0.12	-3.24	<0.01	2.77%
throat PC2	-0.07	0.34	-0.22	0.83	-
belly PC1	0.03	0.11	0.27	0.79	-
belly PC2	-0.09	0.26	-0.36	0.72	-

vent PC1	-0.10	0.07	-1.40	0.16	-
vent PC2	-0.26	0.37	-0.70	0.49	-
Total feeding rate	0.00	0.01	-0.38	0.71	-
Brood size	**0.37**	**0.11**	**3.43**	**<0.01**	**3.09%**
R-squared					0.38

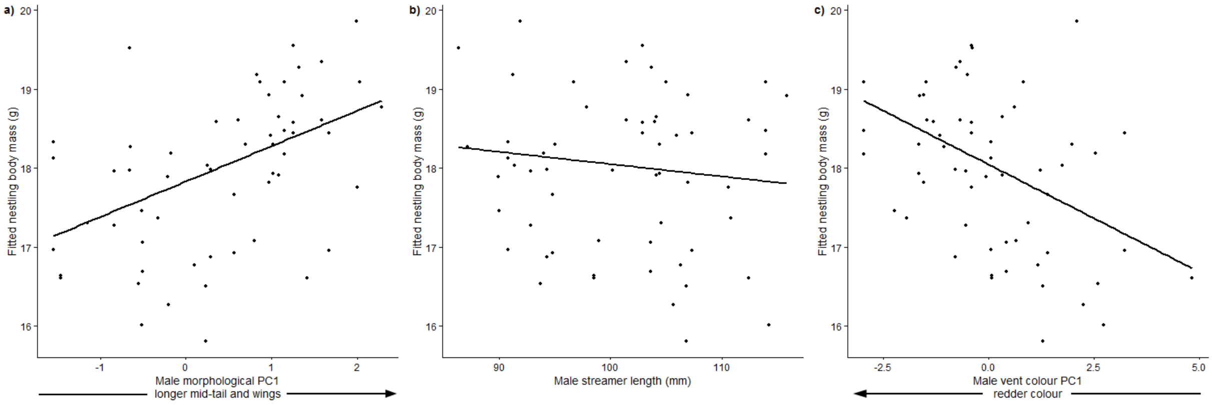


Figure 3.2 The body mass of offspring was positively correlated with a) male morphological PC1, negatively correlated with b) male streamer length and negatively correlated with c) male vent colour PC1 in the minimal GLM model. Fitted values of the nestling body mass from the model and simple regression lines are shown.

Discussion

In my study northeastern Chinese barn swallows have a relatively low ratio of extra-pair offspring: all offspring compared to European or North American barn swallows, but the ratio is within the range of eastern Asian barn swallows (Table 3.3). The existence of EPO in my population of *H. r. mandschurica* did not have a huge influence on the reproductive fitness of male barn swallows and male ventral colour predicted the number of genetic offspring similarly to the number of social offspring excluding EPO (Chapter II). The low ratio of EPO:WPO might be explained by reduced sexual selection pressure, such as lower male-female sex ratio, lower population density or more scattered nesting sites. For instance, one polygamous pair was found during my study with one male mated with two female swallows during one breeding season, indicating that females might have limited access to males of high quality. Also unlike other subspecies which may nest in large groups, northeastern Chinese subspecies seldom nest next to each other (though some pairs nest in neighboring floors of the same stairwell), illustrating different pairs are more separated compared with populations elsewhere.

Table 3.3 The ratio of extra-pair offspring in barn swallow populations from different parts of the world. The length of tail streamers, the colour of ventral plumage and their sexually selected traits are also described here.

Population	Subspecies	Streamer length	Ventral colour	Sexually selected trait	Ratio of EPO	
Europe	*H. r. rustica*	long	pale	tail streamers	18% - 29%	(Turner 2006)
North America	*H. r. erythrogaster*	intermediate	dark	ventral plumage colour	23% - 31%	(Turner 2006)
Japan	*H. r. gutturalis*	short	pale	throat patch	2.7% - 15.6%	(Hasegawa et al. 2010a; Kojima et al. 2009)
northeastern China	*H. r. tytleri* - *H. r. gutturalis* hybrids	short	intermediate	ventral plumage colour	11.6%	(this study)

My results show that male barn swallows with heavier body mass or with darker throat plumage than other males tended to produce more genetic offspring, which is similar with the result of Chapter II when testing with the number of social offspring. Research on *H. r. gutturalis* has shown that male swallows with better body condition can sire more extra-pair offspring, and this is consistent with what I found in northeastern Chinese barn swallows (Kojima et al. 2009). Also, it validates that plumage colour is the sexually selected trait in my study population, as males with more exaggerated trait gain greater breeding success both apparently and genetically. Ventral plumage colour as the sexual signal is consistent with previous research on North American barn swallows, which revealed that for *H. r. erythrogaster* males with dark rusty ventral plumage colour are less cuckolded and males with manually enhanced ventral plumage colour gained higher paternity from their mates (Eikenaar et al. 2011; Safran et al. 2005). For Asian barn swallows, the plumage is under sexual selection is also predicted by phylogenetic relatedness as they are more closely related to North American barn swallows compared to European barn swallows (Dor et al. 2010).

The body mass of offspring was predicted by the mid-tail and wing length and the vent colour of their genetic fathers. With heavier body mass than other nestlings, offspring of males with more exaggerated sexually selected traits are likely to be in better body condition and thus they may survive better. This result supports the view that females select males as their mates to obtain high quality offspring, which can be inherited from the male parent. Further, offspring body mass increased with the number of nestlings in the same brood, indicating that competition for food within the nest is unlikely to be the limitation on nestling body mass. Under this assumption, females are more likely to seek for extra-pair copulations with high quality males to increase the genetic quality of their offspring. But it is also noticed that though explained a relatively low portion of variance (1.77%), male belly colour PC1 is positively correlated with the offspring body mass, which is in the opposite direction to male vent colour PC1. Further ventral colour experiments of separate manipulation on different body regions, e.g. throat, breast, belly and vent may help to explain this inconsistency.

Meanwhile the offspring body mass is negatively correlated with male streamer length, further supporting the view that the tail streamers are not likely to be the sexual signal in northeastern Chinese barn swallows. For European barn swallows, the length of tail streamers is an honest signal indicating the body condition of males (Moller & de Lope 1994). Manipulation of the length of tail streamers affected the survivorship of male barn swallows (Moller & de Lope 1994), and aerodynamic research has shown that on average the natural length of *H. r. rustica* is 10 mm longer than the optimal length for flight (Buchanan & Evans 2000). On the other hand, the sexually selected trait in North American barn swallows, ventral plumage colour, does not seem costly to produce for barn swallow individuals but costly to maintain which may due to the social feedback of this signal (Safran et al. 2008). In this way, the tail streamer length and the ventral plumage colour both fit the assumption of Zahavi's handicap principle respectively in European and North American barn swallows, which supports that they are the sexually selected traits in corresponding barn swallow subspecies. In this study the long tail streamers were likely to be a handicap to male reproductive fitness as long-tailed males sired offspring of lower quality than short-tailed males (Figure 3.2), but they are unlikely to be sexually selected as long-tailed males cannot undertake this cost better than short-tailed ones. It still needs further study to demonstrate why long-tailed swallows tend to reproduce lighter offspring in this population, e.g. whether their foraging ability is impaired or these adults are of low genetic quality themselves.

In conclusion, in northeastern Chinese barn swallows, male plumage colour is considered as the sexually selected trait and males with darker plumage colour have more and higher quality offspring than males with pale plumage. The mechanism of plumage colour being an ‘honest signal’ in sexual selection still needs to be explored, in which manipulation experiments on the sexually selected trait are needed to clarify the function of ventral plumage colour as a sexual signal for male swallows.

Chapter IV. A trait manipulation experiment on the sexually selected trait of northeastern Chinese barn swallows

Abstract

Female animals may adjust their investment in reproduction partially according to the quality of their mates. Thus, if a signal of quality is enhanced in males, females would be expected to allocate more resources in reproduction, e.g. producing more offspring or providing more parental care on offspring. I tested this hypothesis in the barn swallow, whose sexually selected trait is ventral plumage colour in a northeastern Chinese population. I did not find that males with experimentally enhanced plumage colour obtained more offspring or gained greater reproductive fitness, which may due to the weak strength of sexual selection in this population. A larger sample size would be needed to classify the function of the male ventral plumage colour in the sexual selection of this barn swallow population in further experiments.

Introduction

In nature, female animals evaluate the body condition and genetic quality of males through specific traits (Davies et al. 2012). Based on these evaluations, females will prefer to choose mates of the highest perceived quality as their mates. In animals with different mating systems, males with the most exaggerated sexually selected trait may gain an advantage in reproduction in different ways. For polygamous animals, attractive males can have more mates than other males, e.g. the classic research on peacocks *Pavo cristatus* showed that there is a significant positive correlation between the number of mates a male obtains and the number of eye-spots in his elaborate train (Petrie et al. 1991). For monogamous animals, successful males can get mates that are in better condition and so they may sire more offspring, while they can further increase their reproductive success through extra-pair copulations with other females (Andersson

1994). On the other hand, if females are paired with males of low quality, they can seek extra-pair matings with higher quality males to raise the genetic quality of their offspring or lower their investment in reproduction. For instance, blue tit *Parus caeruleus* males that sing longer strophes during the dawn chorus suffer less from cuckoldry and have a lower proportion of extra-pair offspring in their nests (Kempenaers et al. 1997).

The barn swallow is a small, monogamous passerine, and the nestlings are cared for by both parents (Turner 2006). The barn swallow is a classic example of sexual selection as in Europe male barn swallows with long tail streamers are attractive to females and obtain greater breeding success (Møller 1994c). It has also been observed that males with long tail streamers have more extra-pair copulations than those with short streamers (Møller 1992), and they have lower proportion of extra-pair offspring in their nests (Møller & Tegelström 1997). Females with extra-pair nestlings in their nests, on the other hand, experience a loss in parental care from their mates (Møller & Tegelström 1997). It was further shown with streamer length manipulation experiments that males with both naturally long and experimentally elongated streamers have lower extra-pair paternity in own broods and produce more biological offspring during the breeding season (Saino et al. 1997). Unlike European barn swallows, North American barn swallows use the ventral plumage colour as the sexual signal, e.g. the ventral colour is correlated with the number of fledglings produced during the breeding season (Safran & McGraw 2004). After experimental enhancement in ventral plumage colour male barn swallows received greater paternity from their social mates, which was not affected by experimental tail elongation (Safran et al. 2005; Smith et al. 1991).

