Male sexually selected traits of the barn swallow *Hirundo rustica gutturalis* in China

Nana Li

School of biological and chemical sciences

Queen Mary
University of London

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Abstract

The barn swallow (*Hirundo rustica*) is represented by six subspecies and has two well-studied ornamental traits, tail length and ventral plumage colour, which vary geographically among subspecies. Sexual selection on these traits has been suggested to drive speciation. The European subspecies *rustica* has pale ventral feathers and long tail streamers, and females prefer males with longer tail streamers. The North American *erythrogaster* has shorter tails and red ventral plumage and their females use redness of ventral plumage as a mate choice cue. In the Middle East, the subspecies *transitiva* bears long tail streamers and red ventral feathers, both of which have been suggested to show male attractiveness. The Asian subspecies *gutturalis* has a pale belly with short but dimorphic tails. Studies in Japanese populations have suggested that the white spot on the tail feathers and throat patch are sexually selected in males, but this explanation leaves the dimorphism of tail streamers unexplained. To further investigate the sexually selected traits of *gutturalis*, especially the role tail streamers might play, I studied a population of the barn swallow *gutturalis* in China between 2013 and 2015, and conducted a partial cross-fostering experiment in 2015. My data indicate that male tail streamers are sexually selected in the *gutturalis* population in China. Longer-tailed males (with deeper fork tails) were cuckolded less frequently and had a higher reproductive success, they also had mates that invested more in parental care and had a higher total peak body mass of offspring. Both male and female body condition (body mass and tarsus length) influenced offspring growth. Males with larger body mass initiated breeding earlier and their offspring had a larger body mass on day seven after hatching and grew faster as determined by a cross-fostering experiment. Larger females reared offspring that grew faster and reached a higher peak body mass both in original nests and nests with cross-fostered nestlings. Based on feeding rate observations and the cross-fostering experiment, it seems that females obtain indirect benefits rather than direct benefits from mating with more attractive males.

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Chapter 1 General Introduction

1.1 Sexual selection

In many animals, males but not females, have conspicuous traits such as bright feathers, coloured skin, elaborate songs or long horns, examples are the train of the peacock (*Pavo cristatus*) and the lion's (*Panthera leo*) mane. The prevalence of such exaggerated traits in one sex, categorised as secondary sex traits, have attracted the interest of biologists at least back to Darwin because they seem costly but do not enhance an individual's survival (Darwin 1859; Darwin 1871; Smith 1991; Kotiaho 2001).

It has been demonstrated in various species that sexually selected traits often develop beyond the optimum that might be expected under natural selection to be a costly handicap. For instance, experiments have shown a direct fitness cost for sexually selected, bright body-colour patterns in male guppy (*Poecilia reticulata*) in the form of an associated greater risk of mortality to predator blue acara cichlid fish (*Aequidens pulcher*) (Godin and McDonough 2003). In European green lizards (*Lacerta viridis*), males adopt two mating strategies, territorial and floaters, and females prefer males with a brighter nuptial patch. Research has shown that successful territorial males with large territories had a duller nuptial patch than floaters indicating the high cost of bright ornaments (Molnár, Bajer et al. 2016). The notable long tails of birds, i.e. the long central tail feathers of male scarlet-tufted malachite sunbird (*Nectarina johnstoni*) have been proven to be costly to grow and maintain (Evans and Hatchwell 1992; Evans and Thomas 1992). The males with a slightly artificially shortened tail can perform better in flight and catching insects (Evans and Hatchwell 1992).

Male-male competition and female preference

Darwin reasoned that the function of extravagant traits was to win competition among members of the same sex (typically males) to gain mating advantages and/or to attract the members of the opposite sex (typically females) (Darwin 1859). The first process explains that larger or more exaggerated traits (usually weapons) help males win male-male competition and the access to more fertile females. A classic example is that male red deer's (*Cervus elaphus*) antler size is positively related with their fighting ability and reproductive success (Clutton-Brock, Guinness et al. 1982). Another example is the horns of dung beetle of genus *Onthophagus* (Coleoptera: Scarabaeidae) (Emlen, Marangelo et al. 2005). It is ubiquitous that female dung beetles dig burrows into the soil below the dung. Males guard the entrances to these burrows and use their horns to fight with intruding males. Observations in different species have shown that males with longer horns tend to win (Emlen 1997; Moczek and Emlen 2000; Hunt and Simmons 2001) suggesting that the horns are under directional sexual selection (Emlen, Marangelo et al. 2005). The second process describes that the function of the extravagant ornamental traits is to attract females (Andersson 1994). A great number of female preferred traits have been reported, classic examples are: in the trains of Indian peafowl (*Pavo cristatus*), the number of the eye-spots predicts the mating success of males (Petrie, Tim et al. 1991); female widowbird (*Euplectes jaegeri*) prefer males with longer tails (Andersson 1982); female field crickets (*Gryllus integer*) choose males with longer calling-bout durations (Hedrick 1986), and more sexually preferred traits of insects, fishes, birds and mammals etc. have been reviewed by Andersson (1994).

Extravagant traits that preferred by females benefit males not only with mating chances but also other breeding resources as females typically invest more in offspring from the beginning of anisogamy (Robert 1972; Andersson 1994). The competition among males for access to females and other breeding resources has been a force to drive the evolution of conspicuous traits in different mating systems (Andersson 1994). Competition for female preference is obviously fiercer in polygynous species because females provide parental care alone and successful males can obtain multiple females while others have none (Andersson 1994). Male competition also occurs in socially monogamous species because female access can be limited when the sex ratio is skewed for instance the mortality might differ between sexes or when only a few females have arrived at the breeding site. Also male attractiveness can affect females' breeding investment, for instance, in many bird species, males with more exaggerated traits have partners that allocate more maternal effort from egg investment to offspring provisioning (Sheldon 2000). Furthermore, sperm competition often occurs in socially monogamous species especially birds with high frequency extra-matings (Kempenaers, Verheyen et al. 1992; Griffith, Owens et al. 2002). The phenotypes favoured by females can predict success in extra-pair copulations: for instance, the blacker dorsal plumage and larger forehead patches (sexually selected ornaments) was related to the number of extra-pair young sired by male pied flycatchers (*Ficedula hypoleuca*) (Canal, Potti et al. 2011).

Though less common, females will compete for mating with males in the sex-role reversed species where males provide parental care or when the number of males is limited. For instance, in wattled jacanas (*Jacana jacana*) in the Republic of Panama, females are heavier and with more fleshy facial ornamentation and wing spurs than males, males alone hatch eggs and feed chicks, the sex ratio is female dominant (can be 1.43:1 to 2.22:1). A study has found that heavier and more ornamented females were more successful to achieve in mating with multiple males (Emlen, Wrege et al. 2004).

In summary, sexual selection is produced by the differences in reproduction that arise from variation among individuals in traits that affect success in competition over mates and fertilizations (Andersson 1994; Clutton-Brock 2007).

Study of sexually selected traits

To test the sexually preferred traits, trait-related comparative analysis such as selection gradients and differentials have been the most useful approach for demonstration and explanation for taxonomic and other trends of secondary sex traits associating with phylogeny, mating system, life history and other aspects (Andersson 1994). Based on observations under natural condition, causal relationships can be obtained between measurements of sexually selected traits and the corresponding attractiveness of the individual with such traits in mating, female parental care investment and reproductive success. Further treatments are commonly done by artificially manipulating the traits to test its influence on breeding success, examples would be altering the tail length of birds (e.g. tail streamers of barn swallow (*Hirundo rustica*) (Møller 1988a)), dying plumage to change its brightness or colouration (e.g. house finch (*Carpodacus mexicanus*) (Hill 1991)). In the highly polygynous red-collared widowbird (*Euplectes ardens*) population, males have a long graduated tail and red collar plumage. Observations of males' courtship behaviours have demonstrated a strong female preference for long tails rather than the plumage colour (Pryke, Andersson et al. 2001). In a further tail manipulation experiment, tail length had been confirmed as the primary mate choice cue which influenced male reproductive success: longer-tailed control males attracted significantly more nesting females to their territories compared to males with experimentally shortened tails (Pryke and Andersson 2005). A recent manipulation experiment in fish skin was carried out on mosquitofish (*Gambusia affinis*), a species in which males lack colourful ornaments. In the experiment males were painted with blue, red and transparent colourations. The study shows that females prefer blue to transparent ornaments and dislike males with red colours, which might be seen as unhealthy or injured (Casner, Fackelman et al. 2016).

Female choice strategies

Among sexual selection cases (232 cases from 186 species) reviewed by Andersson (1994), 30 cases are male choice (mainly selecting female body size and fecundity) and 167 cases are female choice. Therefore it is common to observe that males maintain conspicuous traits to attract dull females, but meanwhile for females, being choosy can also be costly (Parker 1983). Females commonly adopt best-of-n strategies (decide on the best mate in the available mate pool), or threshold strategies (choose the first one whose quality exceeds a threshold criterion) and adjust strategies in different time of season with changing availability of mates. A study in sand gobies, *Pomatoschistus minutus*, a promiscuous fish with paternal care shows that they are most consistent with a threshold-criterion tactic. Half of the 26 females spawned with the first male encountered and other females accepted the males that courted intensively between two or more males in sequence and never returned to a previously inspected male (Forsgren 1997). In the sex-role reversed pipefish *Syngnathus typhle*, males prefer large females. Manipulation experiments on mate density have demonstrated that males exerted a mate choice only under high mate density. When mates were encountered infrequently, males reduced the costs accepting lower quality females (Berglund 1995). A recent modelling study has also shown that high costs occur most frequently in scramble competition, which constrains females to have a very low acceptance threshold or to sample two individuals at most in the population (Dechaume-Moncharmont, Brom et al. 2016).

Benefits of mate choice

When there is a restricted maximum number of offspring in the season, intensive parental care investment and a costly mate choice process, females would be expected to choose males with reliable signals which guarantee them benefits from mating with such males. Females are the choosy sex most frequently and have been observed to choose mates with various preferences including male body size, male plumage colour, territory quality and other material recourses (Møller and Jennions 2001). Generally, females could obtain direct breeding resources and/or an indirect advantage from heritable good genes from mate choice.

a) Direct benefits of mate choice

Direct benefits of choosing a sexier male might include access to resources or habitat such as food, parenting ability, territories; risk-reduction such as defensive ability, infection avoidance, as described in the “good parent theory” (Hoelzer 1989; Møller and Jennions 2001; Wagner 2011). In several fishes, female prefer to spawn in the nest of a male which already contains eggs showing that the nest might be safe (Noonan 1983; Sikkel 1989). Some birds’ song rates reflect food availability in the nest, e.g. red-winged blackbirds (*Agelaius phoeniceus*) (Searcy 1979) and dunnocks (*Prunella modularis*) (Davies and Lundberg 1984). In some other birds whose males incubate and provision offspring, females select males that can provide better parental care, e.g. male red-winged blackbirds that fed more tend to have larger harems and their parental care influenced the reproductive success (Muldal, Moffatt et al. 1986).

b) Indirect benefits of mate choice

Since epigamic traits are believed to advertise the genetic quality of mates (good genes or compatible genes) (Mays and Hill 2004; Neff and Pitcher 2005), females could have better quality offspring by choosing more attractive males over less attractive ones as indicated by the “good gene theory” (Zahavi 1975; Mead and Arnold 2004; Andersson 2006). Fisherian “sexy sons theory” explains that more attractive males will sire sexier sons that carry the alleles for the trait and daughters carry the alleles for the preference of it (Fisher 1930; Andersson 1994; Kirkpatrick and Hall 2004). This self-reinforcing coevolution between trait and preference was originally known as “runaway theory” (Pomiankowski and Iwasa 1998). Female preferences for good genes (and compatible genes) are more likely to be observed in polygynous species with non-resource-based mating systems in which males only provide sperm or little parental care (Kirkpatrick 1987; Reynolds and Gross 1990). In monogamous species, females also favour males with good genetic quality, and many socially monogamous species have been found to have extra-pair copulations with more preferred males for obtaining better genes (Andersson 1994; Kokko, Brooks et al. 2003; Mead and Arnold 2004; Forstmeier, Nakagawa et al. 2014). For instance, multiple mating showed benefits for female reproduction that increased the hatching success of the cocoons of earthworm (*Eisenia andrei*) (Porto, Velando et al. 2012).

Multiple scenarios can occur together, males with exaggerated traits could provide both direct and indirect benefits to females e.g. the bluest male blue grosbeaks (*Guiraca caerulea*) have the largest body size, maintain the largest territories with the greatest prey abundance, and feed nestlings at the highest rates in the first nest of the season (Keyser and Hill 2000). In partial cross-fostering experiments, casual relations between offspring growth performance (growth rate, body condition and survival) with both original and fostering fathers will be tested to distinguish direct or indirect benefits females obtain from mate choice. For example, freely mating, female zebra finches' (*Taeniopygia guttata*) reproductive success was 37% higher than when they were forced to mate with a limited number of mates and a cross-fostering experiment showed that the offspring survival rate was related to the rearing parent identity (Ihle, Kempenaers et al. 2015).

New researches have been updating the sexual selection theories whilst challenging the existing paradigm and definitions. For instance, some ornaments may once have been weapons that evolved under male-male competition, which suggests that other forms of selection beyond female choice might be overlooked (McCullough, Miller et al. 2016). The red nuptial colouration of the three-spined stickleback (*Gasterosteus aculeatus*) has been shown to function in offspring defence instead of female choice because males expose this colouration while raising offspring after the mating season has finished (Candolin and Tukiainen 2015). Alternatively, the expression of ornamental traits can be environment/season dependent, for example a safer environment would allow males to display more exaggerated ornaments (Heinen-Kay, Morris et al. 2015). Also along with the breeding season the operational sex ratio changes: the choosy sex might lower their standards or adopt the secondary selected trait to fit the best of the situation (Passos, Tassino et al. 2014;

Wacker, Östlund-Nilsson et al. 2016). Examination of trait function and evolution seems to matter to both males and females, and more pluralistic and integrative approaches considering both sexes are still needed to understand the evolution of sexually selected traits through which males and females achieve reproductive success (Kokko, Brooks et al. 2003).

In this thesis, I observed a population of barn swallows (*Hirundo rustica gutturalis*) from 2013 to 2015 in Qingdao China to test the roles that several potential sexually selected male traits play. I first described general morphology and dimorphism of the potential sexually selected traits of this population. Then further examined whether these potential sexually selected traits predicted laying date, parental care investment and reproductive success, and meanwhile related the breeding success behaviour to female traits to test the importance of female ornamental traits and mutual selection. In 2015, I conducted a cross-fostering experiment to test the relationship of phenotype of both fostering and genetic parents with offspring growth, to reveal the benefits (direct and/or indirect) female can obtain from choosing preferred mate.

1.2 Study animal

1.2.1 Barn swallow

The barn swallow (*Hirundo rustica*) is an aerial insectivorous passerine that frequently resides alongside humans. It has a streamlined body shape, long pointed wings and a long forked tail. It weighs about 20 g (Turner 2006). The plumage colour patterns include a metallic blue dorsal side, chestnut forehead, chin and throat patches, and close to black elongated tail streamers with a white spot towards the base of each tail feather (see an example individual in **Figure 1.1**). The colouration of the ventral side of barn swallows varies from pale to dark red among subspecies (Scordato and Safran 2014). The most obvious sexual dimorphism in barn swallows is that males have longer tail streamers and larger body size. Distributed worldwide, often alongside humans (Zink, Pavlova et al. 2006), the barn swallow has been a popular bird in culture as well as scientific studies (Møller and Gregersen 1994; Turner 2006).