In northeastern China there is a population of barn swallows that are hybrids of *H. r. tytleri* and *H. r. gutturalis* (Safran & Scordato, unpublished data). These birds have intermediate streamer length and rusty ventral plumage, and previous studies on this population have shown that the ventral plumage colour seems to be sexually selected (Chapter I, II and III). I tested whether manipulation of plumage colour caused changes in reproductive success or parental investment on the offspring in this population, measured as differences in brood size, number of biological offspring and feeding rate. I expected that by darkening ventral plumage colour, male barn swallows would gain greater reproductive success, and females paired to darker males would increase their investment in parental care.

Methods

Field work methods

I studied a population of barn swallows in Shuangyashan City, Heilongjiang Province, China (46° 35’ N, 131° 14’ E) from June to September in 2015. In 2015, 43 pairs of barn swallows were caught and their breeding success was assessed. In the analysis of parent barn swallow morphological and colour traits, data of swallows caught in 2013 were also included (in total: male: n = 81, female: n = 85). Basic fieldwork methods have been described in Chapter III.

Plumage colour manipulation and measurement

As experience suggested that if parent barn swallows were caught earlier there was a high risk of them abandoning nests, parent barn swallows were caught with mist nets at night during the first five days after their chicks hatched out and were released near their nests at the same night. At capture, male barn swallows were randomly assigned into two groups, a control group and an experimental group. Male barn swallows in the experimental group had their ventral plumage colour enhanced using a marker pen (light walnut colour, Prismacolor, US), which darkens the plumage colour within the natural range of variation (Safran et al. 2005). The manipulation of colour enhancement with this method has been shown to have no effect on barn swallow themselves so a sham control group was not included here (Safran et al. 2005). The method of plumage colour measurement has been described in Chapter II.

Statistical analysis

Morphological traits and colour traits

As morphological traits were intercorrelated, I used principal components analysis (PCA) to collapse morphological traits (body mass, tarsus length, wing length and central tail feather length) into a smaller number of uncorrelated principal components. The PCA was performed using the function 'prcomp' in the R package 'stats', and variables were scaled to have unit variance before the analysis took place. The first three principal components (PC1, PC2 and PC3) explained more than 80% of the variation in these morphological traits (Table 2.1). Thus, these three PC scores were used in the statistical analyses. See Chapter II for the result of all PCA analyses.

With the help of the function ‘t.test’ in the R package ‘stats’, t-tests were performed to test whether there were differences in colour traits between the control group and the experimental group before the manipulation, and whether the manipulation changed the ventral plumage colour of male barn swallows.

The reproductive success of male barn swallows

Several indices were used to assess the reproductive success of male barn swallows: the number of eggs in first or second clutches, the number of nestlings, the number of fledglings, the number of biological offspring of male swallows and the number of EPO sired by other males than the focal male in first or second broods. The number of chicks was recorded on Day 15 after nestlings hatched out. I tested whether there was a significant difference in the reproductive success between the control group and the experimental group with t-tests using the function ‘t.test’ (a *p* value of < 0.05 was considered to be statistically significant), followed by power tests using the function ‘power.t.test’ in the R package ‘stats’ to calculate the sample size needed to obtain a target power. Also, I used analysis of variance (ANOVA) to test the existence of the difference using group, the streamer length and the morphological PC3 values of female swallows as factors. Female streamer length and morphological PC3 values may reflect the female reproductive potential and body condition respectively and were shown previously to influence the number of chicks in a brood (see Chapter II & III).

The parental care of parent swallows

I tested whether the plumage colour manipulation influences parental investment on offspring. I calculated how many times did male or female adult swallows return to their nests and feed their nestlings in an hour’s time on Day 15 with the videos taken during the field observation, and the feeding rate of both male and female adults was used as an index of parental care in t-tests and ANOVA similarly to those described above.
All analyses were conducted with the R software (R Foundation for Statistical Computing, Vienna, Austria.).

Results

Plumage colour enhancement

Before the plumage colour enhancement, there was no difference between the experimental group and the control group in all colour tratis (*theta*, *phi*, *r* and brightness of throat, breast, belly and vent regions: Welch two sample t-tests: T = -0.97 – 0.57, df = 33.21 – 42.00, all *p* > 0.34). After the manipulation, the *r* of throat plumage colour was reduced (Welch two sample t-tests: *r*: T = 3.18, df = 36.72, *p* < 0.01; *theta*, *phi* and brightness: T = -1.24 – 0.86, df = 30.47 – 33.42, all *p* > 0.23; Figure 4.1), while breast, belly and vent regions were changed in similar patterns: the *theta* and brightness were reduced (Welch two sample t-tests: *theta*: T = 3.30 – 5.33, df = 29.15 – 33.87, all $p < 0.01$; brightness: T = 3.48 – 5.32, df = 28.92 – 39.07, all $p < 0.01$), the *phi* was increased (Welch two sample t-tests: T = -4.68 – -2.54, df = 30.299 – 36.17, all $p < 0.02$) and the *r* was not significantly different (Welch two sample t-tests: T = -0.64 – 1.32, df = 36.75 – 39.43, all $p > 0.19$, Figure 4.2).

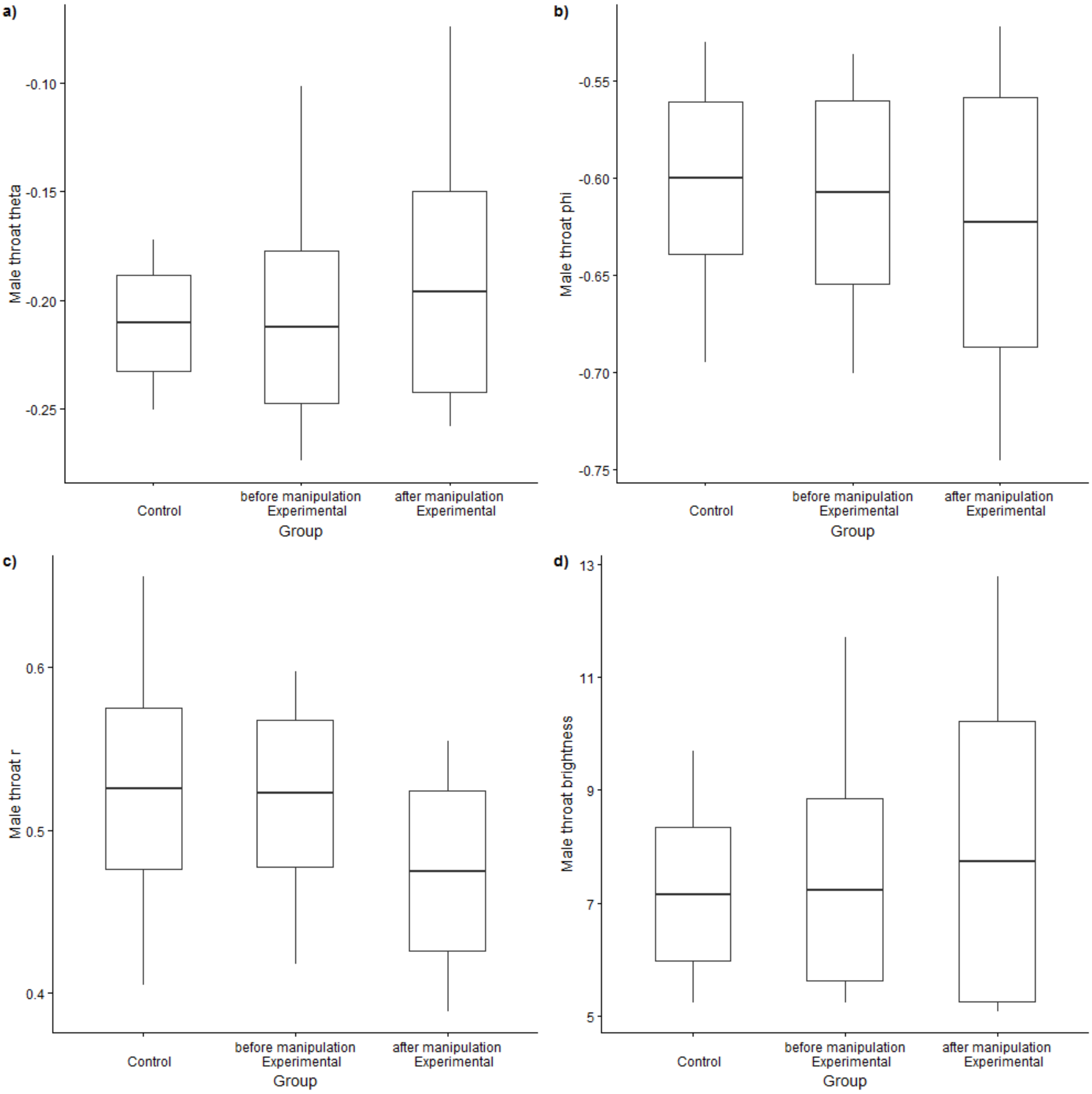


Figure 4.1 Colour traits of throat plumage were significantly changed in *r* after the experimental manipulation. Mean ± SD and the range of the data are shown in box plots.

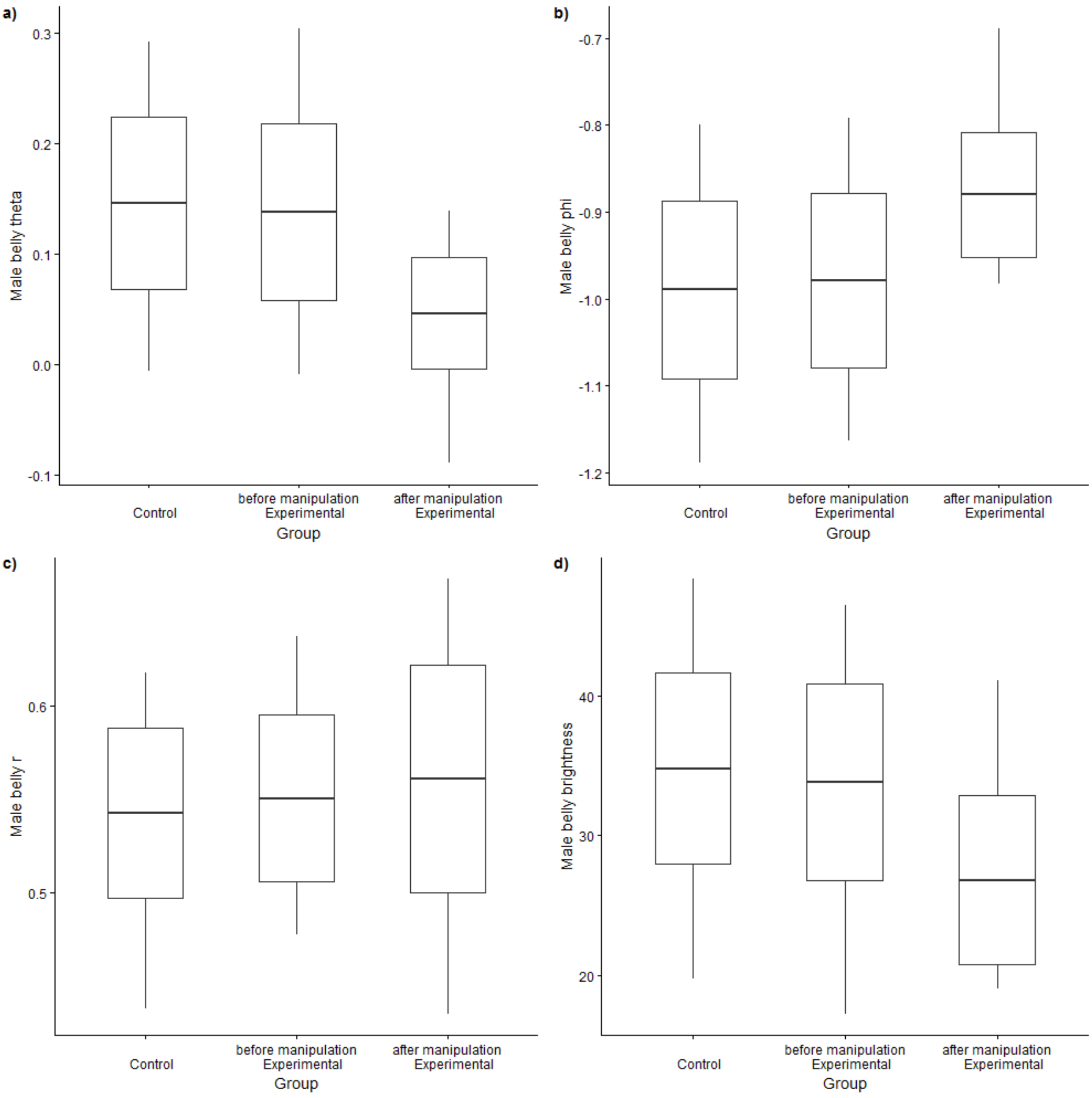


Figure 4.2 Colour traits of belly plumage were significantly changed in *theta*, *phi* and brightness after the experimental manipulation. Mean ± SD and the range of the data are shown in box plots.

Reproductive Success

The manipulation of male plumage colour was made after nestlings of first clutches hatched out, and as expected there were no significant differences in the number of first clutch eggs between the control group and the experimental group. However, there was also no significant effect of manipulation on the number of second clutch eggs (Figure

4.3). The result of analysis of variance (ANOVA) between the number of eggs and the manipulation group, female streamer length and female morphological PC3 values also indicates that the number of eggs were not significantly different between treatments (Table 4.1). Similarly, the difference in the number of eggs between first and second clutches was not significantly different between the control group and the experimental group (Figure 4.4).

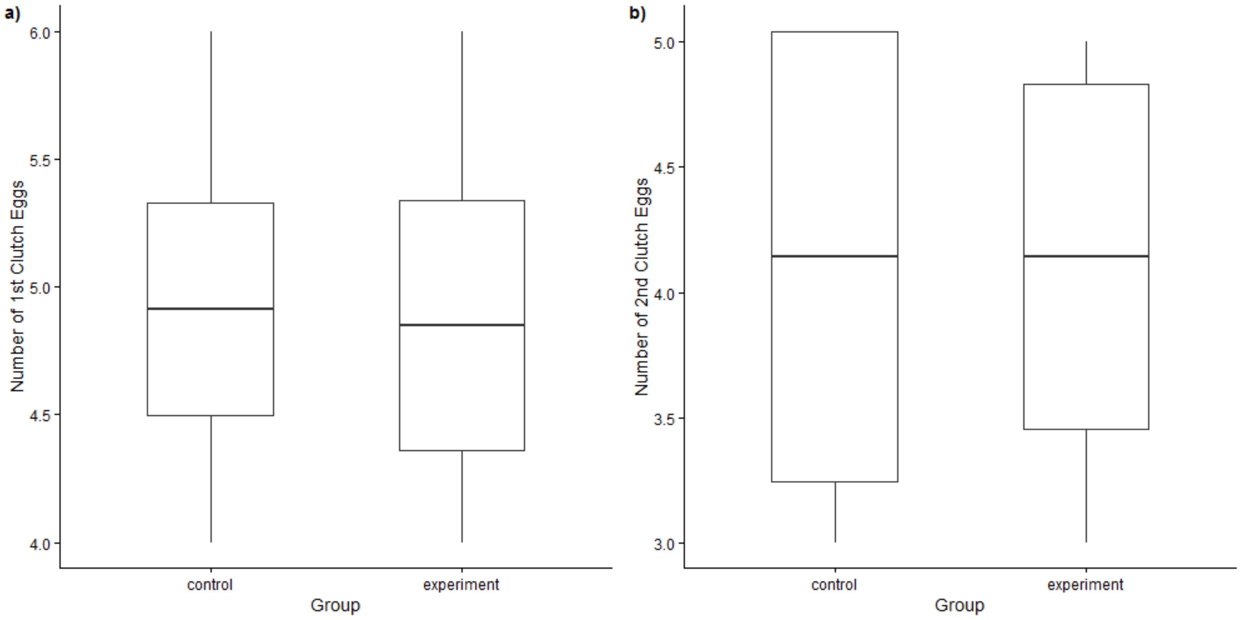


Figure 4.3 Plumage colour manipulation on male barn swallows did not significantly change the number of eggs laid by their mates. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = 0.45, df = 37.62, $p = 0.65$; b) second broods: T = 0, df = 11.24, $p = 1$.

Table 4.1 Relationship between number of eggs and the manipulation group, female streamer length and female morphological PC3 values.

	Sum Sq	Df	F	*p*
First broods				
(Intercept)	5.89	1	31.38	<0.01
Manipulation group	0.20	1	1.04	0.31

Female streamer length	0.23	1	1.24	0.27
Female morphological PC3	0.16	1	0.84	0.37
Residuals	7.13	38		
Second broods				
(Intercept)	0.24	1	0.34	0.57
Manipulation group	0.29	1	0.41	0.54
Female streamer length	0.06	1	0.09	0.77
Female morphological PC3	0.47	1	0.66	0.44
Residuals	6.38	9		

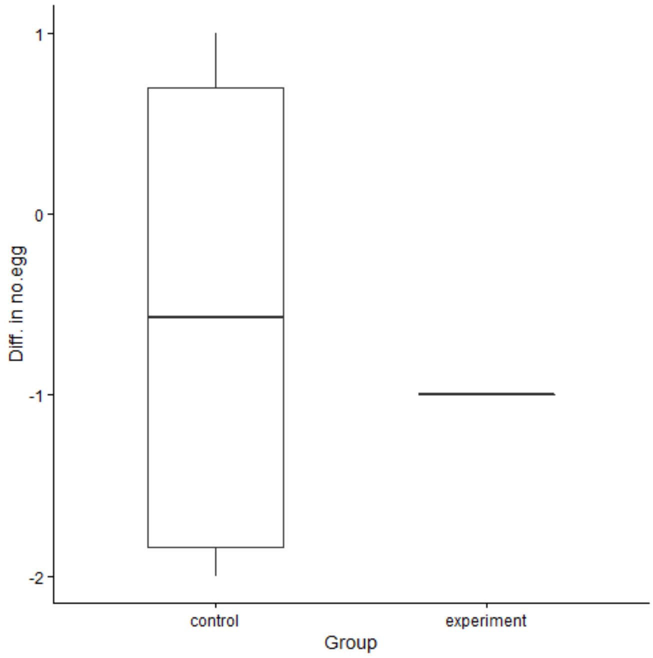


Figure 4.4 Differences in the number of eggs between first and second broods did not change after the plumage colour of male barn swallows was enhanced. Mean ± SD and the range of the data are shown in box plots. t-test: T = 0.89, df = 6, *p* = 0.41. Note that numbers of eggs in the experimental group are the same so there is no variation.

There was also no significant difference in number of nestlings between the two groups in first broods (Figure 4.5). In second broods, on average male swallows in the experimental group had 4.00 nestlings and males in the control group had 3.00

nestlings, but this was not significantly different (Figure 4.5). ANOVA analysis on the relationship between the number of fledglings and the manipulation group, female streamer length and female morphological PC3 values also indicates there was no significant difference between treatments (Table 4.2). The difference in the number of nestlings between first and second broods was also not significantly different between the two groups (Figure 4.6).

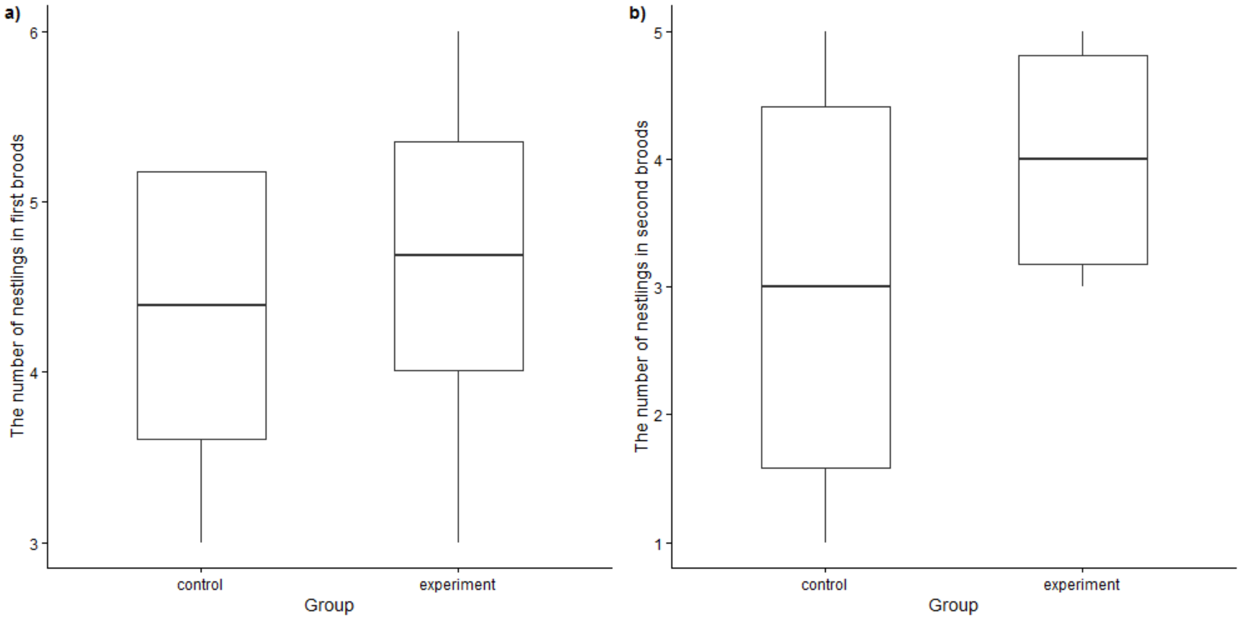


Figure 4.5 The number of nestlings in first and second broods. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = -1.31, df = 39.93, *p* = 0.20; b) second broods: T = -1.62, df = 9.6, *p* = 0.14.