Figure 1.1 An individual barn swallow *H. r. gutturalis* from China.

Research into barn swallow speciation and phylogeny is still updating our knowledge of this species, especially for the mixed breeding populations in the boundary areas of various subspecies (Dor, Safran et al. 2010). Currently, barn swallows are considered to be a single species with six subspecies (**Figure 1.2** (Brown and Brown 1999)). The nominate form, *H. r. rustica*, migrates from sub-Saharan Africa to breed throughout Europe, North Africa and Western Russia. *H. r. tytleri* occurs in the Baikal region of East Russia to South Mongolia wintering in India and Southeast Asia. *H. r. erythrogaster* breeds in North America and overwinters in South and Central America. Two subspecies are distributed in restricted areas in the Middle East and North Africa: *H. r. savignii* in the Nile Valley in Egypt which is resident with no migration, and *H. r. transitiva* which migrates a short-distance to breed in Israel, Jordan, Lebanon, and Syria. The study subspecies, *H. r. gutturalis*, breeds in the Russian Far East, China, Japan and Korea, and migrates to Southeast Asia in winter (Scordato and Safran 2014).

Barn swallows are monogamous, males and females provision offspring together and both have extra-pair mating behaviour (Møller and Gregersen 1994; Turner 2006). They build an open bowl-shaped nest using mud mixed with grass stem inside human buildings such as animal barns, bungalows and hallways, some also dwell under roofs and bridges. Migratory barn swallows spend 3-10 months in the breeding areas. When arriving at the breeding area, they either use the old nest, repairing it, or both members of the pair build a new nest. Males arrive at the breeding site first and will occupy a potential nesting site in order to attract females. They display their outer tail feathers with vigorous singing to attract females which might fly into the area multiple times before deciding whether to stay with the male or not (Møller and Gregersen 1994). Each season, barn swallows complete 1-3 broods in each of which females lay 1-7 eggs and incubate them approximately 14 days before hatching, but at least *erythrogaster* and *gutturalis* males also contribute to incubation (Ball 1983; Smith and Montgomerie 1992); both parents provision the offspring for approximately 20 days to fledge and will feed them some more days (Turner 2006).

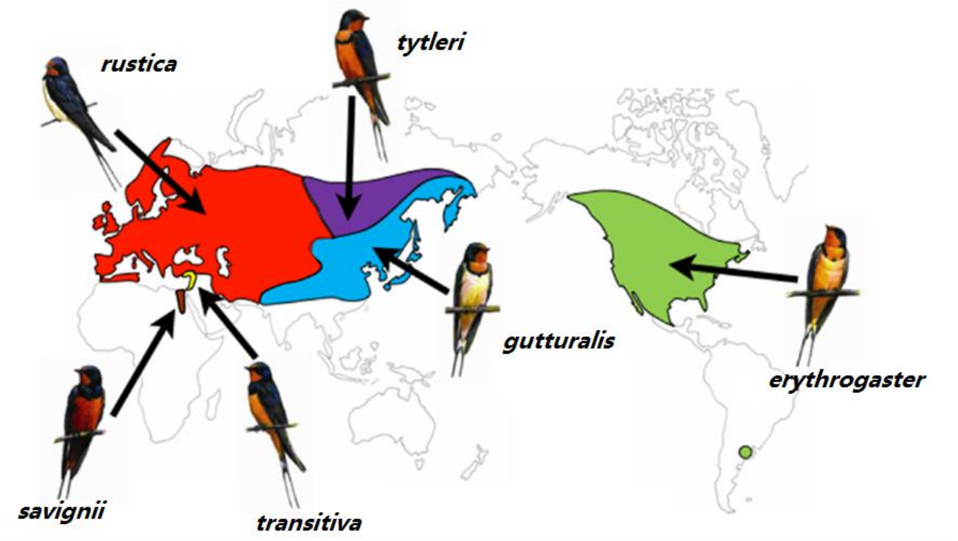

Figure 1.2 Six subspecies of barn swallow thorough the world with one typical male to show various phenotypes especially in tail streamer length and ventral side plumage colouration. From Scordato and Safran (2014).

Phenotype of the barn swallow varies among subspecies, particularly in body size, tail streamer length and ventral plumage colouration (**Table 1.1**). The most well-known subspecies *rustica* has the largest body size, longest tail streamer, and the palest ventral feathers. *H. r. gutturalis* has the smallest body size, the shortest tail streamers and pale mixed with light orange belly feathers. The other four subspecies all have darker red ventral plumage and longer tail streamers than those of *gutturalis* (Scordato and Safran 2014).

1.2.2 Sexual selection of barn swallow

Sexual selection clues that females use to choose males have been shown to vary across subspecies in four subspecies *rustica*, *gutturalis*, *erythrogaster* and *transitiva* (**Table 1.1**) and thus they are suggested to play important roles in phenotypic divergence and speciation of barn swallow (Romano, Costanzo et al. 2016; Safran, Vortman et al. 2016).

Table 1.1 Comparison of breeding regions, phenotypes and sexual selection male traits of six subspecies of barn swallow *Hirundo rustica* (summarised from Scordato and Safran 2014**).**

Subspecies	***rustica***	***tytleri***	***gutturalis***	***savignii***	***transitiva***	***erythrogaster***
Breeding regions	Europe, North Africa and western Russia	Baikal region in eastern Russia and Mongolia	Far East Russia, China, Japan, Korea	Nile Valley of Egypt	Israel, Jordan, Lebanon, and Syria	North America
Body size	largest	intermediate	smallest	large	large	intermediate
Tail streamer	long	intermediate	shortest	long (shorter than ***transitiva***)	long	intermediate
Ventral feather	palest	dark red	pale with orange-brown	dark red (darker than ***transitiva***)	dark red	dark red
Sexually selected male traits	**tail streamer** (Møller 1988a), **tail white spot** (Kose and Møller 1999), **song** (Møller, Saino et al. 1998)	Unknown	**throat patch**, **tail white spot** (Hasegawa, Arai et al. 2010a)	Unknown	**tail streamer**, **ventral plumage redness (Vortman, Lotem et al. 2011)**	**ventral plumage redness** (Safran and McGraw 2004)

H. r. rustica

1) Tail streamers

The barn swallow has been a text-book model bird for sexual selection study since the 1980s with a famous manipulation experiment conducted on *H. r. rustica* showing that male tail streamer length was a predictor of mating success (Møller 1988a). Male barn swallows were put into four groups in which the tail streamers were: elongated, shortened, untreated in a control group, and cut but not changed in length in a sham control group. The result showed that the males with elongated tails attracted females more quickly than males with tails unaltered or shortened. Afterwards, an extensive amount of research has followed to examine the sexual function of the male tail streamers in this small bird (Møller and Gregersen 1994).

Empirical studies on *rustica* have consistently shown that long tail streamers are the trait preferred by females, and that long-tailed males have stronger immune systems, initiate breeding earlier and have higher reproductive success (obtaining more extra-mating opportunities, less commonly being cuckolded by social mates) than males with shorter tails (Smith, Montgomerie et al. 1991; Møller, Chabi et al. 2006). Furthermore, it is believed that females that mate with longer-tailed males obtain indirect benefits, for instance, studies on *rustica* have shown that the offspring of longer-tailed males had better immune function than the offspring of short-tailed males (Møller and Gregersen 1994; Saino, Bertacche et al. 2002). However, more attractive males do not tend to be better fathers because they spend more time seeking extra-pair females and care for their offspring less (Møller 1990b; Møller and Gregersen 1994).

The roles that long tail streamers play in mating and breeding remain unclear. Some experiments have shown that longer tail streamers are handicaps. For instance, tail length manipulation studies have shown that a small part of the tail streamers (12 mm, 9-20%) has evolved under sexual selection which indicates that longer tails (beyond the aerodynamic optimum) could be a handicap but will benefit males by increasing their attractiveness (Evans 1998; Buchanan and Evans 2000; Rowe, Evans et al. 2001). In a *rustica* population it has been found that male survival chance decreased with tail elongation and increased with tail shortening, supporting the assumption that the long tail streamers are costly handicaps (Møller and de Lope 1994). It has also been suggested that barn swallow tail streamers have evolved only under natural selection, functioning as a flight control device, and the variance is showing sex and age instead of an ornament (Norberg 1994). In an aerodynamic performance by manipulating tails into different lengths, it has been found that the optical lengths varies among individuals, but the extra lengths which is supposed to have evolved by mate choice showed no difference among individuals. Therefore the experiment suggests that tail streamers naturally reflect the individual's age and sex rather than being sexually selected (Bro-Jørgensen, Johnstone et al. 2007). Though older swallows have longer tails, significant advantage in breeding success has still been shown when the age factor was being controlled (Møller, Barbosa et al. 1998).

The handicap theory on tail streamers of barn swallow is still under debate (Aparicio and Møller 2012; Evans, Bro-Jørgensen et al. 2012), but after all the length beyond aerodynamic optimum should be restricted within a small range because the energy budget of locomotion is crucial for efficient aerial hunters Hirundines (Evans and Thomas 1992). Further study of within-individual tail streamer manipulations and its effect on flight performance and fitness dynamics will provide more understanding of natural and sexual selection force on tail streamers (Scordato and Safran 2014).

2) Tail white spots

In *rustica*, the white spots on the tails have also been shown to be important in mate choice, possibly because they signal resistance to louse infestation (but see Bush, Kim et al. 2006) or ability to keep it unbroken during display. Males with artificially reduced spot size suffered a decrease in reproductive success (Kose and Møller 1999; Kose, Mänd et al. 1999), though the treatment itself might have affected the female decision because it looked abnormal (Buchanan and Evans 2000). A recent study has shown that the shape and size of the white spot have an association with feather growth during winter moulting which directly reflects body condition, suggesting white spot is a reliable signal of phenotypic quality in socio-sexual communication (Saino, Romano et al. 2015).

3) Song

Barn swallow males sing very complex songs with various social functions dynamically changing within the season (Galeotti, Saino et al. 1997). This makes the signal harder to interpret than heritable and more easily measureable morphological traits like feather colour or tail length, thus fewer studies have been reported on male song compared to other ornamental traits (Scordato and Safran 2014).

Scordato (2014) has reviewed the song functions in mating of male barn swallows and concluded that females prefer males with high quality songs in *rustica*. Males that sing longer songs paired more successfully (Garamszegi, Heylen et al. 2005; Scordato and Safran 2014). Male *rustica* with longer tails and higher song rates had fewer extra-pair young in nest (Møller, Saino et al. 1998). However tail streamer length was a more important trait because shorter-tailed males failed to be as attractive as longer-tailed males by singing at a higher rate (Møller, Saino et al. 1998).

H. r. erythrogaster

In North America, the barn swallow subspecies *erythrogaster* have shorter tail streamers but dark red ventral plumage. There are studies showing that tail streamers predicted mating advantages: males with longer tail streamers had a higher extra-pair success than shorter-tailed males, and they also paired with early breeding females in prime body condition (Obayashi, Hayakawa, et al. 2006). Further, the advantage of tail streamer length was shown to be associated with age that older males with longer tails had higher fertilization success than younger shorter-tailed males (Lifjeld, Oddmund, et al. 2011). However, the conclusion is debated because in other researches, long tail streamers did not confer any advantages in mating, instead, studies of populations in North America show that the redness of the ventral plumage is related to male pairing patterns and seasonal reproductive success (Safran and McGraw 2004). In an egg removal experiment, females increased their social mate's proportion of paternity of their eggs when his feather colouration was artificially enhanced (Safran, Neuman et al. 2005; Neuman, Safran et al. 2007). Consistent with parental care studies with *rustica*, male *erythrogaster* with redder feathers did not provision offspring more indicating that females obtain indirect benefits from attractive males (Maguire and Safran 2010).

The deeper colouration of ventral feathers is unlikely to be an obvious handicap for *erythrogaster*'s survival, because individuals with redder plumage did not seem to be more conspicuous to predators (Scordato and Safran 2014). The eu- and phaeomelanin pigments maintaining the orange-brown throat and red ventral plumage (McGraw, Safran et al. 2005) are not believed to be costly to produce either (Hubbard, Uy et al. 2010), though the allocation of melanocortins to different receptors to show in external tissues may result in a trade-off between pigment deposition and hormonal function (Roulin, Almasi et al. 2008). Specifically, studies in *erythrogaster* have found that darker males had higher levels of circulating testosterone, and males with experimentally darkened plumage colour increased their testosterone levels, which explains that plumage colour is an honest signal in advertising testosterone levels (Safran, Adelman et al. 2008).

H. r. transitiva

Studies in *rustica* and *erythrogaster* inspired research on the Middle Eastern subspecies *H. r. transitiva* which has both dark red ventral plumage and long tail streamers. Recent studies have shown that both male tail streamer length and ventral feather colour play roles in breeding success: longer-tailed males initiated nest earlier and had fewer extra-pair offspring in nest; males with redder ventral plumage had higher reproductive success (Vortman, Lotem et al. 2011; Vortman, Lotem et al. 2013). A comparison study shows that in America, male *H. r. erythrogaster* with darkened ventral colouration and shortened streamers gained paternity between two successive breeding attempts while exaggeration of both traits improved reproductive success of male *H. r. transitiva* in Israel (Safran, Vortman et al. 2016).

H. r. gutturalis

In the Asian subspecies *H. r. gutturalis*, the model subspecies of this thesis, studies on sexually selected traits have been conducted on populations in Japan since 2009 (Kojima, Kitamura et al. 2009). Multiple traits have been tested to determine if they are related to survival and breeding success: tail streamer length, white spots on tail feathers, ventral plumage colour pattern and throat patch area and colour (Kojima, Kitamura et al. 2009; Hasegawa, Arai et al. 2010a; Tazzyman, Seymour et al. 2012; Hasegawa, Arai et al. 2012b). Male tail streamer length has been shown to be related to survival rate but not to breeding success (Hasegawa, Arai et al. 2014b). Males with a less saturated (more colourful) throat and larger white spots in tails bred earlier than others, indicating an advantage in mating (Hasegawa, Arai et al. 2010a). The throat patch length is believed to vary seasonally and an artificially reduced throat patch led to an increase of parental care which suggests it might be a sexually selected ornament and that males might adjust their behaviour in response to ornamental trait change (Hasegawa and Arai 2015). According to multiple studies, males with more exaggerated traits provided less parental care, indicating that female *H. r. gutturalis* obtain indirect benefits rather than direct benefits from mate choice (Hasegawa, Arai et al. 2014a; Hasegawa and Arai 2015).

However stronger evidence is still needed to draw a clear conclusion on sexually selected traits of *H. r. gutturalis*. For instance, incubation time duration was used as one standard for female breeding investment in the Japanese population, resulting in the conclusion that males with larger white tail spots are preferred because their mates spent longer incubating the clutch (Hasegawa, Arai et al. 2012b). However, whether the female adjusted incubation according to male traits is unclear (for instance, incubation investment could be affected by female fecundity, weather, egg number and food resources), and there has been no further study to show whether incubation time is related to reproductive success. Secondly, a study showed that females preferred males with better quality territories (as indicated by the number of old nests), to initiate breeding earlier (inconsistent with the observation in *rustica* (Møller 1990b)). Territory quality was positively related to the colourfulness of throat patch but not to wing length, tail length, or tail white spots (Hasegawa, Arai et al. 2014a). However, the latter three were significantly correlated with laying date (Hasegawa, Arai et al. 2012a; Hasegawa, Arai et al. 2014a).