Table 4.2 Relationship between number of nestlings and the manipulation group, female streamer length and female morphological PC3 values.

	Sum Sq	Df	F	*p*
First broods				
(Intercept)	0.45	1	0.93	0.34
Manipulation group	0.50	1	1.03	0.32
Female streamer length	1.08	1	2.21	0.15
Female morphological PC3	0.32	1	0.64	0.43

Residuals	18.10	37		
Second broods				
(Intercept)	0.27	1	0.15	0.71
Manipulation group	1.97	1	1.11	0.32
Female streamer length	0.00	1	0.00	0.98
Female morphological PC3	0.00	1	0.00	0.96
Residuals	15.99	9		

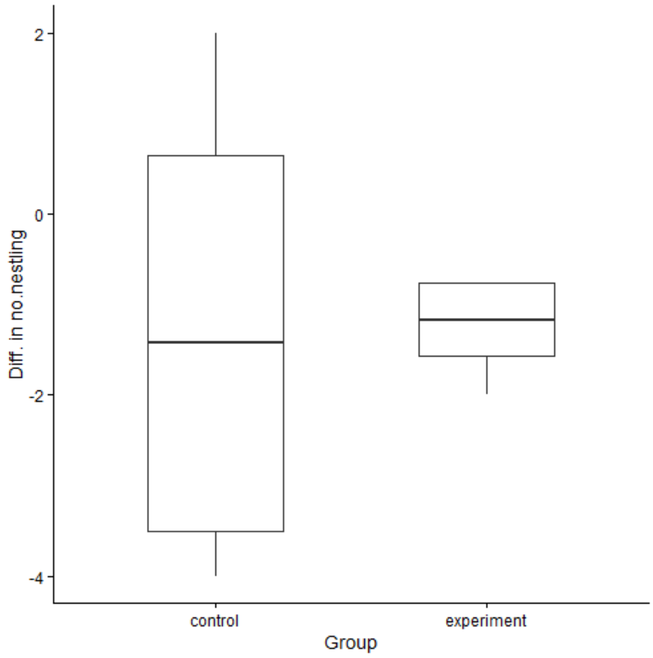


Figure 4.6 Differences in the number of nestlings between first and second broods did not change significantly after the plumage colour of male barn swallows was enhanced. Mean ± SD and the range of the data are shown in box plots. t-test: T = -0.33, df = 6.54, *p* = 0.75.

I tested whether there was a difference in the number of fledglings (Figure 4.7), the number of biological offspring of male barn swallows (Figure 4.8), the number of EPO sired by other male swallows than the focal male (Figure 4.9) between the experimental group and the control group in first and second broods and similarly no significant differences were found.

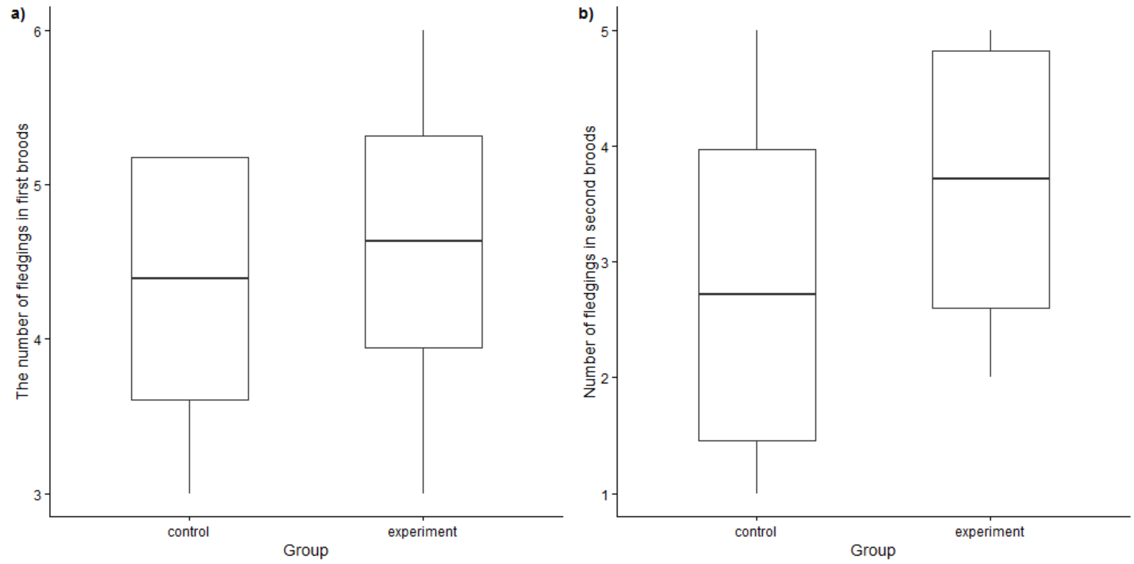


Figure 4.7 The number of fledglings in two groups after plumage colour manipulation. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = -1.06, df = 39.85, *p* = 0.30; b) second broods: T = -1.58, df = 11.83, *p* = 0.14.

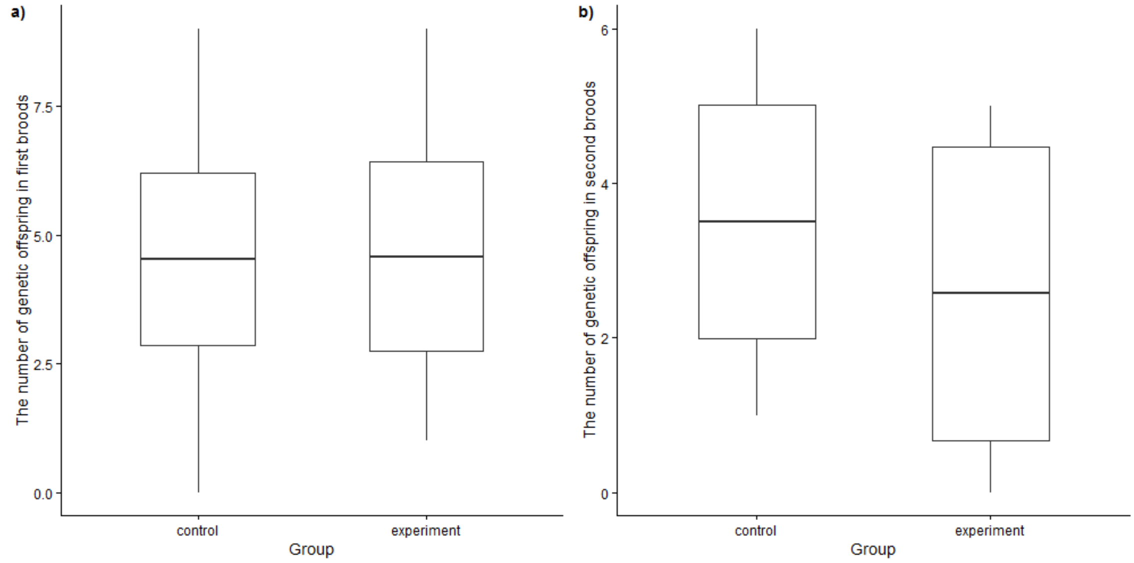


Figure 4.8 The number of biological offspring of male barn swallows in both the control group and the experimental group. Mean ± SD and the range of the data are shown in box plots. t-test: a) first broods: T = -0.10, df = 36.97, *p* = 0.92; b) second broods: T = 1.04, df = 11.46, *p* = 0.32.

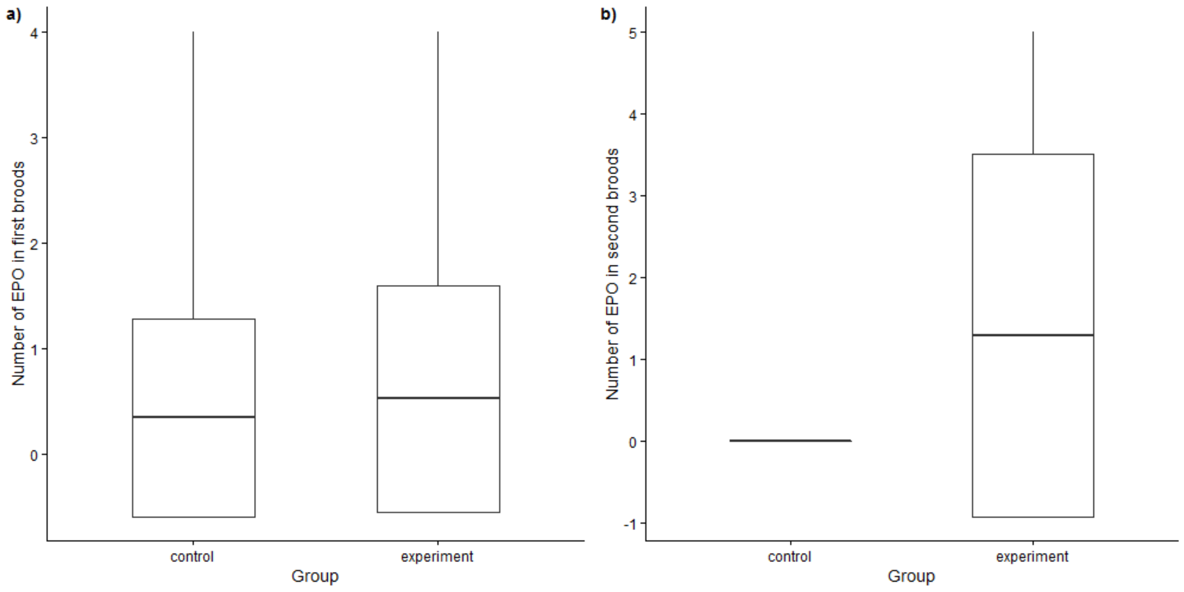


Figure 4.9 The number of EPO sired by other males in first and second broods. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = -0.57, df = 36.04, *p* = 0.57; b) second broods: T = -1.54, df = 6, *p* = 0.18.

Parental care for offspring

The feeding rate of female adults was not significantly different between the two groups in both first and second broods (Figure 4.10). Similarly, colour manipulation did not significantly change the feeding rate of male adult barn swallows (Figure 4.11).

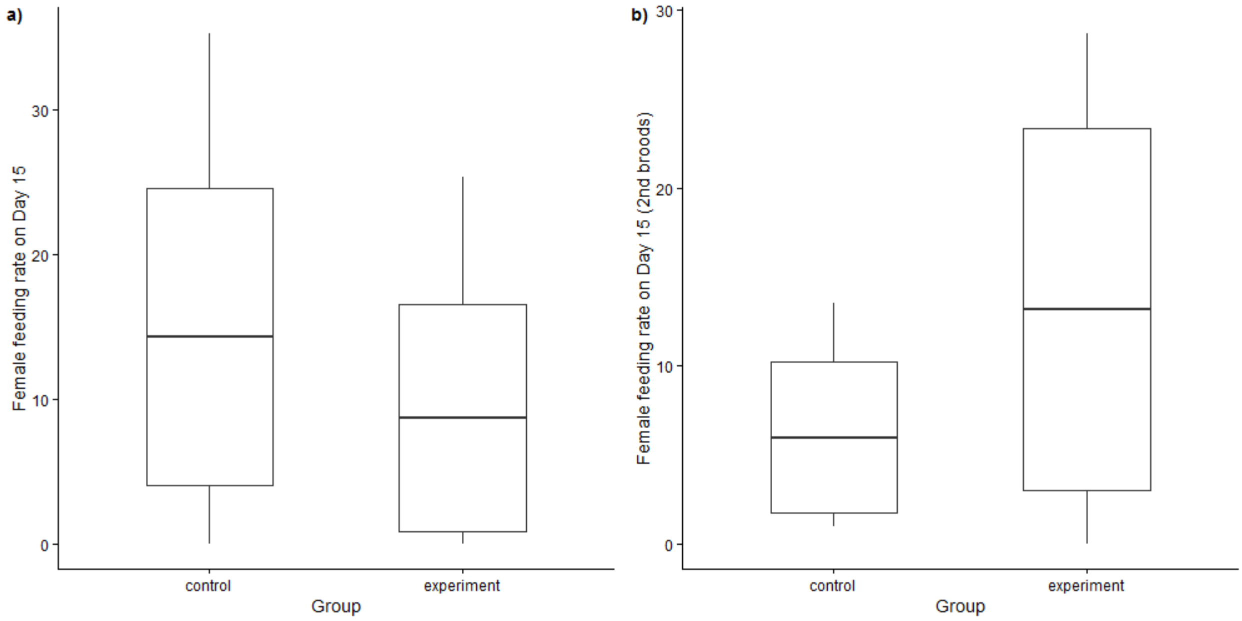


Figure 4.10 The feeding rate of female barn swallows on Day 15. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = 1.96, df = 37.04, $p = 0.06$; b) second broods: T = -1.59, df = 6.69, $p = 0.16$.

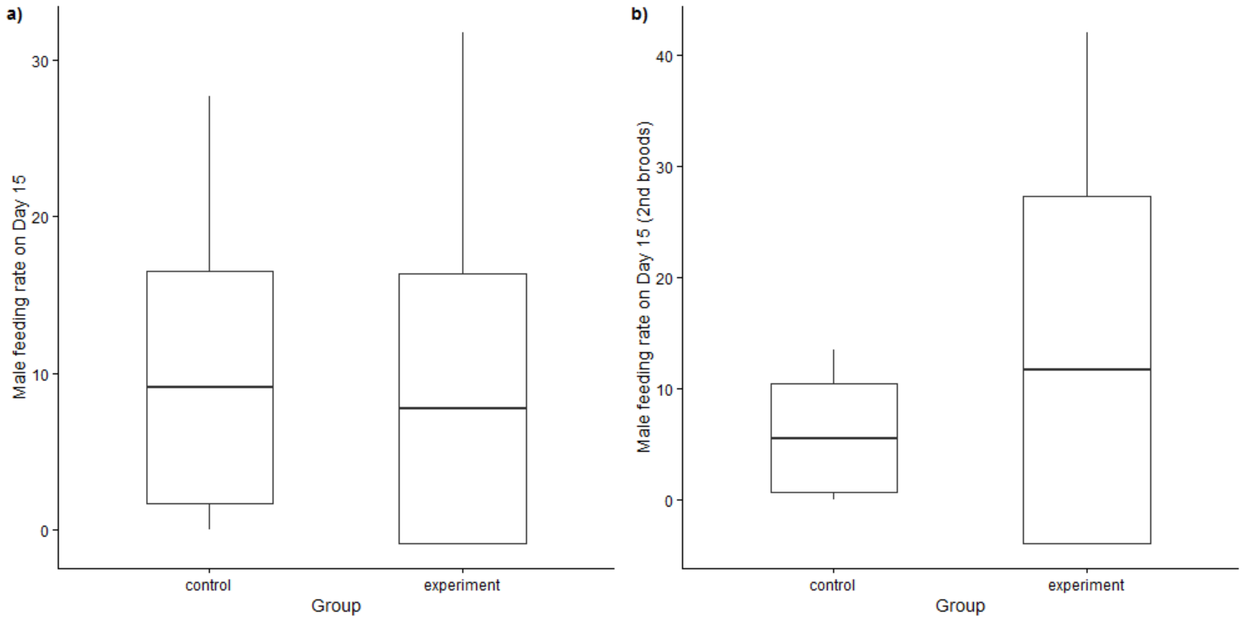


Figure 4.11 Plumage colour manipulation did not change the feeding rate of male barn swallows. Mean ± SD and the range of the data are shown in box plots. a) first broods: T = 0.54, df = 35.85, $p = 0.60$; b) second broods: T = -0.92, df = 5.97, $p = 0.39$.

Discussion

In this study, I tested the reproductive success of male barn swallows after their ventral plumage colour was enhanced, which was identified as the primary sexually selected trait in my previous empirical research (Chapter II & III). However, I did not find that enhancement of ventral colour on male barn swallows significantly changed their reproductive success. Considering that the strength of sexual selection might be weak in this population, e.g. male ventral plumage colour traits (throat colour PC2 and belly colour PC1) explained 18.87% of the variation in annual offspring number (Chapter II), a larger sample size is needed to test whether manipulation on the ventral colour of male adults could change their reproductive fitness. For instance, when testing whether the number of nestlings in second broods is different between the control group and the experimental group with a test, a power test shows that when the statistical power was 0.8 and significance level was 0.05, the sample size would need to be over 25 nests in both groups to result in a significant difference in a t-test with an effect size such as shown in my experiment. When using the number of fledglings in second broods as the dependent variable, the sample size would need to be increased to over 26 nests to show a significant difference with this effect size with a statistical power of 0.8 and a significance level of 0.05. Repeating the experiment to increase sample size would help confirm whether the plumage colour manipulation could cause change in the brood size of male barn swallows.

Similarly, for testing whether the colour manipulation could change the paternity males gained from their social mates, the sample size is still the limitation here, e.g. the result would be statistically significant if the sample size was over 27 for the number of EPO sired by other males in a brood, with a statistical power of 0.8 and a significance level of 0.05. The phenotypic signal of quality could influence the paternity of male animals, like a previous experiment on American barn swallows *H. r. erythrogaster* which showed that males whose plumage colour was enhanced received a greater share of paternity from their social mates (Safran et al. 2005). On the other hand, it was also shown in sugarbirds *Promerops cafer* that enhancement in the sexually selected trait could increase their success in gaining extra-pair offspring, but losing within-pair paternity meanwhile, indicating different breeding strategies could be used by male animals depending on their ornamentation (McFarlane et al. 2009). Thus, a fuller analysis covering all breeding pairs in the research area is needed to exclude the possibility that males in the experimental groups gain extra-pair offspring in broods elsewhere.

In European barn swallows *H. r. rustica*, experimental manipulation of the sexually selected trait of males affected the reproductive effort by females, e.g. the seasonal number of clutches and the feeding rate (De Lope & Møller 1993). In this study, the result shows that colour manipulation cannot significantly change the feeding rate of female adult barn swallows in neither first nor second broods (Figure 4.10). Similarly, male feeding rate was not significantly different between the two groups in either first broods or second broods (Figure 4.11). Thus, it remains unclear whether enhancement in the ventral plumage colour changed female reproductive effort in our study population, and further research would be needed to establish the relationship between parent feeding rate and the brood size.

The barn swallow subspecies are recently derived, and the extent to which their sexual behaviour is different between different subspecies remains unknown (Dor et al. 2010). Sexual selection, as a potential driver for speciation, may exist in different forms in barn swallows, different populations may use different traits as the sexual signal or respond differently to the exaggeration of the sexual ornaments (Safran et al. 2013). The northeastern Chinese population is an intermediate type in two potential sexual signals of barn swallows: the streamer length and the ventral plumage colour. Though empirical study has shown that the ventral plumage colour is the sexually selected trait in this population, it remains unknown how females will respond to the change in ventral colour of their mates. In conclusion, in this study I manipulated the sexually selected trait of northeastern Chinese barn swallows, expecting to see a change in their reproductive success. Though after manipulation male barn swallows were in the direction of having a larger brood size and gaining greater reproductive fitness, no statistical difference was found, potentially restricted by the relatively weak sexual selection strength.

General Conclusions

In the last a few decades the barn swallow has been considered as a model animal in the research of sexual selection. Considerable work has been done to demonstrate the relationship between the length of tail streamers and the reproductive success in European barn swallows, while ventral plumage colour has been shown to be the sexually selected trait in North American barn swallows. As the barn swallow consists of at least six different subspecies, which vary extensively in morphology and are broadly distributed all over the world, it is also suitable to study how sexual selection may drive speciation in this particular species. Thus a systematic study on the sexual selection behaviour of various barn swallow populations, including the sexually selected trait is essential for further research.

During my PhD study I worked on barn swallows in China, especially those in the northeastern region. Firstly, I conducted a comprehensive analysis on the morphology of Chinese barn swallows, aiming to explore the phenotype variation between different populations. I found that Chinese barn swallow populations in different regions of China are different in both morphological and colour traits and can be separated using morphology, including populations in the northeastern part of China (referred to as *H. r. mandschurica*), which have intermediate length of streamers and intermediate plumage colour among the barn swallow subspecies complex. For one of the northeastern populations, both observational and experimental studies were conducted to explore the sexual selection strategy of these barn swallows. Based on the results of the empirical study, I have found that ventral plumage colour, not the length of tail streamers, is the sexually selected trait in northeastern Chinese barn swallows. The ventral plumage colour can predict the reproductive success of a male, for instance male swallows with darker or redder ventral plumage bred earlier and produced more offspring than males with pale plumage colour. The ratio of EPO to all offspring is relatively low in this population compared to other barn swallow populations (11.6% in my study), but the ventral colour of male barn swallows can predict the number of their biological offspring and the body condition of their offspring. The manipulation experiment shows that manual enhancement of ventral plumage did not significantly change the reproductive success of male barn swallows, nor did it change the provisioning rate of their mates. This non-significant result may be due to weak sexual selection in this population, and enlarging the sample size of this experiment may help to clarify this tentative result.