1.3 Purpose of study

Studies in the barn swallow *H. r. rustica* have demonstrated that the female preference for long tails is a strong sexual selection force driving the males to have longer tail streamers than females, for instance the length difference was 14 mm (males 105 mm and females 91 mm) in a population in Northern Europe (Møller and Gregersen 1994). The Asian subspecies *H. r. gutturalis*, has a more similar phenotype to *rustica* than to other subspecies, with a pale belly and dimorphic tails as shown in a Japanese population, the length difference was 15 mm based on data collected in 2005 (males 94 mm, females 79 mm) and 13 mm in 2006 (males 93 mm, females 80 mm) (Hasegawa, Arai et al. 2010a). We would expect the male tail streamers in this subspecies to serve as a sexually selected cue. Currently, multiple male traits have been reported to be sexually preferred by females in the Asian subspecies *gutturalis* in Japanese population, but the sexual dimorphism in tail streamers remains unexplained.

Here we present work that has been done on a population of *gutturalis* breeding in eastern central China, investigating their sexually selected traits to contribute more potential understanding for this subspecies.

1.3.1 Aims

1) Describe *H. r. gutturalis*' morphology and dimorphism of potential traits that might be sexually selected.

2) Identify the male traits of *gutturalis* that predict the mating success and reproductive success.

3) Identify the advantage of female ornamental traits in reproductive success and mutual selection on such traits.

4) Explore the benefits that female *gutturalis* obtain from mating with more attractive males than with less attractive ones.

5) Examine the relationship between females' reproductive allocation and male attractiveness.

1.3.2 Objectives

1) Collect data of morphology of barn swallow *gutturalis* and present their dimorphism including body mass, tarsus length, wing length, tail length (outermost tail feathers and short tail), tail white spot length and feather colouration (throat, breast, belly and vent areas), to determine potential traits being sexually selected.

2) Correlate the potential sexy traits to mating success (pair up early) and reproductive success for both males and females.

3) Determine male traits that are preferred in copulations. Examine the male traits that relate to paternity maintenance in social nest and that preferred by other females in extra-pair mating.

4) Test the correlation of male attractiveness and female reproduction investment in number of eggs and parental care.

5) Explore direct and/or indirect benefits that females obtain to mate with more ornamented males. Determine the genetic and parental care effects on offspring growth performance in a cross-fostering experiment.

1.4 Study site

The study was conducted from April to August between 2013 and 2015 in a village near Qingdao, which lies in Shandong Peninsula in the east coast of central China, adjacent to the Yellow Sea (N 36 ° 36′, E 120° 13′) (**Figure. 1.3**). Qingdao has a temperate, four-season, monsoon-influenced climate that lies in the transition between the humid subtropical and humid continental regimes. The annual mean temperature is 12.6 ℃, extreme temperature range since 1951 has been recorded from -15.5 ℃ on 16 January 1958 to 38.9 ℃ on 15 July 2002 (https://en.wikipedia.org/wiki/Qingdao). Winter is cool to cold with the coldest month January with an average temperature of -1.5 ℃. Neighbouring the ocean, it experiences a one-month delayed spring compared to most inland areas. Summer is generally hot and humid, the hottest month is July with an average temperature of 25.3 ℃ (http://www.qdsn.gov.cn/n16/n1114/n1145/n1160/index.html).

Figure 1.3 The study site of barn swallow *H. r. gutturalis* is in a village in Qingdao of Shandong Province on east coast of China.

Barn swallows that breed in Qingdao, arrive at this site in early March and leave in late September completing 1 or 2 broods. Like in Japan (Hasegawa, Arai et al. 2010a), they build mud nests around 2.5 meters high under long eaves or inside the concrete hallway buildings along the street separately rather than in groups, usually one nest and occasionally two per building (**Figure 1.4**). The red-rumped swallows (*Cecropis daurica*) and barn swallows share the same habitat locally (the red-rumped swallow has a much smaller population than the barn swallow). They used to build nests inside the main bungalows where people live resulting in them being well protected from extreme weather and predators like magpies (*Pica pica*). However, in the last two decades, local people have started using window nets to keep flies and mosquitoes out which block entry for barn swallows.

Figure 1.4 The hallway buildings barn swallows nest in. They prefer to stay inside (a), but can also build the nest just under the eaves (b).

1.5 General methods

1.5.1 Field work methods

Capture

Parent barn swallows were captured using mist nets while they visited the nest on day seven after their nestlings hatched. The mist net was set on one side of the entrance of the hallway building and covered quickly when they flew in to feed, they were caught when they hit the net. At capture, they were marked with two colour rings on their legs to aid individual identification.

Trait measurements

Birds were weighed with an accuracy of ± 0.25 g with spring scale (Pesola, Switzerland). Tail length (both tail streamers and short tail in the middle) and right tarsus length, the longest length of white spots on the right two outermost tails were measured using a electronic digital vernier caliper (Jazooli, UK) with an accuracy of ± 0.1 mm. Both right and left wing lengths were measured using a stopped ruler accurate to ± 0.1 cm.

In 2014 and 2015, the first three eggs of each brood were photographed with a graph paper under them and egg width and breadth were obtained from the photo to determine egg volume (mm^3), see the detailed method in Chapter 4.

In 2013, the total mass of chicks on day 10 and day 15 after hatching was weighed (accurate to ± 0.25 g). In 2014 and 2015, the body mass of each chick on each day between day 7-16 was taken to obtain the growth rate of nestlings (nearest to 0.01g).

Sample collections

In 2013, around five feathers samples from throat, belly, and vent were collected for colour analysis and in 2014, feathers from throat, breast, belly and vent parts were collected. The feathers were stuck on a paper card with individual ID and collection date and kept in a box at room temperature. The spectral reflectance was measured within four months since collection.

In 2014 and 2015, approximately 20 μl blood samples were also taken from all adults at capture and chicks of 10 days old for paternity test and sexing.

Observation

After mid-April, when barn swallows started building nests, I checked on the progress of their breeding season, when they paired and their nest building every other day. This enabled me to determine the day on which the first egg was laid. It typically took 14 days for eggs to start hatching, I therefore stopped visiting for about 10 days during incubation then started to visit regularly every day to record the date of hatching. For fledging date, I observed for a couple of minutes daily to check if any offspring had left the nest. The number of eggs in the clutch, the number of chicks that hatched and fledged were recorded for each brood. I used binoculars (concealing myself with plastic sheet) to record the number of visits parent barn swallow made in one hour when chicks were 10 and 15 days old.

1.5.2 Experiments

Paternity test

Blood samples were processed for sexing the parents and paternity test to obtain the genetic reproductive success for both parents, the detailed method is described in the Methods of Chapter 3.

Cross-fostering experiment

In 2015, among broods that hatched on the same day, some of the nestlings were swapped and all chicks were marked with coloured strings on their legs so that I could identify them, and the body mass of every chick was recorded from day seven to day 16 after hatching.

1.5.3 Data analysis

Linear mixed effects models (LMER) for Gaussian data were calculated in R (R Core Team 2015) using adult morphology as fixed effects, and year as the random effect to correlate to breeding success and parental care to examine sexually selected traits. Binomial data including chance of having EPY or a second brood and feeding percentage were run in generalised linear mixed models (GLMM).

Detailed methods and results are presented in the following chapters:

Chapter 2) The morphology and dimorphism of adults in this population were described, including body mass, tarsus length, wing length, tail streamer length, white spot size in tail feathers, short tail length, feather colour from throat, breast, belly and vent areas. PCA were conducted on one individual's body mass, tarsus length, wing length and short tail length to demonstrate correlations among body traits of a barn swallow individual. Potential sexually selected traits were tested by examining their correlations with breeding initiation date and reproductive success in social nest for both males and females.

Chapter 3) Paternity of offspring was tested to determine the extra-pair mating behaviour and genetic reproductive success of individuals. Investment in parental care (provisioning rate) was related to male and female traits to explore relationship of breeding investment (or benefit their partner receive) and attractiveness.

Chapter 4) Offspring growth was observed in natural nests and in a cross-fostering experiment to examine genetic and environmental effects (relatedness with traits of both genetic parents and fostering parents) on chick growth to distinguish either direct or indirect benefits females could obtain by selecting specific males.

Chapter 5) General discussion.

Chapter 2 Morphology and Social Breeding Success

Abstract Sexually selected traits, such as the extravagant plumage colouration and long tails of some birds, are suggested to benefit individuals via increasing reproductive success since the costly traits do not seem to help survival. Signals that have evolved via sexual selection may diverge among populations or subspecies of the same species. Studies in barn swallows (*Hirundo rustica*) have shown various ornamental traits that are sexually selected such as the long tail streamers of European subspecies *H. r. rustica* and dark red ventral plumage of North American subspecies *H. r. erythrogaster*. The Middle Eastern subspecies *H. r. transitiva* has red ventral plumage and long tail streamers, both of which have been demonstrated to play roles in male reproductive success. The Asian subspecies, *H. r. gutturalis*, has the shortest tail streamers (although they are dimorphic) and smallest body size of all the subspecies. Studies in a Japanese population have indicated that females prefer males with larger and less colour saturated throat patches and larger white spots on their tail feathers, while the dimorphism of tail streamers remains unexplained. I studied a population of *gutturalis* in Qingdao, China to further investigate the preferred ornamental traits in this subspecies by testing relationships between the morphology of both males and females and their reproductive success. In this chapter, the results show that ventral plumage of *gutturalis* was pale and unlikely to be either sexually dimorphic or selected. In social pairs, smaller (shorter tarsus) and longer-tailed females had more successful first broods and higher annual reproductive success. Apart from male body mass predicting laying date, no direct correlations between male ornamental traits and social reproductive success was found.

2.1 Introduction

Sexual selection is believed to be an important force driving the evolution of many extravagant ornaments such as the antlers of deer (*Cervus elaphus*), trains of peacock (*Pavo cristatus*), and bright colour patterns found in other bird feathers and fish skin. They are believed to be reliable signals indicating good quality, therefore individuals with more exaggerated traits are preferred by the opposite sex and will gain more mating chances (Darwin 1888; Andersson 1994). There are a number of theories explaining mate choice processes and development of the selected traits. 1) The "handicap mechanism" explains that the traits are preserved and favoured because they relate to the quality of the males and only good quality individuals are able to afford and display them though they can be costly (Zahavi 1975). 2) The traits preferred by females are believed to be honest advertisements indicating the males' possession of good genes e.g. for better viability or immune system, as suggested by the "good gene theory" (Pomiankowski 1988). 3) The "runaway theory" and the "sexy son theory" suggest that more attractive individuals are able to benefit their partners by having sexy sons that get more mating chances while their daughters prefer the same attractiveness of males (Fisher 1930; Weatherhead and Robertson 1979). 4) Apart from the genetic aspect, the good parent theory suggests that males with exaggerated traits can provide direct benefits to their mates, with high quantity parental care and a decreased likelihood of transmitting parasitic infections to their mates or offspring (reviewed by Møller and Gregersen 1994). These theories have been shown to be compatible with each other on the evolution of male ornamental traits from evidence provided by observation, comparison experiment and modelling methods on various species. However in some species, the functions of specific traits need further exploration for example some showy traits could be weapons as well

(McCullough, Miller et al. 2016), so evolution of which might be driven by natural rather than sexual selection; instead of being sexy, the successful males might be just the most adapted individuals to specific environment (Seddon 2005; Seehausen, Terai et al. 2008).

Birds' tails have been classic examples of sexually selected ornamental traits (Andersson 1994), and the monogamous hirundine - the barn swallow (*Hirundo rustica*) has been a model for investigating mate choice by the manipulation of its tail streamers (Møller 1988a). Most subspecies of barn swallow are migratory and breed both in colonies and solitarily. Six subspecies are generally recognised among which the ornamental traits vary (Turner 2006). The subspecies are mostly distinguished by body size, tail streamer length and ventral feather colouration (Scordato and Safran 2014). Although European *H. r. rustica* has a large body and long tail streamers while South Asian *H. r. gutturalis* has the smallest body size and shortest tail streamer among the six subspecies, these two subspecies are considered closer in phenotype because of the pale belly feathers, whilst the other four subspecies have dark reddish ventral plumage. *H. r. tytleri*, which breeds in North China, Mongolia and Russia, has dark reddish belly feathers with medium body size and tail streamer length. Another two geographically close sedentary subspecies, *H. r. savignii* and *H. r. transitiva* found in Egypt and Middle East respectively, are similar in phenotype with common traits including large body size, long tail streamers and dark red ventral plumage. The North American subspecies *H. r. erythrogaster* has dark red belly plumage and medium length tail streamers (Smith and Montgomerie 1991; Scordato and Safran 2014).

Having subspecies that vary in both tail streamer length and colouration makes barn swallows an excellent model for investigations into sexual selection and speciation

(Møller and Gregersen 1994; Safran 2010; Scordato and Safran 2014). The best known subspecies is *H. r. rustica*, on which Møller (1988) conducted the original manipulation experiments on male tail streamers showing that female preferred males with longer tail streamers even when they were artificially elongated because longer-tailed males paired up more quickly than shortened-tailed males. In another experiment, the tail streamer symmetry was changed by cutting the tail streamer, and males with more symmetrical tails found mates faster (Møller 1993b; Møller and Gregersen 1994; Møller 1994a). The white spots on the tail feathers have also been proposed to be sexually selected because in one population, males were found to have fewer offspring in the season when their tail white spots were covered by black ink (Kose and Møller 1999). These conclusions have been challenged because cutting and fixing manipulation might also impair their flying ability and the painting might make the tails look abnormal, affecting the males’ performance and the females' decision (Buchanan and Evans 2000).

In artificial enhancement experiments on feather colouration and tail streamers of males, it was determined that females of *H. r. erythrogaster* prefer males with darker red plumage coloration but not longer tail streamers (Safran, Neuman et al. 2005; Neuman, Safran et al. 2007), though extra-mating advantage was observed for long-tailed males in one population (Obayashi, Hayakawa, et al. 2006; Lifjeld, Oddmund, et al. 2011).

In the Middle Eastern subspecies *H. r. transitiva*, which has both dark ventral plumage and long tail streamers, both male tail streamer length and ventral feather colour play roles in breeding success (Vortman, Lotem et al. 2011; Vortman, Lotem et al. 2013). Males with longer tail streamers initiated nesting earlier and had fewer extra-pair offspring; and males with darker ventral plumage had more total chicks in the season (Vortman, Lotem et al. 2011; Vortman, Lotem et al. 2013).

The Asian subspecies *H. r. gutturalis* has slightly elongated tail streamers and a pale belly with some individuals having light orange-red feathers on it. In Japan, researchers working on *gutturalis* have reported that multiple traits are related to individual survival and breeding success (Kojima, Kitamura et al. 2009; Hasegawa, Arai et al. 2010a; Tazzyman, Seymour et al. 2012). They have suggested that white spot length and throat patch size are all dimorphic in this subspecies, and females prefer males with larger white spots in tails and with more colourful and larger throat patches (Kojima, Kitamura et al. 2009; Hasegawa, Arai et al. 2010a; Tazzyman, Seymour et al. 2012). Though tail streamer length is also dimorphic with those of males' being much longer, its length is only related to the survival rate but not breeding success and females did not tend to get benefits from choosing long-tailed males (Hasegawa, Arai et al. 2014b).