Overall my study fills a gap in our knowledge about Chinese barn swallows, including their morphological variation and sexual selection strategy. To understand the formation of the phenotype variation in Chinese barn swallow populations, a high-resolution analysis on the genetic structure of these populations will be useful. Meanwhile though I have shown that the ventral plumage colour seems to be the sexually selected trait in northeastern Chinese barn swallows, the mechanism of sexual selection in this population is still not clear. To solve this problem more trait manipulation experiments with larger sample sizes are needed, as well as understanding the physiological basis of the ventral plumage colour expression. In the future with a full understanding of how sexual selection works in different barn swallow populations and the genetic variation between each of them, it will help to answer the question of how natural selection, sexual selection or both drive speciation in the barn swallow subspecies complex.

Appendix Data

Table S1 Pearson's *r* correlation coefficients matrix between mean values of morphological traits and colour traits from all populations of the barn swallow. *P* values are shown in brackets. Sample size is 209 – 213 for males and 216 – 217 for females. a) morphological tratis; b) colour traits.

a)

	Wing Length	Streamer Length	Body Mass	Tarsus Length	Bill Length	Bill Depth
Males						
Streamer Length	0.57 (<0.01)					
Body Mass	0.71 (<0.01)	0.45 (<0.01)				
Tarsus Length	0.31 (<0.01)	0.27 (<0.01)	0.35 (<0.01)			
Bill Length	0.40 (<0.01)	0.23 (<0.01)	0.31 (<0.01)	0.13 (0.06)		
Bill Depth	0.24 (<0.01)	0.29 (<0.01)	0.18 (<0.01)	0.04 (0.57)	0.24 (<0.01)	
Bill Width	0.25 (<0.01)	0.20 (<0.01)	0.29 (<0.01)	0.12 (0.08)	0.42 (<0.01)	0.24 (<0.01)
Females						
Streamer Length	0.63 (<0.01)					
Body Mass	0.52 (<0.01)	0.40 (<0.01)				
Tarsus Length	0.38 (<0.01)	0.21 (<0.01)	0.39 (<0.01)			

Bill Length	0.21 (<0.01)	0.11 (0.12)	0.03 (0.68)	0.16 (0.02)		
Bill Depth	0.20 (<0.01)	0.21 (<0.01)	0.24 (<0.01)	0.15 (0.03)	0.21 (<0.01)	
Bill Width	0.25 (<0.01)	0.24 (<0.01)	0.28 (<0.01)	0.17 (0.01)	0.23 (<0.01)	0.21 (<0.01)

b)

		Throat			Breast			Belly			Vent		
		brightness	hue	chroma	brightness	hue	chroma	brightness	hue	chroma	brightness	hue	chroma
Males													
Throat	brightness												
	hue	-0.33 (<0.01)											
	chroma	-0.36 (<0.01)	0.30 (<0.01)										
Breast	brightness	0.36 (<0.01)	-0.05 (0.46)	0.11 (0.11)									
	hue	-0.23 (<0.01)	0.17 (0.01)	0.04 (0.59)	-0.26 (<0.01)								
	chroma	-0.28 (<0.01)	0.08 (0.25)	-0.05 (0.47)	-0.83 (<0.01)	0.26 (<0.01)							
Belly	brightness	0.24 (<0.01)	-0.05 (0.45)	0.04 (0.52)	0.62 (<0.01)	-0.22 (<0.01)	-0.61 (<0.01)						
	hue	-0.01 (0.91)	0.09 (0.19)	0.00 (0.99)	-0.03 (0.65)	0.30 (<0.01)	0.02 (0.72)	-0.07 (0.30)					
	chroma	-0.28 (<0.01)	0.14 (0.05)	-0.01 (0.85)	-0.70 (<0.01)	0.25 (<0.01)	0.78 (<0.01)	-0.77 (<0.01)	-0.04 (0.57)				
Vent	brightness	0.32 (<0.01)	0.00 (0.98)	0.09 (0.18)	0.73 (<0.01)	-0.22 (<0.01)	-0.71 (<0.01)	0.56 (<0.01)	0.09 (0.21)	-0.63 (<0.01)			
	hue	-0.15 (<0.01)	0.12 (0.07)	0.03 (0.64)	-0.37 (<0.01)	0.24 (<0.01)	0.41 (<0.01)	-0.31 (<0.01)	0.20 (<0.01)	0.38 (<0.01)	-0.47 (<0.01)		
	chroma	-0.33 (<0.01)	0.06 (0.35)	0.03 (0.66)	-0.68 (<0.01)	0.19 (0.01)	0.74 (<0.01)	-0.52 (<0.01)	-0.06 (0.37)	0.66 (<0.01)	-0.91 (<0.01)	0.45 (<0.01)	

		Throat			Breast			Belly			Vent		
		brightness	hue	chroma	brightness	hue	chroma	brightness	hue	chroma	brightness	hue	chroma
Females													
Throat	brightness												
	hue	-0.49 (<0.01)											
	chroma	-0.58 (<0.01)	0.38 (<0.01)										
Breast	brightness	0.29 (<0.01)	-0.18 (0.01)	-0.06 (0.34)									
	hue	-0.01 (0.86)	0.11 (0.11)	0.02 (0.73)	-0.23 (<0.01)								
	chroma	-0.28 (<0.01)	0.23 (<0.01)	0.18 (0.01)	-0.77 (<0.01)	0.16 (0.02)							
Belly	brightness	0.21 (<0.01)	-0.13 (0.06)	-0.04 (0.55)	0.57 (<0.01)	-0.20 (<0.01)	-0.48 (<0.01)						
	hue	-0.01 (0.90)	0.00 (0.98)	-0.13 (0.06)	-0.10 (0.15)	0.33 (<0.01)	0.05 (0.49)	-0.18 (0.01)					
	chroma	-0.30 (<0.01)	0.25 (<0.01)	0.22 (<0.01)	-0.61 (<0.01)	0.19 (0.01)	0.75 (<0.01)	-0.59 (<0.01)	-0.08 (0.26)				
Vent	brightness	0.23 (<0.01)	-0.15 (0.02)	0.00 (0.98)	0.66 (<0.01)	-0.19 (0.01)	-0.67 (<0.01)	0.56 (<0.01)	-0.13 (0.06)	-0.59 (<0.01)			
	hue	-0.14 (0.05)	0.07 (0.28)	-0.04 (0.59)	-0.38 (<0.01)	0.37 (<0.01)	0.36 (<0.01)	-0.33 (<0.01)	0.34 (<0.01)	0.30 (<0.01)	-0.39 (<0.01)		
	chroma	-0.25 (<0.01)	0.22 (<0.01)	0.15 (0.02)	-0.62 (<0.01)	0.15 (0.03)	0.76 (<0.01)	-0.46 (<0.01)	0.03 (0.67)	0.66 (<0.01)	-0.85 (<0.01)	0.29 (<0.01)	

Table S2 Mean values of morphological traits in different populations and the groups divided by Tukey's test. For each trait, populations that are not significantly different are divided into the same group (α = 0.05).

Wing Length								Streamer Length							
Male				Female				Male				Female			
population	mean	SD	group	population	mean	SD	group	population	mean	SD	group	population	mean	SD	group
DH	127.26	0.79	a	WLMQ	127.53	3.04	a	WLMQ	120.23	12.41	a	WLMQ	98.02	9.96	a
GEM	127.17	NA	ab	GT	127.00	NA	ab	DH	116.63	11.05	a	GT	95.75	NA	ab
WLMQ	126.90	3.08	ab	DH	125.56	3.17	ab	GT	115.00	NA	ab	YM	93.39	5.00	ab
JQ	125.58	2.07	ab	GEM	125.08	2.95	ab	ZY	110.99	9.07	ab	ZY	91.94	4.63	ab
ZY	124.31	2.76	ab	YM	123.44	1.71	ab	GEM	108.50	NA	ab	JQ	91.09	6.43	ab
GT	124.17	NA	abc	JQ	122.40	2.88	ab	BT	108.29	6.79	ab	DH	90.84	5.43	ab
YM	123.81	2.88	abc	ZY	122.10	2.95	ab	WW	108.08	6.37	ab	GEM	86.58	14.02	ab
WW	123.04	2.96	abc	WW	120.36	2.82	bc	YM	105.02	6.65	ab	XA	86.36	7.41	b
YC	120.80	2.44	bc	YC	120.02	3.03	bc	JQ	103.19	7.09	b	BT	86.26	5.51	b
BT	120.61	3.10	bc	LZ	119.75	2.37	bcd	YC	101.41	5.50	b	YC	85.43	7.13	b
LZ	120.04	3.61	bc	XA	118.36	2.31	bcde	LZ	101.40	11.26	b	CC	85.04	6.03	b
XA	119.67	1.74	bc	BT	117.32	2.55	bcdef	CC	100.53	6.86	b	LZ	84.40	6.74	b
HK	118.62	2.12	bcd	HK	117.18	3.19	bcdef	BJ	99.46	13.12	b	SY	84.35	2.55	b
ZZ	118.36	1.86	bcd	ZZ	116.99	2.07	bcdef	QQHE	99.46	6.64	b	WW	83.94	3.93	b
BJ	117.92	2.33	bcd	QQHE	116.23	3.08	bcdef	ZZ	98.31	8.19	b	SYS	83.79	5.13	b
CS	117.82	2.43	bcd	CS	116.19	1.97	bcdef	HEB	98.04	5.24	b	BJ	83.68	4.76	b
QQHE	116.99	1.55	cd	BJ	114.75	2.33	cdef	SYS	97.27	7.92	b	QQHE	82.55	5.44	b
CC	116.79	2.68	cd	CC	114.74	3.09	def	SY	96.02	7.52	b	QHD	81.63	6.18	b
HEB	116.36	1.35	cd	NN	114.60	3.74	def	XA	94.93	6.02	b	HEB	81.30	4.72	b
NN	116.07	2.44	cd	QHD	114.31	2.81	ef	CS	94.90	9.32	b	CS	80.72	4.74	b
QHD	115.70	2.03	cd	SY	113.90	2.53	ef	QHD	94.44	9.07	b	ZZ	80.53	6.11	b
SYS	115.33	2.58	cd	SYS	113.68	4.89	f	HK	90.98	4.65	b	HK	78.78	4.66	b
SY	114.13	3.15	d	HEB	113.50	2.76	f	NN	90.25	6.38	b	NN	78.47	4.12	b