Objectives

Sexual selection studies in *gutturalis* have been reported only from the Japan population leaving the dimorphism of tail streamers unexplained. To further study the sexual preference in this subspecies, a population of *gutturalis* in China was observed for three years. If a male trait is sexually preferred, males with the more exaggerated trait will be expected to be more preferred by females which results in earlier breeding. Additionally, a recent meta-analysis on sexual selection strength in barn swallows has shown that sexual selection effect is stronger before egg deposition than other stages in breeding season (Romano, Costanzo et al. 2016). Starting earlier, more ornamented males are more likely to have a second brood and achieve a higher reproductive success than the less attractive males in the same breeding season. Meanwhile, their superior genetic quality with potentially more reproductive investment allocated by females should help their young to survive better. In this chapter, the objectives are:

1) Determine the potential sexually selected traits of barn swallow *gutturalis* by their dimorphism among a range of traits including body size (body mass, tarsus length and wing length), plumage colouration (throat, breast, belly and vent areas), and plumage size (tail streamer length, short tail length and tail white spot size).

2) Further test the potential sexy traits in prediction of mating success which should be an earlier breeding initiation date.

3) By relating the traits to the number of eggs from the social nests which shows female reproductive investment, male attractiveness can be further determined.

4) Potential sexually preferred traits will be tested in prediction of the successful fledglings in the first brood, chance of having a second brood, and the number of annual successful fledglings.

2.2 Methods and materials

2.2.1 Capture and measurements

Capture

From late April to May in 2013, 40 male and 37 female adults were captured using mist nets before the females laid eggs. Seven pairs abandoned their nests after capture, they were later found to have constructed new nests in other locations nearby. In the following two years, barn swallow parents were captured on day seven after the chicks hatched and no birds abandoned their nests. In 2014, 32 males and 32 females were measured including five pairs and one individual from year 2013 was measured again (recaptured for the blood sample extraction); and in 2015, another 25 males and 27 females were caught, ringed and measured.

Measurements

At capture (**Figure 2.1**), a unique combination of two plastic colour rings was placed on legs (one ring on each leg) to allow individual identification. Birds were weighed to an accuracy of ±0.25 g, using a spring scale (Pesola, Switzerland) within a plastic fan-shaped bag. Tail length (both tail streamers and short tail in the middle) and right tarsus length, the longest length of white spots on the right two outermost tails were measured by electronic digital vernier caliper (Jazooli, UK) with an accuracy of ± 0.1 mm (**Figure 2.2**), the white spot size is defined as the sum of the two lengths (Hasegawa, Arai et al. 2010a). Both right and left wing lengths were measured using a stopped ruler accurate to ±0.1 cm.

Figure 2.1 A male and a female barn swallow *H. r. gutturalis* at capture in Qingdao China. Both male (a) and female (b) barn swallows had pale belly feathers, male tail streamers were longer than female's and the female had an obvious brood patch.

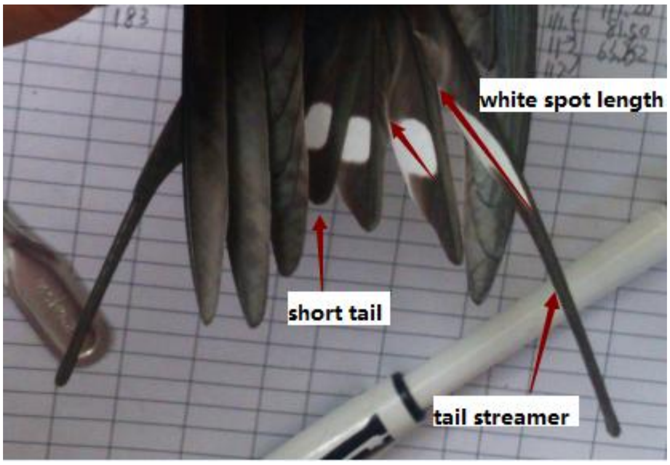

Figure 2.2 Short tail, tail streamer and measured tail white spot lengths of barn swallows *H. r. gutturalis* in Qingdao China. The two spot lengths were added together to describe tail white spot size.

Plumage colouration measurements

In 2013, feather patches (around five feathers) were plucked from the throat, belly and ventral regions of the adults. In 2014 breast feathers were collected in addition. Feathers were stuck to a card with the swallow ID number and collection date, and stored in a box. When the breeding season finished after four months, the feather reflectance spectra were measured using a USB2000 spectrometer in 2013 and a USB4000 in 2014 (light source: PX-2, Ocean Optics). Reflectance measurements were calibrated with black and white cloths provided as standards to define 0% and

100% reflectance. Before measurements, the feather ends were stuck together on the ID card to make the feather patch appearance close to that on the bird’s body. During measurement, the card was put flat on table, the probe was held vertically to attach to the feather on the part shown to others, then another two measurements were taken by moving the beam slightly within the area. For each sample a mean value of the three measurements was used in analysis. Between the wavelengths 300-700 nm, 1149 reflectance values were recorded for the samples of year 2013 and 1999 reflectance values were recorded for samples collected in year 2014 for each region.

Sexing

In 2013, sex was assigned by the fact that normally the female has an obvious brood patch (**Figure 2.1**). In 2014 and 2015, 20 μl blood was taken from the brachial vein of each individual, and each sample was preserved in either 1 ml of lysis buffer with 2% sodium dodecyl sulfate or 97% ethanol (White, Densmore III et al. 1992). DNA was extracted from each blood sample using Tiangen DNA Extraction Kits (Tiangen Biotech, Beijing). Then P2/P8 primers were used in polymerase chain reaction (PCR). Gel electrophoresis was used to separate DNA fragments in agarose gels. The agarose gel was prepared by heating 1% w/v agarose in 1x Tris/Acetic Acid/EDTA (TAE) buffer. Ethidium bromide DNA gel stain was added to a final concentration of 1:20,000. Samples were mixed with 5x loading buffer. An appropriate DNA ladder was run with samples. Sex was determined by the number of bands on the DNA gel, females have two and males have one (Griffith, Owens et al. 2002).

2.2.2 Observations

Each nest was checked daily after nest construction was finished and the dates when the female laid the first egg, when the chicks hatched and when the brood fledged were recorded; along with the number of eggs laid, the number of chicks at day 10 after hatching (Vortman, Lotem et al. 2011) and the number of nestlings that survived to fledge. The breeding behaviour of 30, 32 and 34 pairs were observed in 2013-2015, respectively (including the pairs being studied more than one year).

2.2.3 Statistical analysis

The earliest initiation day in sample nests was recorded as natural number one, the later date numbers for other nests increased along with number of days they spent to start laying eggs (Earliest initiation date, 2013, 2nd of May; 2014, 23rd of April; 2015, 27th of April). The date numbers were counted separately each year, with year as a random effect in the linear mixed effects model. Some individuals were caught in more than one year. In these cases, feather and blood samples from the earliest year were used, and other data from 2014 (as this year had more second brood data available) were used in the analysis to avoid pseudoreplication. Chicks that survived to day 10 were defined as successful chicks and the annual number of successful fledglings was obtained by adding the nestling in two broods together which survived to fledge.

All the data processing and figures were conducted using R (version i386 3.2.2) program (R Core Team 2015). To examine the relationship between morphology and breeding success, linear mixed effects models were built (LMER) in R using libraries lme4 and lmerTest. There were 10 fixed effects including both sexes' body mass, tail streamer, short tail, wing and tarsus lengths, with year as a random effect. Breeding initiation date, the number of eggs in the first brood, number of successful chicks and annual successful fledglings were used as dependent variables. The statistics of the model were given by summary command and the non-significant factor with the highest *p* value was deleted in turn until the simplest model containing significant variables was obtained. Linear regressions were calculated using linear model function (lm) in the library of lme4 in R. The test of the effect of the tail white spot contains the white spot lengths in male and female as fixed effects and year as a random effect. *P* values of significant variables in tables were marked in bold, and the factors with *p* values <0.05 were marked with one star "*", marked with two stars "**" when the *p* values are <0.01. In the figures that show factors and correlations for the models (e.g. **Figure 2.10**), the rectangles on the left show all the dependent variables used in the linear mixed model or generalised linear mixed model, the year in oval shape means that it is a random factor in the model and dependent variable were in square shape on the right. Factors with arrows were fixed effects in the final model: solid arrows show positive relationships and dash arrows mean negative ones. The weight of coefficient of predictive factors was represented by the relative width of arrows which was calculated using the factor's coefficient to be divided by the sum of coefficients of all factors in the final model. Significant factors were with stars. The fixed effects with no arrows were excluded from the model, and all of their statistics are shown in separate result tables.

Two years' feather reflectance data was analysed separately due to the use of different devices. For each year's sample data, principal component analysis (PCA) was performed on reflectance values including 1149 (year 2013) or 1999 (year 2014) different wavelengths between 300-700 nm on each feather region of the individuals.

2.3 Results

2.3.1 Morphology and dimorphism of barn swallow *gutturalis*

Descriptive statistics for the six measurements of adult morphology are shown in **Table 2.1**.

Table 2.1 Trait measurements (mean ±standard deviation [SD]**) and *t* test between two sexes in a population of barn swallow *H. r. gutturalis* in Qingdao, China.** Significant *p* values are in bold.

Traits	Male	Female	Welch Two Sample t-test		
			df	*t*	*p*
Tail streamer (mm)	96.18 ±8.54	79.88 ±7.90	155.87	12.45	**<0.0001**
Short tail (mm)	43.47 ±2.86	44.16 ±2.91	155.24	-1.51	0.133
Wing (mm)	114.0 ±2.58	111.5 ±3.03	149.40	5.56	**<0.0001**
Body mass (g)	16.97 ±1.12	17.40 ±1.59	98.34	-1.69	0.093
Tarsus (mm)	9.15 ±0.99	9.28 ±0.94	156.00	-0.84	0.400
White spots (mm)	35.63 ±6.79	25.93 ±4.14	66.16	7.80	**<0.0001**

Dimorphism

Tail streamer This *H. r. gutturalis* population was sexually dimorphic in tail streamer length, on average tail streamers were 16.0 mm or 16.95% longer in males than in females (**Table 2.1, Figure 2.3**).

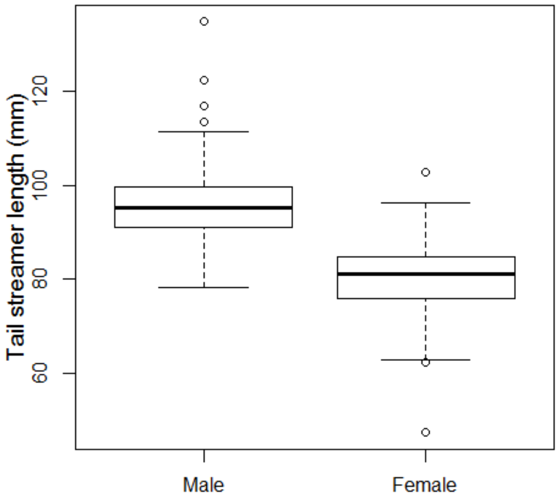

Figure 2.3 Tail streamer length was sexually dimorphic in a population of barn swallow *H. r. gutturalis* in Qingdao, China (statistics results in **Table 2.1).**

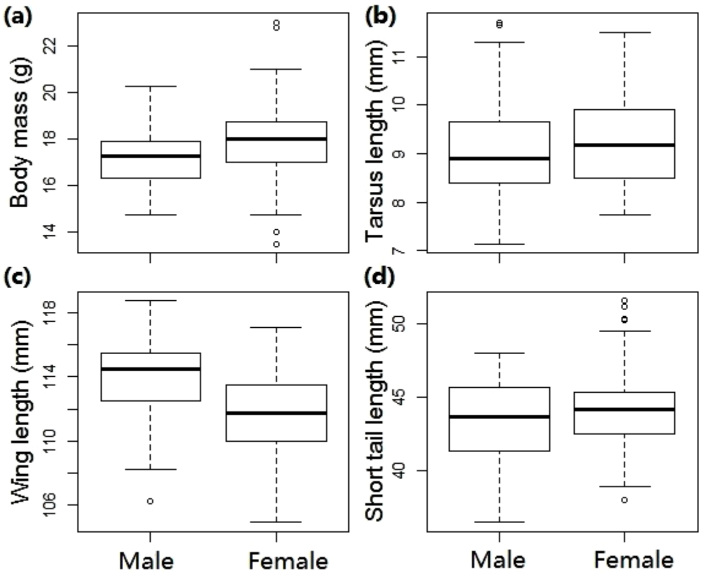

Figure 2.4 Comparison of body mass (a), tarsus (b), wing (c) and short tail (d) lengths between male and female in a population of barn swallow *H. r. gutturalis* in Qingdao, China (statistics results in **Table 2.1).**

Wing, short tail, body mass and tarsus Males had slightly longer wing than females, and had similar body mass, short tail and tarsus lengths with females (**Figure 2.4**). Male wings were on average 2.5 mm (2.19%) longer than those of females, which is consistent with the difference of tail streamers but the degree is much smaller. Therefore, in this study site, male barn swallows tend to have longer tail streamers and slightly longer wings, but otherwise rather similar body sizes to females.

Correlation of the same trait of two sexes in a pair

No significant correlation patterns were found in lengths of tail streamers and wings of two members of a social pair (**Figure 2.5, Table 2.2**). For both body mass and tarsus length, the two sexes showed positive correlation in measurements (**Figure 2.6, Table 2.2**).

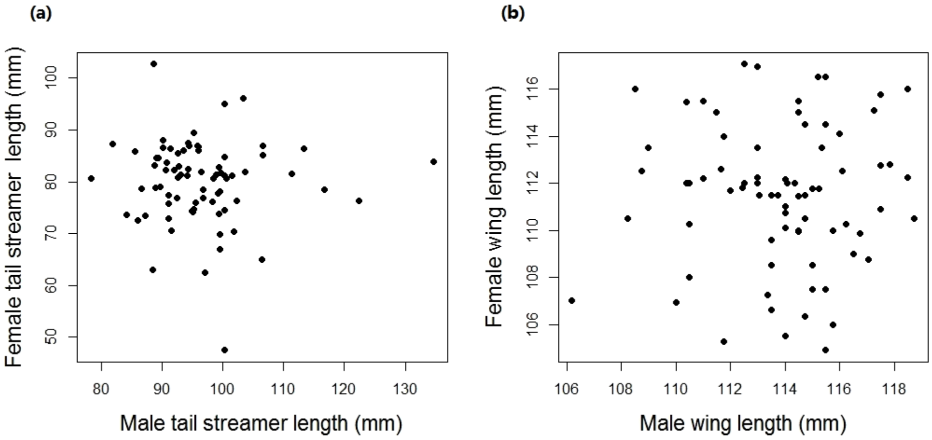

Figure 2.5 No significant linear correlations were found in tail streamer length (a) and wing length (b) between sexes of one social pair in a population of barn swallow *H. r. gutturalis* in Qingdao, China (statistics results in **Table 2.2).**

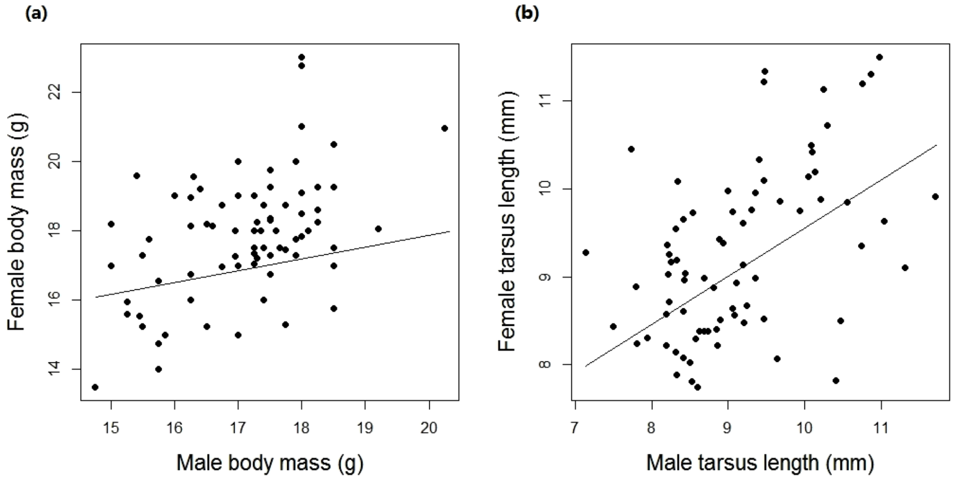

Figure 2.6 There was a significant association between the members of a pair in body mass (a) and tarsus length (b) in a population of barn swallow *H. r. gutturalis* in Qingdao, China (statistics results in **Table 2.2).**

Table 2.2 Regression statistics results of four traits between sexes of a social pair in a population of barn swallow *H. r. gutturalis* in Qingdao, China. Significant *p* values are in bold.

Between two sexes	*df*	F	*p*	R^2	coefficient	interpret
Tail streamer	81	0.26	0.613			
Wing length	81	0.50	0.483			
Body mass	57	16.15	**0.0002**	0.22	0.34	11.04
Tarsus length	81	33.97	**<0.0001**	0.29	0.55	4.07

Tail white spots

In males, the white spot length on the streamer feather was on average 23.61 ± 4.84 mm and it was positively related to the tail streamer length (**Figure 2.7**). They were 7.23 mm (30.62%) longer than those in female tail streamers which were averagely 16.38 ± 2.67 mm. The second white tail spot of males and females were respectively 12.02 ± 2.54 mm and 9.56 ± 1.92 mm, so those of males were averagely 2.46 mm (20.46%) longer. Adding together as total white spot length, tail white spot of males were 35.63 ± 6.19 mm and females were 25.94 ± 4.14 mm (**Table 2.1**).

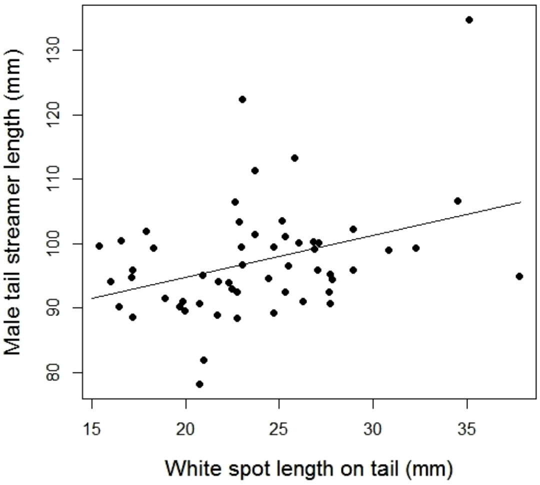

Figure 2.7 White spot length in the outermost tail feather of males could predict the length of the tail feather in a population of barn swallow *H. r. gutturalis* in Qingdao, China (statistics result in **Table 2.3).**

Table 2.3 Statistics table of length correlation of outermost tail white spot and its tail streamer in a population of barn swallow *gutturalis* in Qingdao, China. Significant *p* values are in bold.

	Estimate	*df*	*t*	*p*	F	R-squared
Male	0.184	39	2.347	**0.024**	5.510	0.101
Female	0.009	41	0.150	0.881	0.023	-0.024

The large white spot on the outermost tail was related to the tail streamer length in males (**Figure 2.7**, **Table 2.3**), hence the white spots were not used as an independent variable in the further modelling and analysis, but the analysis on tail white spot length with the reproductive success were done as a comparison to the research that has been done in Japanese population.

Interaction of traits of each individual

PCA were conducted among body mass, tarsus length, wing and short tail lengths to determine the interaction of characteristics of an individual. The first PC contributed 36% of data variance and the second PC contributed 31.5%. The last two PCs shared similarly 17.1% and 15.4% of the data variance, respectively (**Table 2.4**). Due to lack of a dominant PC representing the individual body characteristics, the four traits were all used in model building in reproductive success prediction.

Table 2.4 PCA results of body mass, tarsus, wing and short tail lengths of individuals in a population of barn swallow *gutturalis* in Qingdao, China (n=157**).**

	PC1	PC2	PC3	PC4
Body mass (g)	-0.635	-0.163	-0.703	0.274
Tarsus (mm)	-0.641	-0.233	0.392	-0.618
Wing (mm)	0.004	-0.763	0.378	0.524
Short tail (mm)	0.431	-0.580	-0.457	-0.518
Standard deviation	1.200	1.122	0.827	0.784
Proportion of Variance	0.360	0.315	0.171	0.154
Cumulative Proportion	0.360	0.675	0.846	1

Plumage colouration

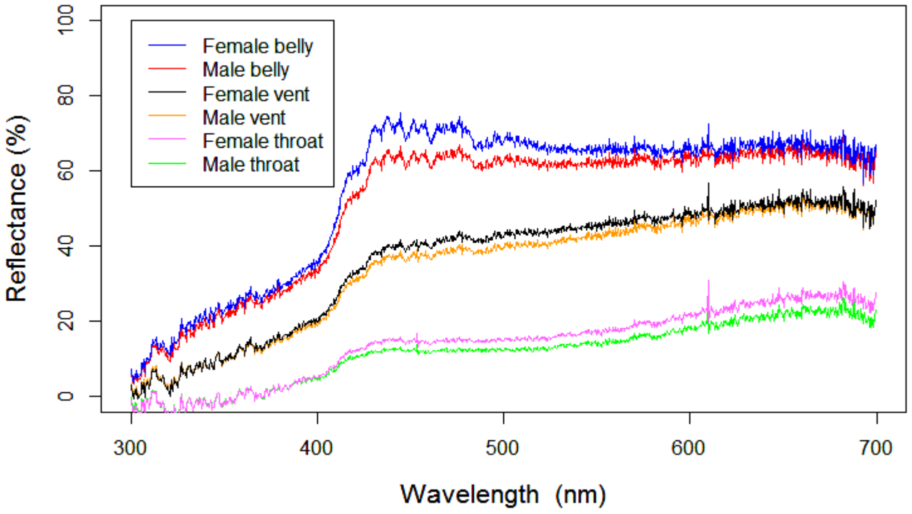

Figure 2.8 Mean reflectance spectra (wavelength 300-700 nm**) for feathers from throat, belly and vent regions of male and female barn swallow *H. r. gutturalis* from Qingdao, China (**all regions, n=28**).**

Figure 2.8 shows the mean reflectance spectra for wavelengths between 300 and 700 nm from the plumage regions examined. Both sexes showed very similar reflectance patterns (2013 data). Between 300 nm and around 400 nm wavelength (UV light range), the feathers' reflectance was low, predominantly below 20%. For the human visible region (around 400 nm to 700 nm), all plumage regions reflect similar amount of light across the wavelength range. Little variation reflectance across the range of wavelengths means that the feathers will not appear colourful to any animal with visual sensitivities in this range (which would include humans and birds).

The PCA results show that PC1 (indicating brightness) on average accounted for 91% of the variance in the data of 2013, with PC2 and PC3 values explaining less than 5% (**Table 2.5**). Also, **Figure 2.9** shows that in all six regions, low PC1 values distributed evenly along the whole wavelength range without much variance. The fact that it was flat across the range of wavelengths means that it will represent changes in overall brightness but not any colour. **Table 2.5** shows the PCA results from 2014 samples demonstrating that for feathers from the belly, throat, vent and breast regions, PC1 explained more than 97% of the variance in the reflectance spectra of these samples which strengthened the conclusion from data in 2013.

Table 2.5 Proportion of the variance explained by three PCs of plumage reflectance in a population of barn swallow *H. r. gutturalis* in Qingdao, China (2013, all regions n=28; 2014, male and female breast, n=32, other regions n=24**).**

Year	Sex	Feather region	PC1	PC2	PC3
2013	Male	Throat	0.964	0.025	0.006
		Belly	0.826	0.117	0.034
		Vent	0.937	0.041	0.011
	Female	Throat	0.945	0.027	0.015
		Belly	0.869	0.085	0.020
		Vent	0.915	0.050	0.016
2014	Male	Throat	0.998	0.002	0.000
		Breast	0.978	0.022	0.000
		Belly	0.982	0.017	0.001
		Vent	0.981	0.019	0.000
	Female	Throat	0.998	0.002	0.000
		Breast	0.990	0.009	0.001
		Belly	0.987	0.012	0.001
		Vent	0.982	0.017	0.000

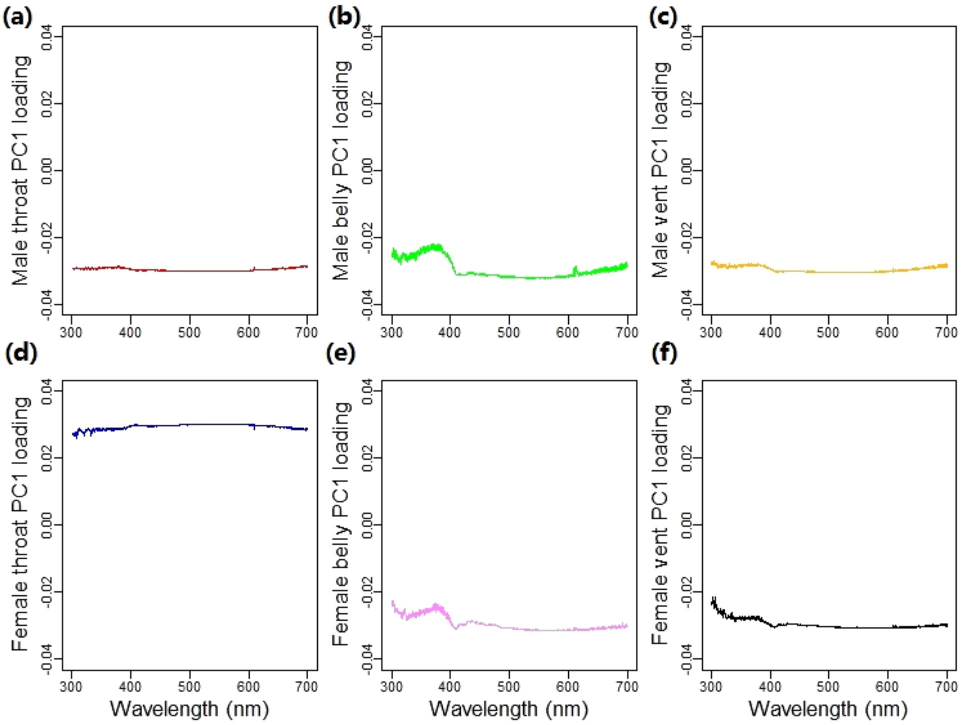

Figure 2.9 PC1 loadings of feather reflectance within the wavelength between 300-700 nm in a barn swallow *H. r. gutturalis* population in Qingdao, China. Feathers from six regions of barn swallow *gutturalis*: (a) male throat, (b) male belly, (c) male vent, (d) female throat, (e) female belly and (f) female vent (samples from 2013**).**

Table 2.6 *T* test of the PC1 of the feather reflectance between two sexes in a population of barn swallow *H. r. gutturalis* in Qingdao, China.

Year	Feather region	Welch Two Sample t-test between sexes		
		t	*df*	*p*
2013	Throat	-3.22E-10	53.999	1
	Belly	-4.10E-09	54.000	1
	Vent	8.12E-09	53.841	1
2014	Throat	-0.000956	44.332	0.999
	Breast	0.01475	51.595	0.988
	Belly	-0.004206	45.309	0.997
	Vent	-0.005261	44.997	0.996

Comparison of PC1 of the same region between two sexes showed that male and female barn swallow *H. r. gutturalis* did not show different patterns in their plumage brightness (PC1) for all the four regions sampled (**Table 2.6**). As PC2 and PC3 represented only a small amount of the variance, no further analysis was conducted on these principal components.

2.3.2 Reproductive success

First brood initiation date

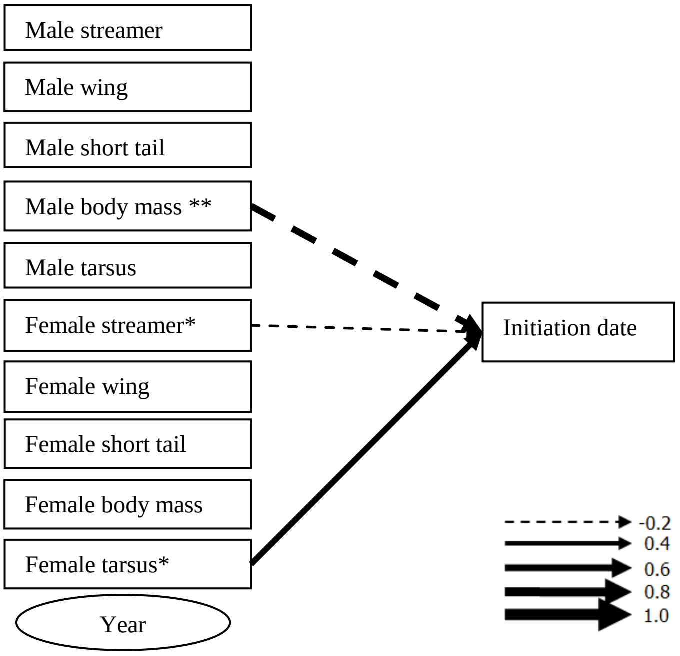

Figure 2.10 First brood initiation date number was negatively related to male body mass and female tail streamer length and positively related to female tarsus length in a population of barn swallow *H. r. gutturalis* in Qingdao China. Larger numbers mean later in dates (plots in **Figure 2.11** and statistics results in **Table 2.7**).

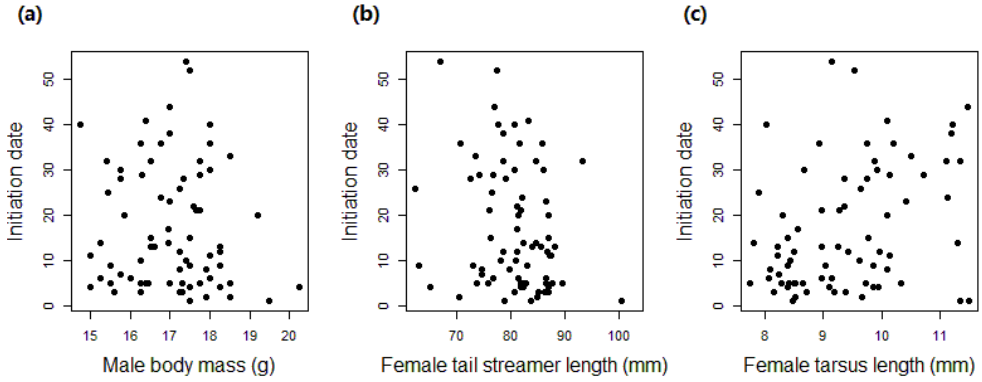

Figure 2.11 Plots of male body mass (a), female tail streamer (b) and female tarsus length (c) versus initiation date number in a population of barn swallow *gutturalis* in Qingdao, China. Larger number means later in dates.