Body Mass								Tarsus Length							
Male				Female				Male				Female			
population	mean	SD	group	population	mean	SD	group	population	mean	SD	group	population	mean	SD	
WLMQ	19.07	0.61	a	YM	21.77	1.78	a	GEM	11.27	NA	a	WLMQ	11.25	0.42	a
DH	18.19	0.67	a	WLMQ	20.83	1.67	ab	WLMQ	11.02	0.49	a	WW	10.86	0.29	ab
GT	17.97	NA	ab	GEM	19.43	1.58	abc	YM	10.96	0.22	a	YM	10.82	0.25	ab
JQ	17.94	0.78	ab	DH	19.41	2.44	abc	WW	10.94	0.48	a	QQHE	10.76	0.39	ab
GEM	17.92	NA	abc	JQ	19.19	2.45	abc	LZ	10.79	0.15	ab	DH	10.75	0.31	ab
ZY	17.67	1.14	abc	GT	19.16	NA	abcd	JQ	10.68	0.29	ab	JQ	10.73	0.29	ab
YM	17.61	0.52	abc	ZY	19.13	1.69	abcd	DH	10.65	0.37	ab	GEM	10.70	0.24	ab
YC	16.69	0.80	bc	WW	18.95	1.49	abcd	ZY	10.57	0.25	ab	YC	10.66	0.28	ab
WW	16.64	0.76	bc	XA	18.60	1.60	bcd	SY	10.56	0.29	ab	SY	10.62	0.34	ab
LZ	16.42	0.56	bc	QQHE	18.05	1.77	bcd	YC	10.54	0.35	ab	GT	10.60	NA	ab
XA	16.28	0.94	bc	ZZ	17.68	0.94	cd	BT	10.53	0.36	ab	BT	10.58	0.37	ab
ZZ	16.04	0.59	bcd	HEB	17.36	1.31	cd	XA	10.50	0.45	ab	ZY	10.57	0.24	ab
BT	16.03	1.05	bcd	YC	17.19	1.39	cd	QQHE	10.47	0.36	ab	XA	10.49	0.24	ab
HK	15.58	0.65	bcde	LZ	17.16	1.13	cd	GT	10.47	NA	ab	ZZ	10.48	0.37	b
SYS	15.54	0.88	bcde	HK	17.03	1.60	cd	CS	10.40	0.57	ab	HEB	10.43	0.53	b
CS	15.25	0.90	bcde	BT	16.68	1.64	cd	NN	10.40	0.40	ab	SYS	10.43	0.26	b
BJ	15.06	1.27	bcde	SYS	16.58	1.21	cd	HEB	10.39	0.18	ab	HK	10.42	0.90	b
QQHE	14.90	0.95	bcde	CS	16.36	1.39	cd	SYS	10.33	0.34	ab	LZ	10.37	0.55	b
CC	14.77	0.57	ce	SY	16.16	1.60	cd	ZZ	10.30	0.26	ab	CS	10.33	0.35	b
HEB	14.64	0.95	de	BJ	16.05	1.87	cd	CC	10.17	0.33	ab	QHD	10.31	0.38	b
QHD	14.46	0.80	e	CC	15.62	1.56	cd	BJ	10.13	0.09	ab	NN	10.24	0.35	b
SY	14.29	0.47	e	QHD	15.38	1.22	cd	QHD	10.07	0.35	ab	CC	10.23	0.48	b
NN	14.28	0.70	e	NN	14.46	1.40	d	HK	10.06	1.54	ab	BJ	10.22	0.39	b

Bill Length							
Male				Female			
population	mean	SD	group	population	mean	SD	group
GEM	6.17	NA	a	GT	6.38	NA	a
DH	6.00	0.19	a	QHD	5.92	1.01	a
WW	5.88	0.32	ab	WLMQ	5.84	0.31	ab
YM	5.84	0.31	abc	ZY	5.83	0.36	ab
QHD	5.83	0.33	abc	WW	5.79	0.34	ab
JQ	5.74	0.26	abcd	HK	5.73	0.22	ab
WLMQ	5.72	0.42	abcde	SY	5.72	0.26	ab
ZY	5.72	0.26	abcde	JQ	5.70	0.24	ab
GT	5.70	NA	abcde	DH	5.68	0.32	ab
CS	5.66	0.27	abcde	GEM	5.65	0.26	ab
LZ	5.64	0.17	abcde	YM	5.64	0.32	ab
CC	5.59	0.33	abcde	NN	5.61	0.25	ab
HK	5.55	0.19	abcde	YC	5.61	0.23	ab
HEB	5.52	0.39	abcde	CC	5.54	0.29	ab
SY	5.50	0.33	abcde	ZZ	5.53	0.18	ab
ZZ	5.50	0.26	abcde	HEB	5.53	0.38	ab
YC	5.49	0.24	abcde	LZ	5.51	0.32	ab
BT	5.48	0.28	abcde	CS	5.50	0.30	ab
BJ	5.44	0.14	abcde	BJ	5.46	0.28	ab
QQHE	5.39	0.23	acde	BT	5.43	0.21	ab
XA	5.34	0.19	ade	QQHE	5.41	0.26	ab
NN	5.34	0.35	ade	XA	5.35	0.24	ab
SYS	5.28	0.24	ae	SYS	5.33	0.25	ab

Table S3 Mean values of colour traits in different populations and the groups divided by Tukey's test. For each trait, populations that are not significantly different are divided into the same group (α = 0.05).

Throat Brightness								Breast Brightness							
Male				Female				Male				Female			
population	mean	SD	group	population	mean	SD	group	population	mean	SD	group	population	mean	SD	group
YM	11.51	3.74	a	QHD	14.78	8.27	a	JQ	56.68	7.73	a	GT	66.17	NA	a
CC	10.27	2.07	ab	WLMQ	13.94	2.10	ab	ZY	56.44	8.88	a	ZY	57.97	4.90	a
ZY	10.26	2.75	ab	JQ	12.50	3.70	ab	QHD	55.23	7.38	ab	WW	57.13	8.40	a
HEB	9.63	2.46	abc	GT	12.18	NA	abc	DH	54.83	6.96	abc	JQ	56.83	7.18	a
JQ	9.54	2.29	abc	WW	12.12	2.60	abc	YM	54.06	8.63	abc	YM	56.73	5.02	a
WLMQ	9.42	2.64	abc	CC	11.63	3.45	abc	WLMQ	53.16	5.42	abc	WLMQ	53.82	9.69	a
SY	9.40	1.82	abc	ZY	11.55	1.39	abc	CS	49.43	4.86	abcd	QHD	48.60	7.04	a
WW	9.33	2.56	abc	HEB	11.27	3.48	abc	SY	47.91	16.29	abcde	CS	48.51	5.12	ab
DH	9.10	1.84	abc	YM	11.25	1.22	abc	GEM	47.85	NA	abcdef	DH	48.33	4.49	ab
QHD	9.06	1.91	abc	GEM	11.22	1.22	abc	XA	46.28	5.07	abcdef	SY	48.26	10.44	ab
GEM	9.00	NA	abc	DH	10.50	1.62	abc	CC	45.24	14.58	abcdef	HK	47.65	11.24	ab
GT	8.61	NA	abc	SY	10.24	1.18	abc	HK	44.77	5.59	abcdef	XA	46.49	6.14	ab
YC	8.42	1.28	bc	YC	9.93	1.78	bc	WW	44.65	6.91	abcdef	ZZ	44.92	5.57	ab
XA	8.18	1.15	bc	XA	9.81	1.92	bc	ZZ	44.41	5.47	bcdef	NN	44.57	8.17	ab
HK	8.05	1.52	bc	LZ	9.67	2.85	bc	GT	43.33	NA	bcdef	BT	44.13	6.32	ab
SYS	7.82	1.73	bc	BJ	9.59	1.62	bc	NN	41.69	8.62	bcdef	GEM	43.98	0.27	abc
BJ	7.64	1.42	bc	BT	9.55	1.25	bc	HEB	40.88	5.85	bcdef	CC	43.87	9.06	abc
BT	7.53	1.69	c	NN	9.10	1.86	bc	LZ	39.98	7.89	cdef	LZ	41.89	6.98	abc
CS	7.20	0.89	c	HK	9.09	1.67	bc	YC	38.05	7.12	def	YC	41.25	10.85	abc
LZ	7.19	0.92	c	ZZ	9.03	1.68	bc	BJ	37.45	5.32	def	BJ	41.06	4.05	abc
ZZ	7.05	1.07	c	CS	8.85	1.80	bc	BT	37.16	7.47	ef	HEB	39.68	7.57	abc
NN	7.03	1.36	c	QQHE	8.70	1.24	bc	SYS	33.99	9.09	ef	SYS	37.80	6.53	bc
QQHE	6.95	1.05	c	SYS	8.06	1.18	c	QQHE	30.86	6.28	f	QQHE	32.30	7.89	c

Throat Hue								Breast Hue							
Male				Female				Male				Female			
population	mean	SD	group	population	mean	SD	group	population	mean	SD	group	population	mean	SD	group
GT	685.43	NA	a	HK	678.75	9.20	a	NN	652.87	32.88	a	GEM	638.55	3.56	a
BJ	681.88	5.72	a	QQHE	678.58	9.29	a	SYS	642.49	28.18	a	QQHE	634.30	28.44	a
QQHE	680.45	11.39	a	ZZ	677.57	13.04	a	ZZ	639.81	34.85	a	SY	631.09	33.36	a
HK	679.01	6.50	a	CS	677.17	8.09	ab	QQHE	634.17	31.25	a	SYS	629.91	25.17	a
ZZ	678.69	9.66	a	SYS	676.54	9.34	ab	GEM	634.16	NA	a	GT	628.35	NA	ab
SYS	678.68	9.16	a	XA	674.45	14.41	ab	SY	627.87	32.48	a	WLMQ	627.16	27.93	ab
WLMQ	678.42	8.05	a	GEM	674.43	19.79	ab	BT	620.81	20.59	a	QHD	626.32	22.93	ab
CS	678.14	11.31	a	GT	673.75	NA	ab	GT	620.13	NA	a	BJ	624.31	22.75	ab
BT	677.99	9.22	a	BJ	673.56	14.41	ab	YC	619.05	32.06	a	ZZ	622.68	27.62	ab
NN	676.42	9.21	a	LZ	671.76	19.27	ab	BJ	618.40	25.19	a	NN	618.54	38.12	ab
QHD	675.05	13.36	a	NN	671.23	10.93	ab	WLMQ	617.50	7.86	a	HK	618.18	34.73	ab
WW	674.53	13.01	a	SY	670.99	9.02	ab	LZ	615.83	28.28	a	JQ	616.25	33.67	ab
LZ	673.90	8.72	a	YC	670.63	10.46	ab	ZY	613.85	21.89	a	CC	615.98	22.51	ab
GEM	673.06	NA	a	WLMQ	669.54	7.95	ab	CC	613.28	25.32	a	YC	612.55	29.28	ab
XA	672.68	13.43	a	DH	668.77	17.26	ab	HK	612.77	29.99	a	LZ	612.39	27.16	ab
ZY	672.42	13.71	a	ZY	668.24	20.75	ab	XA	611.72	18.24	a	HEB	611.09	15.17	ab
YM	671.12	14.12	a	BT	665.47	10.16	ab	CS	611.64	41.56	a	BT	610.79	16.70	ab
DH	670.84	13.57	a	JQ	665.23	17.86	ab	WW	611.26	19.47	a	XA	604.93	20.44	ab
JQ	670.61	19.83	a	WW	664.27	12.12	ab	QHD	608.46	31.79	a	CS	603.80	23.66	ab
SY	669.85	16.73	a	YM	661.77	12.83	ab	DH	607.87	34.91	a	DH	602.54	28.25	ab
YC	665.13	11.02	a	CC	661.27	17.65	ab	JQ	607.35	28.49	a	WW	598.88	45.15	ab
HEB	663.80	6.53	a	HEB	659.75	8.62	ab	HEB	601.22	16.77	a	ZY	596.63	14.35	ab
CC	663.77	17.79	a	QHD	658.48	20.90	ab	YM	598.95	20.84	a	YM	573.20	20.60	ab