Table 2.7 Statistics results of linear mixed effects model predicting the first brood initiation date using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, breeding initiation date was related to female tail streamer length, male body mass and female tarsus length. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold. Factors showing negative relations to the date numbers predicted earlier initiation dates.

Traits in final model			
Fixed effects	*df*	*t*	*p*
Female tail streamer	68.95	-2.275	**0.026**
Male body mass	69.77	-3.178	**0.002**
Female tarsus	51.96	2.186	**0.033**
Random effect	Groups	Variance	SD
Year	3	37.53	6.126
Traits excluded	*df*	*t*	*p*
Male wing	62.98	0.001	0.999
Female wing	61.76	-0.117	0.908
Female body mass	64.96	0.277	0.783
Male tail streamer	64.00	0.302	0.764
Male short tail	66.97	0.479	0.634
Female short tail	67.96	-0.523	0.603
Male tarsus	66.50	-0.724	0.454

The linear mixed effects model shows that first brood initiation date was negatively related to male body mass and female tail streamer length and positively related to female tarsus. Therefore, pairs with heavy males, long-tailed females, and small females started their first broods earlier (**Figures 2.10 & 2.11, Table 2.7**).

Number of eggs and chicks in the first brood

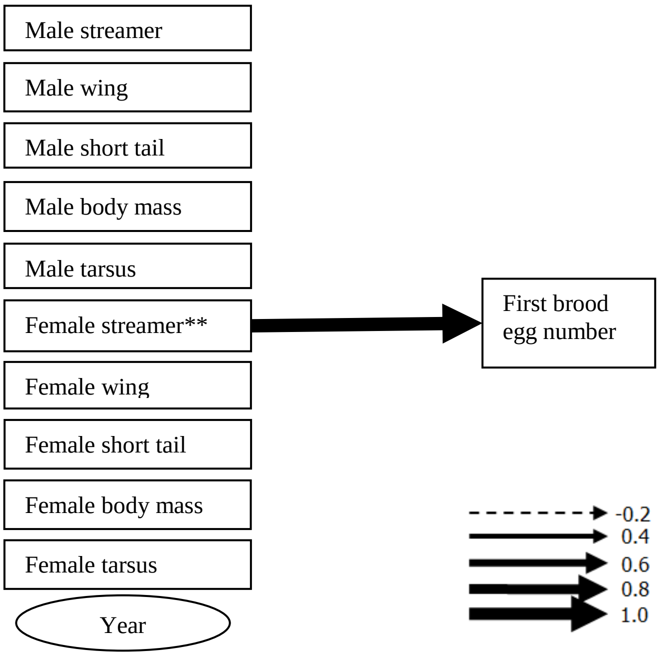

Figure 2.12 The number of eggs in the first brood was positively related to female tail streamer length in a population of barn swallow *H. r. gutturalis* in Qingdao China (plots in **Figure 2.13** and statistics results in **Table 2.8).**

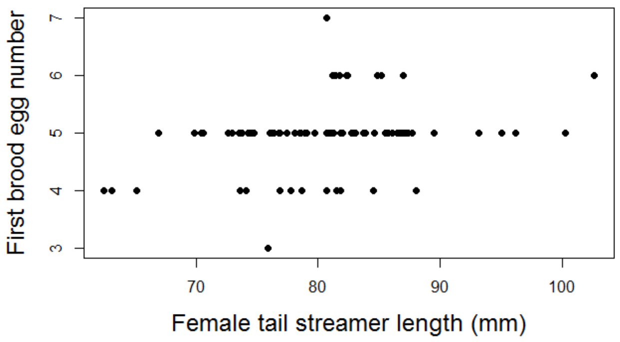

Figure 2.13 Plots of female tail streamer with the number of eggs in the first brood in a population of barn swallow *gutturalis* in Qingdao, China.

Table 2.8 Statistics results of linear mixed effects model predicting the number of eggs in the first brood using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, the first brood egg number was related to female tail streamer length. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*t*	*p*
Female tail streamer	78.17	2.933	**0.004**
Random effect	Groups	Variance	SD
Year	3	0.020	0.141
Traits excluded	*df*	*t*	*p*
Male tail streamer	69.57	0.077	0.939
Male body mass	70.25	0.051	0.960
Male tarsus	57.05	0.159	0.874
Female wing	71.93	-0.156	0.876
Male wing	74.78	-1.085	0.282
Female short tail	74.78	1.003	0.319
Female body mass	73.51	1.046	0.299
Female tarsus	32.63	1.310	0.199
Male short tail	76.78	1.727	0.088

In the first brood, females with longer-tailed streamers laid more eggs (**Figure 2.12** & **2.13, Table 2.8**), while no significant results were found with the number of successful chicks surviving to 10 days old (**Table 2.9**).

Table 2.9 Statistics results of linear mixed effects model predicting the first brood successful chicks using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, no parent trait was significantly related to the number of successful chicks in the first brood.

Traits excluded			
Fixed effects	*df*	*t*	*p*
Male short tail	61	-0.300	0.766
Female tarsus	62	0.47	0.640
Male body mass	63	-0.516	0.608
Female body mass	64	0.348	0.729
Male tarsus	65	-0.58	0.564
Female tail streamer	66	0.831	0.409
Female wing	67	-1.57	0.121
Male wing	68	-1.651	0.103
Male tail streamer	69	1.256	0.213
Female short tail	58	1.93	0.058
Random effect	Groups	Variance	SD
Year	3	0.834	0.913

Annual successful fledglings

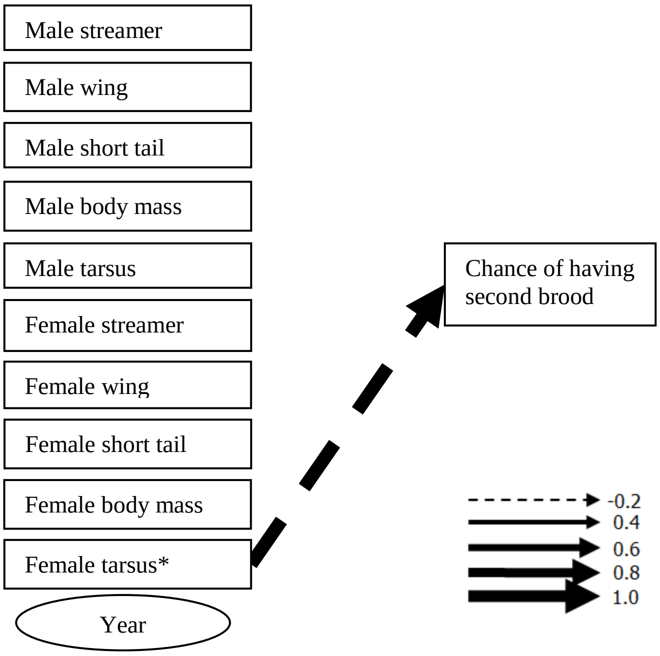

Figure 2.14 Females with longer tarsi had lower chance of having the second brood in a population of barn swallow *gutturalis* in Qingdao, China (plots in **Figure 2.15** and statistics results in **Table 2.10).**

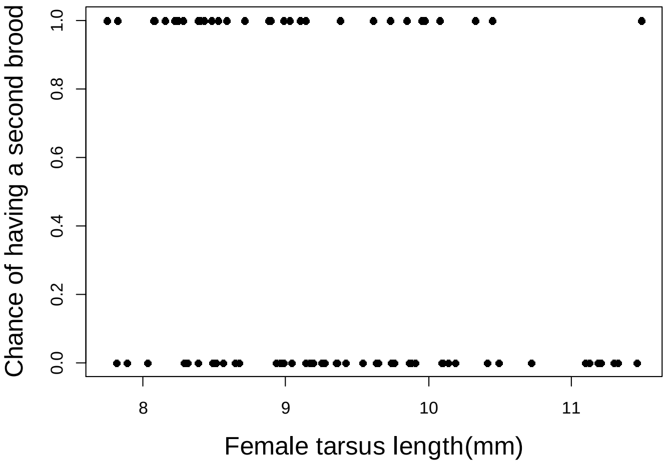

Figure 2.15 Plots of female tarsus length and chance of having the second brood in a population of barn swallow *gutturalis* in Qingdao, China.

Table 2.10 Statistics results of generalised linear mixed model predicting the chance of having a second brood using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, females with shorter tarsi had a higher chance of having a second brood. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*z*	*p*
Female tarsus	78	-2.27	**0.023**
Random effect	Groups	Variance	SD
Year	3	0.002	0.050
Traits excluded	*df*	*z*	*p*
Female short tail	69	0.108	0.914
Male wing	70	0.319	0.750
Female body mass	71	0.408	0.683
Male short tail	72	0.476	0.634
Male tarsus	73	-0.52	0.603
Male tail streamer	74	-0.619	0.535
Female tail streamer	75	0.748	0.454
Female wing	76	1.172	0.241
Male body mass	77	1.556	0.120

Females with longer tarsi had a lower chance of having a second brood (**Figure 2.14** & **2.15, Table 2.10**). None of the measured male or female traits were significantly related to reproductive success either in the first brood or annually (**Table 2.11 & 2.12**).

Table 2.11 Statistics results of linear mixed effects model predicting the number of fledglings in the first brood using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, no parent trait was significantly related to the number of successful fledglings in the first brood.

Traits excluded			
Random effect	Groups	Variance	SD
Year	3	0.83359	0.913
Fixed traits excluded	*df*	*t*	*p*
Male short tail	61	-0.300	0.766
Female tarsus	62	0.470	0.640
Male body mass	63	-0.516	0.607
Female body mass	64	0.348	0.729
Male tarsus	65	-0.580	0.564
Female tail streamer	66	0.831	0.409
Female wing	67	-1.570	0.121
Male wing	68	-1.651	0.103
Male tail streamer	69	1.256	0.213
Female short tail	58	1.930	0.058

Table 2.12 Statistics results of linear mixed effects model predicting the number of annual successful fledglings using male and female traits. In the population of barn swallow *H. r. gutturalis* in Qingdao China, no parent trait was significantly related to the number of successful fledglings in a season.

Traits excluded			
Random effect	Groups	Variance	SD
Year	3	4.826	2.197
Fixed traits excluded	*df*	*t*	*p*
Female short tail	54	-0.192	0.848
Male body mass	55	0.345	0.731
Male short tail	56	-0.394	0.695
Male tail streamer	57	0.374	0.710
Female tail streamer	58	0.368	0.714
Male wing	59	0.762	0.449
Male tarsus	60	-0.653	0.516
Female wing	61	-0.937	0.353
Female body mass	62	1.024	0.310
Female tarsus	63	-1.957	0.055

White spot and reproductive success

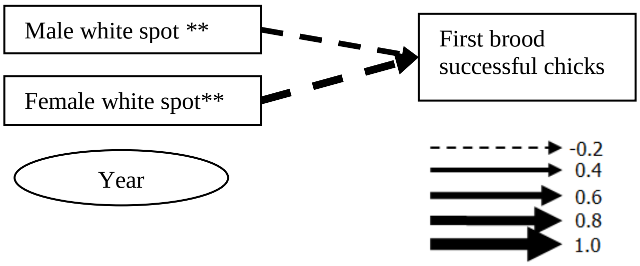

Figure 2.16 The number of successful chicks in the first brood was negatively related to male and female tail white spot lengths in a population of barn swallow *H. r. gutturalis* in Qingdao China (statistics result in **Table 2.13).**

Table 2.13 Statistics results of tail white spot lengths predicting the number of successful chicks in the first brood in a population of barn swallow *H. r. gutturalis* in Qingdao China. With year as a random effect, both male and female tail white spot sizes showed negative relations to the number of successful chicks in the first brood in linear mixed effects model. Significant *p* values are in bold.

Fixed effects	*df*	*t*	*p*
Male tail white spot	37	-2.239	**0.031**
Female tail white spot	37	-3.818	**<0.001**
Random effect	Groups	Variance	SD
Year	2	0.680	0.825

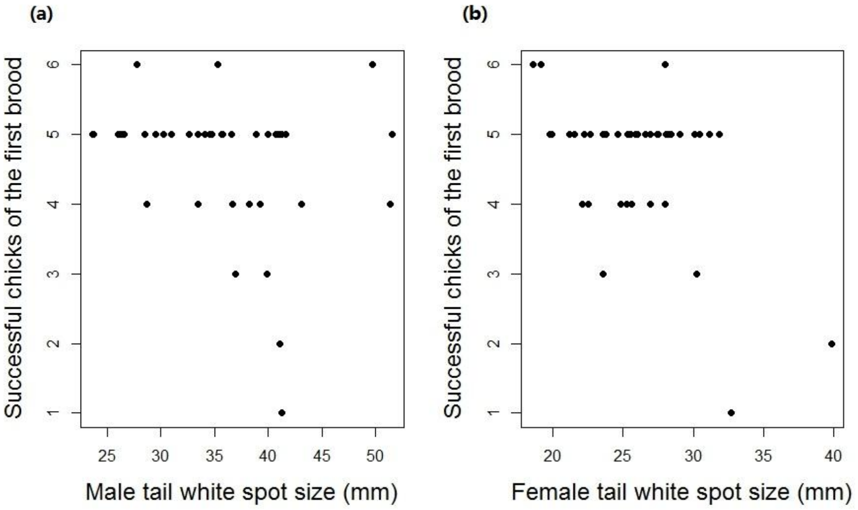

Figure 2.17 Plots of number of successful chicks with male tail white spot length (a) and female tail white spot length (b) in a population of barn swallow *H. r. gutturalis* in Qingdao China.

The number of nestlings in the first brood was also negatively related to both male and female tail white spot length (**Figure 2.16 & 2.17, Table 2.13**). No significant results were found in models correlating white spots with initiation date or total fledglings (**Table 2.14 & 2.15**).

Table 2.14 Statistics results of tail white spot predicting first brood initiation date in the linear mixed effects model. With year as the random effect, neither of white spot size of males or females showed significant relation to the breeding initiation date in a population of barn swallow *H. r. gutturalis* in Qingdao, China.

Traits excluded			
Fixed effects	*df*	*t*	*p*
Male tail white spot	45.95	-0.747	0.459
Female tail white spot	47.51	-1.787	0.080
Random effect	Groups	Variance	SD
Year	2	144.86	12.04

Table 2.15 Statistics results of tail white spot predicting number of annual fledglings in the linear mixed effects model. In the population of barn swallow *H. r. gutturalis* in Qingdao China, white spot size of both males and females showed no significant relation to the number of annual fledgling. Year is the random effect.

Traits excluded			
Fixed effects	*df*	*t*	*p*
Female tail white spot	47.97	0.09	0.929
Male tail white spot	48.66	-0.68	0.500
Random effect	Groups	Variance	SD
Year	2	0.377	0.614

2.4 Discussion

2.4.1 Phenotype and dimorphism of *H. r. gutturalis*

In this study population, *H. r. gutturalis* had similar mean tail streamer length (96 mm in males and 80 mm in females), to that recorded in the Japanese population in 2005 where mean streamer length was males 94 mm, females 79 mm, and in 2006 males 93 mm and females 80 mm (Hasegawa, Arai et al. 2010a). These are both much shorter compared to a population of *H. r. rustica* with 105 mm and 91 mm for males and females, respectively (Møller 1988a). The difference in tail length between males and females is 16 mm for this population of *H. r. gutturalis*, which is even larger than the difference in *H. r. rustica* which was 14 mm. Given that *gutturalis* has shorter tails, therefore their tail streamer dimorphism degree is higher than that in *rustica* suggesting that the tail streamers in *gutturalis* are also likely to be sexually selected.

The white spot on the tail feathers were sexually dimorphic. When adding the two spots on the right of the tail together, males' were approximately 36 mm and females' were 26 mm on average, slightly shorter than that found in one Japanese population which were approximately 38 mm and 29 mm in males and females, respectively (Hasegawa, Arai et al. 2010a).

Generally, the ventral feathers were not colourful in the *H. r. gutturalis* population. The reflectance spectra showed that the throat, breast, belly and vent reflected less than 20% of light in the UV region, and reflected a similar amount of light along the human visible spectrum without much variance. Additionally, both sexes showed a similar reflectance pattern along all wavelengths, with no significant difference between sexes for the main component (averagely > 90%) of the light reflectance data, so the ventral plumage in this population did not seem to be dimorphic. With the high reflectance, we can confirm that the *H. r. gutturalis* has pale belly feathers. Reflectance of the vent part was lower which means they are not as white as belly, but the colour pattern was rather dull. Therefore, although a few individuals (approximately 1-2 in 60 adults) with dark orange-brown colour ventral plumage can be observed (see **Figure 2.18**), the ventral plumage of *gutturalis* is generally pale with very light orange-brown ventral feathers, but not colourful nor dimorphic. Unlike *erythrogaster* and *transitiva* males in which the ventral colour is dark red, colourful and dimorphic, the ventral feather colouration of *gutturalis* is unlikely to be a sexual signal and so *gutturalis* seems to be closer to subspecies *rustica* in terms of phenotype (Scordato and Safran 2014).

Figure 2.18 White underparts were most common but occasionally birds with darker feathers were found. Two barn swallows at capture showing the orange colouration: (a) an individual with light orange-brown colour, (b) an individual with rare dark orange-brown plumage colour in a population of *H. r. gutturalis* in Qingdao, China.

Male *gutturalis* have longer tail streamers and slightly longer wings than those of females, while the two sexes tended to have similar body mass, skeletal size and short tail length. Within a pair, heavier individuals tended to pair with heavier partners, and individuals with longer tarsi tended to pair with individuals with longer tarsi, though these traits were not found to significantly predict breeding success. In *rustica* female tail streamer length has been reported to be related to that of their mate (Møller 1993a), while no such pattern has been found for the feather traits including tail streamer, short tail and wing lengths in this *gutturalis* population.

2.4.2 Traits predicting breeding success

Unlike findings in *rustica* (Møller, Barbosa et al. 1998), that females prefer males with longer-tails and such males then had higher reproductive success, male tail streamer length of *gutturalis* was not directly related to either the brood initiation date or the number of offspring in social nests.

Males with larger body mass started the first brood earlier than males with lower body mass which might indicate that larger males are preferred by females, or that they return from migration earlier. Body mass is important for survival especially for migratory birds (see case studies on common loons *Gavia immer* (Gray, Paruk et al. 2014) and dunlins *Calidris alpina alpina* (Schwemmer, Voigt et al. 2016)) and the importance of body mass has been shown in courtship and breeding initiation when they arrive at the breeding site (i.e. captive mallards *Anas platyrhynchos* (Pattenden and Boag 1989)). Body mass is also crucial for determining female energy allocation on laying eggs and incubation in birds (i.e. common eider *Somateria mollissima* (Hanssen, Engebretsen et al. 2002)). The result also has shown correlation in body mass of a pair, which might indicate that it is a cue for both sexes selecting partner at the beginning of the season. However, body mass is more often used to represent body condition in general for example studies on *Hirundo rustica* has suggested that parasite load affects body mass (Barbosa, Merino et al. 2002). Male body mass alone has not been suggested as important in sexual selection as a direct signal in barn swallows, in one case study on *rustica*, it was unrelated to song performance of males which is believed to show attractiveness to females (Møller 1991; Galeotti, Saino et al. 1997).

Females with longer tail streamers had a larger clutch size for the first brood (more eggs) which might support the hypothesis that the tail streamer is a sexually selected trait. In subspecies *rustica* and *transitiva*, in which male tail streamers are sexually selected, females with longer tail streamers have been found to start laying eggs earlier and had more offspring in a season (Møller 1993a; Vortman, Lotem et al. 2011), and *rustica* females with longer tails survived better than that with shorter tails (Møller and Szép 2002).

There are studies in both *rustica* and *gutturalis* demonstrating the importance of the white spots on the male tails due to the fact that they are shown to the females during the courtship especially in dim light conditions, and they also reflect their parasite burden because feather lice favour this area (Kose, Mänd et al. 1999; Hasegawa, Arai et al. 2010a). In the studied population, longer male tail spot did not predict earlier initiation date but predicted fewer successful chicks in the first brood, unlike in the Japanese population where the male white spot in tails shows attractiveness in terms of an earlier initiation date (Hasegawa, Arai et al. 2010a). Further study can be done on more comprehensive white spot features to explore its function in signalling body condition and in sexual selection. For instance, white spot shape matters for female choice because it was found that females prefer a pointed white spot (Møller 2016). Features including perimeter and area also have been demonstrated to directly reflect tail feather growth (growth bar width) during moult (Saino, Romano et al. 2015), but also see that growth bars on tail feathers of collared flycatchers (*Ficedula albicollis*) were shown not to predict the size of the forehead white patch which is sexually selected (Hargitai, Hegyi et al. 2012).

In summary, it is unlikely that plumage colouration was sexually selected in the study population of *gutturalis*. In social pairs, males with larger body mass started breeding earlier which might indicate female preference of heavier males. Females with longer tail streamers and females with shorter tarsus length also showed advantage in breeding. No more direct correlation was found between male traits and social reproductive success. Due to the extra-pair mating phenomenon, the actual genetic reproductive success can be different to the social one for both males and females. Offspring paternity test data will aid further exploration on offspring paternity in nest and genetic breeding success.

Chapter 3 Extra-pair Mating and Parental Care

Abstract Sex differences exist both in dimorphic traits and reproductive strategies. Typically males increase the number of mates while females invest more parental care in a restricted number of offspring and improve the genetic quality of offspring by being choosy in mate choice including extra-pair mates. The amount of parental care females invest in their offspring can be seen as an indication of male attractiveness, and extra-pair mates of females are considered to have a higher genetic quality than their social mates. Barn swallows (*Hirundo rustica*) are monogamous with extra-pair mating behaviour, they raise offspring together and more attractive males are less likely to be cuckolded with their mates allocating more maternal care. My study on a population of *gutturalis* in Qingdao China shows that around a quarter of nests contained extra-pair young. Longer-tailed males had a higher percentage of paternity of the chicks in social nests and also achieved higher reproductive success. The two sexes shared roughly equally the task of feeding offspring, but females tended to take a greater share if their mate had longer tail streamers. From this analysis it seems that tail streamers play a role in male attractiveness of *H. r. gutturalis* and female behaviour can be explained by the "differential allocation theory".

3.1 Introduction

It is a basic tenet of natural selection that all individuals should aim to maximize their reproductive success and this is likely to result in the two sexes adopting different reproductive strategies over mating and parental care (Parker 2006; Davies, Krebs et al. 2012). For example, females are normally the choosy sex seeking a good quality mate, and guarantee their reproductive success by raising good quality offspring with a high certainty of maternity. By mating with multiple females, males in many species are able to sire more offspring but will not necessarily invest in additional parental care. Competition for access to females for mating and parental care among males is a driving component in sexual selection in different mating systems (Darwin 1871; Johnstone, Reynolds et al. 1996).

Most mammals are polygamous (95%), and males do not provide parental care but they increase their fitness by copulating with more females (Andersson 1994). Selection can be intense on males of polygynous species because the more successful males can have multiple females while others will end up without having any mate (Andersson 1994). The situation is more obvious when the females are clustered and the breeding season is long (Emlen and Oring 1977). The competition for parental care also drives the evolution of female trait in sex-role reversed species (Clutton-Brock and Vincent 1991). Observations on Bornean smooth guardian frogs (*Limnonectes palavanensis*) have shown that females call more frequently to attract males, in which species females leave the clutch after laying eggs leaving males to provide parental care (Hopwood, Moore et al. 2015).

Monogamous species usually have the same mate throughout the whole of one or more breeding seasons and usually both members of a pair raise offspring together.

However reproductive strategies still differ between the two sexes, for instance, females in many monogamous birds invest heavily in reproduction including laying large energy-rich eggs and investing more in parental care, while male contributions can be rather limited (Andersson 1994; Sheldon 2000). Compared to the restricted number of offspring females can have in a season, males can achieve higher reproductive success via extra-pair copulations to sire more offspring without investing more parental care (Andersson 1994). Apart from male biased operational sex ratio (Price 1984; Kvarnemo, Moore et al. 2007) and the competitions for high-quality females (Kirkpatrick, Price et al. 1990), male competition for extra-pair copulations has been suggested as an important force to drive sexual selection in monogamous species, for instance, it has been shown to be the most important mechanism that drives sexual selection in male splendid fairy-wren but not others (*Malurus splendens*) (Webster, Tarvin et al. 2007).

This basic inequality in reproductive investment has important consequences for females. They should prefer a high quality mate to increase their fitness either directly or indirectly (Andersson 1994; Møller and Gregersen 1994; Kokko, Brooks et al. 2003). Direct benefits include breeding resources such as nests, territories and guard, food and other gifts, male parental care and absence of contagious parasites as suggested by the “good parent theory” (Hoelzer 1989); More research has found that females seek good genes from mating with more attractive males to have offspring with heritable sexual attractiveness and viability (Møller and Thornhill 1998). Though males with more exaggerated traits are considered to carry more excellent genes, they often behave more actively in extra-pair copulations to sire more young, not benefiting females with more parental care than less attractive males (Møller and Thornhill 1998). However, the “differential allocation theory” suggests that females have the ability to allocate their effort differentially based on the quality of their mate, and increase their investment when their partner is more attractive which can guarantee them high quality offspring (Burley 1986; Sheldon 2000; Horváthová, Nakagawa et al. 2012). For example when male blue tits' (*Parus caeruleus*) UV attractiveness is reduced by manipulation, females reduced the parental effort (feeding rates) accordingly (Johnsen, Delhey et al. 2005). Also, the "reproductive compensation theory" which can be seen as a different aspect of the "differential allocation theory", explains that to reach higher reproductive success, individuals paired with a poor quality mate, have to input more parental care into offspring to guarantee reproductive success (Saino, Bertacche et al. 2002; Bolund, Schielzeth et al. 2009; Harris and Uller 2009). While females invest more parental care when they mate with poor mates might be because they have involved with extra-pair copulation as it is a common strategy for females to achieve both direct benefit from social mate and better indirect benefit from extra-pair mates to best increase fitness. House wrens (*Troglodytes aedon*) are socially monogamous, and females increase their fitness via extra-pair mating because extra-pair offspring were more likely to return as breeding adults to the local population than within-pair offspring (Bowers, Forsman et al. 2015). In paired analyses of tree swallows (*Tachycineta bicolour*), breeding in Wisconsin, successful extra-pair sires were found to be more experienced breeders, heavier, had fewer lice holes in their feathers, had longer wings and tended to be in better condition than the male they cuckolded (Whittingham and Dunn 2014). Therefore, studies in extra-pair mating and parental care help explain female preferences and the attractiveness of males (Webster, Pruett-Jones et al. 1995; Whittingham and Dunn 2016).

The barn swallow is socially monogamous and they can keep a pair bond for multiple years. Both the barn swallow parents are involved in nest building and offspring provisioning with females generally contribute more than their partners (Anthony and Ely 1976; Møller and Gregersen 1994; Turner 2006). Both sexes have been shown to have high extra-pair copulation frequency leading to intense sperm competition (Saino, Primmer et al. 1997; Safran, Neuman et al. 2005; Vortman, Lotem et al. 2013), though in *gutturalis* the extra-pair copulation rate was found to be low (Hasegawa, Arai et al. 2010b). Males only help normally one social partner to raise chicks, and they can achieve a higher reproductive success by copulating with extra-pair females without feeding the extra-pair young. Though it has been reported that parasitic females can lay their eggs into other swallow nests (Petrželková, Michálková et al. 2015), the majority of females only have a limited number of offspring. Beside investing more in parental care, their most common strategy to enhance reproductive success is to pair with a high quality mate (Møller 1985; Turner 2006). In barn swallow subspecies *rustica* (Møller 1988b) and *erythrogaster* (Safran and McGraw 2004), it has been demonstrated that females preferred males with more exaggerated traits than their social pair in extra-pair copulations.

In Denmark, the subspecies *rustica*, studies have shown that extra-pair copulations compromise about 10% of all copulations with last-male advantage (Møller 1985; Birkhead, Møller et al. 1992). Generally two thirds of all extra-pair copulations were found with the nearest neighbours and they occurred most frequently between earlier breeding males and later breeding females (Møller 1985). Females paired with medium- and short-tailed males preferentially have extra-pair copulations with long-tailed males, thus longer-tailed males are more successful in extra-pair copulations and will have overall higher genetic reproductive success (Møller 1988a).

In North America, subspecies *erythrogaster* has tail streamers that are shorter than *rustica*, and much redder ventral plumage coloration. In this subspecies, males with experimentally elongated tail streamers do not gain an advantage from siring more chicks. In the non-manipulated control male group, male tail streamer length had a negative effect on the number of extra-pair young (EPY) in their social nests (Smith 1991; Smith and Montgomerie 1991). It is believed that the aerodynamic disadvantages of streamer breakage that result from incubation attendance are at least partly responsible for the shorter streamer length of males in North America (Smith and Montgomerie 1991). More recent studies have shown that the plumage colouration is a more significant mate choice preference: males with redder breast and belly feathers paired earlier and sired more offspring (Safran and McGraw 2004; Neuman, Safran et al. 2007). An experiment showed that females adopted a dynamic strategy based on the ornamental trait change of their social partner. Before the experiment, the number of young sired by the social mate did not differ. Then the males were divided into three groups randomly, in one group the ventral plumage of males were darkened within natural range and one group of males were sham manipulated as control and another control group of males stayed untreated. Then the eggs were removed after they completed their clutches leaving them time to assess again the signal quality of males before the second attempt. After assessing the paternity of two successive clutches, the results showed that males with enhanced plumage redness gained paternity compared to the two control groups in which the paternity level remained the same (Safran, Neuman et al. 2005).

In general, current evidence tends to support the differential allocation hypothesis that female barn swallows allocate more parental care when they socially mate with a better quality male to achieve high overall fitness which pays off the parental care compensation (Møller 1985; Turner 2006; Maguire and Safran 2010). In *rustica*, long-tailed males provide less parental care than short-tailed males, both when considering natural and experimentally manipulated tail length (De Lope and Møller 1993). Observations and testosterone implant experiments have demonstrated that high circulating levels of testosterone in long-tailed males are causally related to reduced male feeding rates (Saino, Møller et al. 1995). Another explanation for this observation might be that the preferred males are more likely to copulate with other females or protect more than one female in his territory though only very few males are socially polygynous in regions where data has been collected (Spain 1%, Canada 2%, respectively (Turner 2006)). The parental care research on *erythrogaster* shows that females invest more when they mate with males with darker belly feathers, and good quality fathers did not input more effort in parental care (Maguire and Safran 2010).