Throat Chroma								Breast Chroma							
Male				Female				Male				Female			
population	mean	SD	group	population	mean	SD	group	population	mean	SD	group	population	mean	SD	group
CS	0.54	0.02	a	DH	0.55	0.01	a	QQHE	0.43	0.04	a	QQHE	0.41	0.04	a
GT	0.54	NA	ab	HK	0.54	0.02	a	SYS	0.42	0.04	ab	SYS	0.39	0.03	ab
QQHE	0.53	0.02	ab	CS	0.54	0.02	ab	GT	0.38	NA	abc	HEB	0.37	0.05	bc
DH	0.53	0.02	ab	LZ	0.54	0.03	ab	BT	0.38	0.03	bc	LZ	0.36	0.02	bc
HK	0.52	0.03	ab	SYS	0.53	0.02	ab	HEB	0.38	0.02	bc	GEM	0.36	0.02	bc
NN	0.52	0.01	ab	QQHE	0.53	0.04	ab	NN	0.38	0.04	bc	HK	0.36	0.04	bc
ZY	0.52	0.02	ab	SY	0.53	0.02	abc	YC	0.37	0.03	c	CC	0.36	0.03	c
LZ	0.52	0.02	ab	ZY	0.52	0.02	abc	LZ	0.37	0.04	c	YC	0.35	0.02	c
YM	0.52	0.03	ab	YM	0.52	0.02	abc	CC	0.37	0.04	c	CS	0.35	0.02	c
BT	0.52	0.02	ab	GEM	0.52	0.01	abcd	SY	0.36	0.06	c	BT	0.35	0.01	c
SYS	0.52	0.02	ab	BT	0.52	0.02	abcd	ZZ	0.36	0.02	c	ZZ	0.35	0.02	c
JQ	0.51	0.03	ab	GT	0.52	NA	abcd	HK	0.36	0.01	c	DH	0.35	0.01	c
XA	0.51	0.02	ab	ZZ	0.52	0.01	abcd	WLMQ	0.35	0.02	c	XA	0.35	0.02	c
QHD	0.51	0.01	ab	JQ	0.51	0.02	abcd	CS	0.35	0.01	c	SY	0.35	0.03	c
GEM	0.51	NA	ab	NN	0.51	0.02	abcd	DH	0.35	0.02	c	BJ	0.34	0.02	c
ZZ	0.51	0.02	b	XA	0.51	0.02	abcd	BJ	0.35	0.01	c	NN	0.34	0.02	c
WW	0.50	0.03	b	YC	0.50	0.01	abcd	WW	0.34	0.02	c	WLMQ	0.34	0.02	c
YC	0.50	0.02	b	HEB	0.50	0.03	abcd	GEM	0.34	NA	c	YM	0.34	0.01	c
HEB	0.50	0.02	b	WW	0.50	0.01	abcd	XA	0.34	0.02	c	QHD	0.33	0.02	c
BJ	0.50	0.01	b	BJ	0.50	0.03	abcd	YM	0.34	0.02	c	ZY	0.33	0.01	c
CC	0.50	0.03	b	CC	0.50	0.03	bcd	JQ	0.33	0.02	c	JQ	0.33	0.01	c
WLMQ	0.50	0.03	b	QHD	0.49	0.04	cd	QHD	0.33	0.02	c	GT	0.32	NA	c
SY	0.49	0.01	b	WLMQ	0.46	0.07	d	ZY	0.33	0.02	c	WW	0.32	0.01	c

Table S4 Maximal GLMM models analyzing reproductive success in relation to morphological traits and potential sexually selected traits in barn swallow.

	Hatching date					Number of eggs					Number of fledglings				
	r	SE	df	T	*p*	*r*	SE	df	T	*p*	*r*	SE	df	T	*p*
Morphological Traits															
male															
PC1	2.29	1.01	46	2.25	0.03	0.05	0.36	30	0.15	0.88	-0.21	0.33	29	-0.65	0.52
PC2	1.96	0.95	46	2.05	0.05	-0.71	0.36	30	-1.97	0.06	-0.77	0.33	29	-2.36	0.03
PC3	4.30	1.53	46	2.81	0.01	0.20	0.54	30	0.37	0.72	0.27	0.54	29	0.51	0.62
female															
PC1	2.69	0.92	46	2.94	0.01	-0.16	0.33	30	-0.50	0.62	0.00	0.30	29	0.00	1.00
PC2	1.93	0.82	46	2.35	0.02	-0.36	0.31	30	-1.14	0.26	-0.33	0.29	29	-1.13	0.27
PC3	2.69	0.83	46	3.22	<0.01	-0.78	0.26	30	-2.99	0.01	-0.44	0.24	29	-1.84	0.08
Streamer Length															
male	-0.10	0.12	46	-0.89	0.38	0.02	0.04	30	0.41	0.68	0.03	0.04	29	0.92	0.37
female	-0.70	0.19	46	-3.70	<0.01	0.13	0.06	30	2.10	0.04	0.15	0.06	29	2.53	0.02
Colour Traits															
male															
throat PC1	-1.44	1.63	46	-0.88	0.38	0.28	0.52	30	0.54	0.60	0.09	0.48	29	0.19	0.85
throat PC2	-3.90	2.10	46	-1.85	0.07	1.08	0.71	30	1.51	0.14	0.92	0.70	29	1.31	0.20
belly PC1	-0.13	0.71	46	-0.18	0.86	-0.46	0.23	30	-1.98	0.06	-0.34	0.21	29	-1.63	0.11

	belly PC2	-0.90	2.08	46	-0.43	0.67	-0.29	0.71	30	-0.41	0.68	-0.36	0.65	29	-0.55	0.59
	vent PC1	-1.12	0.80	46	-1.40	0.17	-0.01	0.22	30	-0.05	0.96	0.00	0.20	29	0.01	1.00
	vent PC2	0.76	2.27	46	0.33	0.74	0.05	0.73	30	0.07	0.94	0.01	0.68	29	0.02	0.98
female																
	throat PC1	1.72	1.55	46	1.11	0.27	0.05	0.47	30	0.11	0.91	-0.02	0.43	29	-0.06	0.96
	throat PC2	1.08	1.32	46	0.82	0.42	0.22	0.43	30	0.52	0.61	0.10	0.40	29	0.24	0.81
	belly PC1	-1.53	0.84	46	-1.81	0.08	0.33	0.29	30	1.16	0.26	0.41	0.27	29	1.53	0.14
	belly PC2	-0.17	1.66	46	-0.10	0.92	-0.50	0.60	30	-0.83	0.41	-0.57	0.67	29	-0.85	0.40
	vent PC1	0.93	0.83	46	1.12	0.27	-0.15	0.28	30	-0.53	0.60	-0.15	0.26	29	-0.57	0.57
	vent PC2	3.16	2.81	46	1.13	0.27	1.04	0.86	30	1.22	0.23	1.02	0.85	29	1.20	0.24

Table S5 The maximal GLM model analyzing the number of genetic offspring of male barn swallows in first broods in relation to male morphological traits and potential sexually selected traits. GLM model: df = 41.

	Number of genetic offspring			
Male Traits	*r*	SE	T	*p*
Morphological Traits				
PC1	-0.47	0.28	-1.69	0.10
PC2	0.32	0.28	1.14	0.26
PC3	-1.10	0.42	-2.63	0.01
Streamer Length	0.01	0.03	0.33	0.74
Colour Traits				
throat PC1	1.00	0.51	1.95	0.06
throat PC2	1.79	0.61	2.91	0.01
belly PC1	0.56	0.25	2.26	0.03
belly PC2	0.43	0.61	0.71	0.49
vent PC1	-0.12	0.22	-0.53	0.60
vent PC2	-0.30	0.73	-0.42	0.68

Table S6 The maximal GLM model analyzing the body mass of nestlings in relation to the morphological traits and potential sexually selected traits of both parents, parental feeding rate and the brood size. GLM model: df = 202.

	Nestling body mass			
	r	SE	T	*p*
Morphological Traits				
male				
PC1	0.64	0.13	4.97	<0.01
PC2	0.36	0.13	2.82	0.01
PC3	-0.15	0.22	-0.68	0.50
female				
PC1	-0.22	0.14	-1.60	0.11
PC2	0.28	0.13	2.23	0.03
PC3	-0.47	0.17	-2.87	<0.01
Streamer Length				
male	-0.07	0.02	-4.17	<0.01
female	0.05	0.03	1.83	0.07
Colour Traits				
male				
throat PC1	0.87	0.32	2.73	0.01
throat PC2	0.95	0.36	2.64	0.01
belly PC1	0.24	0.13	1.82	0.07
belly PC2	-0.06	0.38	-0.15	0.88
vent PC1	-0.33	0.10	-3.46	<0.01
vent PC2	0.06	0.53	0.11	0.91
female				
throat PC1	-0.48	0.56	-0.86	0.39
throat PC2	-0.10	0.50	-0.20	0.84
belly PC1	0.10	0.19	0.50	0.62
belly PC2	-0.10	0.27	-0.37	0.71
vent PC1	-0.14	0.14	-0.97	0.33
vent PC2	-0.30	0.48	-0.63	0.53
Total feeding rate	0.00	0.01	-0.29	0.78
Brood size	0.26	0.14	1.87	0.06

References

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