In the model subspecies *gutturalis*, current evidence from Japan shows that males gain reproductive advantages from multiple traits such as having larger white tail spots and larger throat patches (Hasegawa, Arai et al. 2010a). However, possibly due to the low density, this population performs very low rate of extra-pair copulations with only 5% EPY reported (Hasegawa, Arai et al. 2010b). Thus no male traits preferred in extra-pair copulation has been further investigated. Female *gutturalis* in the Japanese population tends to obtain indirect benefit rather than direct benefit from mating with more attractive males, because males with larger throat patches invested less in parental care than males with smaller throat patches (Hasegawa, Arai et al. 2014a). In a further experiment it was demonstrated that males increased parental investment when their throat patch size was reduced by manipulation, which shows evidence that males could adjust their behaviour based on trait exaggeration change (Hasegawa and Arai 2015). Though tail streamer is not recognised as a sexual selection trait, *gutturalis* males with longer tail streamers did not feed their young in nest more in the Japanese population (Kojima, Kitamura et al. 2009).

Objectives

In the last chapter, I presented that feather colouration is unlikely to be sexually selected in the study population of *gutturalis*. Male body mass predicted an earlier initiation date, but no direct correlation was found between male ornamental traits and social reproductive success. In this chapter, potential sexually preferred traits are further examined in extra-mating and parental care behaviour of both sexes.

1) Preferred male ornamental traits will be determined by their performance in extra-pair mating and genetic reproductive success. Based on the paternity test, the paternity percentage of offspring for both males and females in social nest can be determined. Potentially part of the EPY will be reassigned to their genetic father in the study population to generate genetic reproductive success for these males. More sexually preferred males are more likely to have higher paternity of offspring in nest and gain extra offspring from extra-pair females; the less attractive males might lose paternity by being cuckolded by their social mate.

2) Male attractiveness will further be confirmed based on female parental care allocation. Ornamental traits of both males and females will be related to feeding rate to determine their prediction in parenting ability and related to female feeding ratio to reveal female reproductive strategy responding to ornamental traits.

3.2 Methods

Field work methods

The capture and measurements of parent birds, recording of events, and the recording of the number of eggs, chicks and fledglings, are all described in detail in Chapter 2. In 2014 and 2015, blood samples were taken from all adults at capture and chicks of 10 days old. Around 20 μl blood sample was taken from the brachial vein of each individual, and each sample was preserved in either 1 ml of lysis buffer with 2% sodium dodecyl sulfate or 97% ethanol (White, Densmore III et al. 1992). In 2014, blood samples were taken from 64 adults and 195 young (146 from the first brood, 49 from the second brood) from 32 nests. In 2015, blood samples were taken from 66 adults and 218 chicks (158 from the first brood, 60 from the second brood) from 33 nests. In total, 550 samples were collected including seven eggs that failed to hatch.

Parent feeding rate (visits per hour) was used to represent feeding effort (Møller 1988b). Feeding times were recorded in one hour on day 10 and day 15 between 6 am to 5 pm using binoculars and birds were identified by their colour rings. During observation, I used a plastic sheet to conceal myself so that the barn swallow parents would behave normally without noticing my presence. Also I avoided extreme weather like extremely hot hours or heavy rain when the adults' foraging behaviour was obviously restricted.

Molecular methods and paternity analysis

DNA was extracted from each blood sample using Tiangen DNA Extraction Kits (Tiangen Biotech, Beijing). Then using polymerase chain reaction (PCR), seven microsatellite loci were amplified (Escu6 (Hanotte, Zanon et al. 1994), Ltr6

(McDonald and Potts 1994), POCC6 (Bensch, Price et al. 1997); Hir11, Hir17, and Hir20 (Tsyusko, Peters et al. 2007); Hru6 (Primmer, Møller et al. 1995)). Individual Escu6, Ltr6, Hru6 and Hir20 PCR reactions were combined into multiplex, Hir11, Hir17, and POCC6 were amplified as single locus. PCR amplification conditions were as follows: initial denaturation at 95 ℃ for 15 min or 1 min 40 s (multiplex and single loci, respectively); 10 cycles of denaturing at 94 ℃ for 30 s, annealing at 55 or 60 ℃ (55 ℃ for multiplex, Hir11 and Hir17; 60 ℃ for POCC6) for 30 s and extension at 72℃ for 45 s; another 25 cycles of denaturing at 87 ℃ for 30 s, annealing at 55 or 60 ℃ (55 ℃ for multiplex, Hir11 and Hir17; 60 ℃ for 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 using Genemapper v3.7 (Applied Biosystems, USA) and scored by visual examination.

I assigned paternity using methods implemented in Cervus V 3.0.7 (Marshall, Slate et al. 1998). The combined exclusion probability of seven loci for the first parent was 0.99190134, 0.99953541 for the second parent, and 0.99999793 for the parent pair. Offspring were assigned as extra-pair young if at least two mismatches between the genotype of the offspring and the social father were found and if the social father was not one of the two most likely fathers.

During 2014, on the second day after hatching, one or two chicks (one for the brood of three nestlings, two for brood of 4-6 nestlings) were swapped for the broods that hatched on the same day for a cross-fostering experiment. In the second brood and total paternity analysis, those nests were not excluded because the swapping did not affect female extra-pair mating choice (yes or no) and the further extra-pair young percentage (paired *t* test, n =9, $t_{1,8}$ =1, p = 0.3466). For the calculation of annual number of fledglings, the swapped nests were excluded to avoid the possible manipulation effect on offspring survival. There was one male with two social mates in 2014, the earliest brood was counted as his first brood because in such way, the first brood initiation date can reveal his real breeding onset time. And the second social nest was counted as part of his second brood breeding success (the second brood with the first social female was the other part) as it occurred when the first social brood were fledging.

Data analysis

Due to the fact that females' social and genetic reproductive success did not differ, in this chapter, the models predicting genetic reproductive success were only run with male traits. Linear mixed effects models were used to test the relationships between male morphological traits (fixed effects: male body mass and lengths of tail streamer, short tail, wing and tarsus) and the number of the extra-pair young and the genetic reproductive success in package lme4 and lmerTest in R (R Core Team 2015), with year as a random effect. Generalised mixed effects models were used to predict the chance of having at least one EPY (0 or 1) and paternity ratio (genetic number of chicks divided by number of all chicks in social nest) using glmer command with binomial data family. The statistics of the model were given by summary command in R (R Core Team 2015). Non-significant variables were sequentially removed from the model starting with the largest *p* value until the simplest model was left with significant factors. *P* values of significant variables in tables were marked in bold, and the factors with *p* values < 0.05 were marked with one star "*", and marked with two stars "**" when the *p* values are < 0.01. For figures showing factors and their correlations with dependent variables in the final models (e.g. **Figure 3.1**), the detailed explanation is listed in Methods of Chapter 2.

Female feeding ratio was calculated as the times the female fed divided by the sum of both parents' feeding visits. The generalized linear mixed effects model was conducted in R using glmer as binomial data type. Year was used as a random effect, with both male and female body traits as fixed factors (the same with the traits have been used in Chapter 2), models were simplified as described in the previous paragraph.

3.3 Results

3.3.1 Extra-pair mating behaviour

Distribution of extra-pair young

Table 3.1 EPY distribution of barn swallow *gutturalis* in Qingdao in 2014 and 2015. They are represented as "number of EPY (total chicks in nest)" and nests with EPY are in bold. Nest number 5 was the second social mate of the male in nest number 9.

Year	**Nest Number**	**First brood**	**Second brood**
2014	**1**	**2(5)**	0(2)
	2	**3(3)**	0(4)
	3	**2(5)**	0(4)
	4	**1(3)**	NA
	5	**2(5)**	NA
	6	**2(4)**	NA
	7	0(5)	**3(4)**
	8	0(5)	**1(4)**
	9	0(5)	**2(5)**
	10	0(2)	**1(1)**
	11	0(5)	**1(1)**
	12	0(4)	**1(3)**
	Total EPY	12	9
	Total offspring	146	49
	Nests with EPY	6	6
	Nests without EPY	26	10
2015	**13**	**6(6)**	**3(3)**
	14	**1(5)**	**3(3)**
	15	**1(4)**	NA
	Total EPY	8	6
	Total offspring	158	60
	Nests with EPY	3	2
	Nests without EPY	30	15

Maternity All the tested chicks were the genetic offspring of the mother in the nest.

Paternity Based on **Table 3.1**, 12 nests were found with extra-pair mating young, and 36 nests were without EPY in 2014. 12 out of 146 first brood chicks (8.22%, from six nests) and nine out of 49 second brood chicks (18.37%, from another six nests) were EPY. In total, 21 out of 195 of the chicks (10.77%) were EPY, 12 out of 32 females (37.5%) had at least one extra-pair offspring in nest and no female had extra-pair offspring in both broods. Specially, there was one male who had two social mates in this year, he socially stayed with the female in nest number 9 (**Table 3.1**) and had a first brood of offspring without EPY. Then he had an extra brood with the other female in nest number 5 in which there were two EPY out of five chicks. He further provisioned a second brood with the first social mate and had two EPY out of five chicks. Therefore, this male provisioned three broods and had 11 genetic chicks and four EPY in a year.

In 2015, three females (9.1%) had at least one EPY and 6.42% of all chicks were EPY. Eight out of 154 chicks (5.19%) were EPY from three out of 32 nests (9.37%) in the first brood. In the second brood, there were six EPY out of 60 chicks (10%), from two out of 17 nests (11.76%) which both contained EPY in their previous broods.

For the two years together, 15 out of 65 females (23.07%) had at least one EPY in the social nest, but only two of them had EPY in both of their breeding attempts, one of which had 100% of her chicks as extra-pair offspring with more than one extra-pair mate. In the females who had EPY in social broods, three of them had EPY in the first brood but not in the second brood and six of them did not have EPY in the first brood but did in the second one. In total, there were 17 broods (17.34%) with 35 offspring as EPY (8.47 %) in the population.

Male traits and the likelihood of having extra-pair young

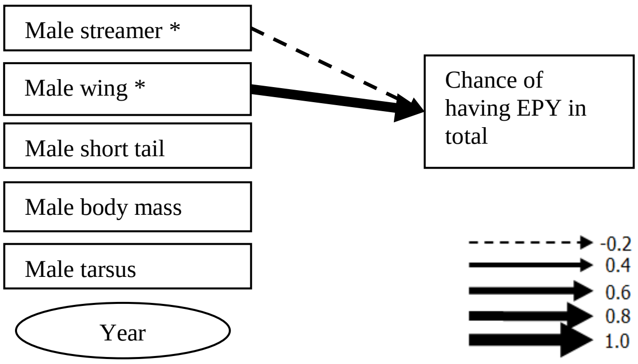

Figure 3.1 Males with shorter tail streamers and longer wings were more likely to have extra-pair young in social nests in a population of barn swallow *H. r. gutturalis* in Qingdao China (plots in **Figure 3.2** and statistics results in **Table 3.2).**

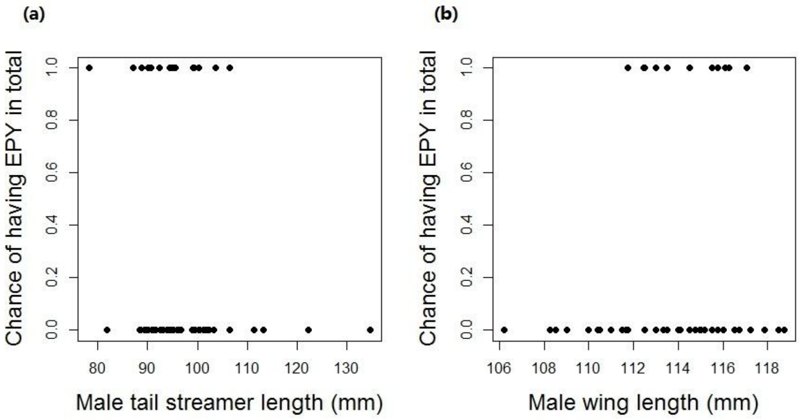

Figure 3.2 Plots of male tail streamer (a) and wing length (b) against probability of having at least one EPY in social nests in a population of barn swallow *H. r. gutturalis* in Qingdao China.

Based on all broods they had in a season, the probability that a male was cuckolded was related to the male tail streamer and wing lengths. Males with longer tail streamers and shorter wings were unlikely to be cuckolded (**Figure 3.1** & **3.2, Table 3.2**).

Table 3.2 Statistics results of generalised linear mixed model predicting males' chance of having EPY in total using male traits. In the barn swallow *gutturalis* population in Qingdao China, male tail streamer length and wing length showed significant correlations with the chance of having EPY in social nests. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*z*	*p*
Male tail streamer	55	-2.378	**0.017**
Male wing	55	2.447	**0.014**
Random effect	Groups	Variance	SD
Year	2	1.516	1.231
Traits excluded	*df*	*z*	*p*
Male short tail	52	0.176	0.860
Male body mass	53	0.289	0.773
Male tarsus	54	1.014	0.310

Paternity ratio of the social chicks

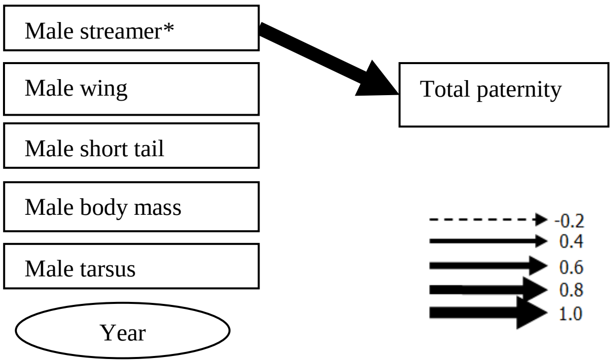

Figure 3.3 Longer-tailed males had higher paternity ratio in nest in a population of barn swallow *H. r. gutturalis* in Qingdao China (plots in **Figure 3.4** and statistics results in **Table 3.3**).

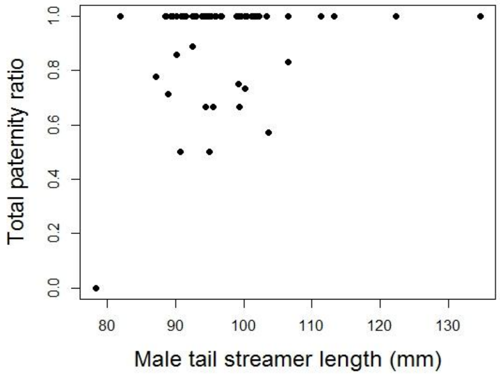

Figure 3.4 Plots of male tail streamer length and offspring paternity ratio in nest in a population of barn swallow *H. r. gutturalis* in Qingdao China.

Table 3.3 Statistics results of generalised linear mixed model predicting paternity of total offspring using male traits. In the Qingdao population of barn swallow *gutturalis*, male tail streamer had a positive correlation with the paternity of offspring in nest. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*z*	*p*
Male tail streamer	56	2.079	**0.038**
Random effect	Groups	Variance	SD
Year	2	0	0
Traits excluded	*df*	*z*	*p*
Male tarsus	52	1.046	0.296
Male short tail	53	-0.935	0.350
Male body mass	54	-0.895	0.371
Male wing	55	-1.420	0.156

On average, the male paternity ratio for first brood nestlings was 0.93 ± 0.21 (mean ± sd, n=58); it was 0.81 ± 0.36 (n=30) for second brood, and in total it was 0.92 ± 0.18 (n=59).

The paternity ratio of all the social chicks in one season was directly related to the male tail streamer length: the longer the tail streamers they had, the higher percentage of chicks were their genetic offspring (**Figure 3.3** & **3.4**, **Table 3.3**).

3.3.2 Male genetic reproductive success

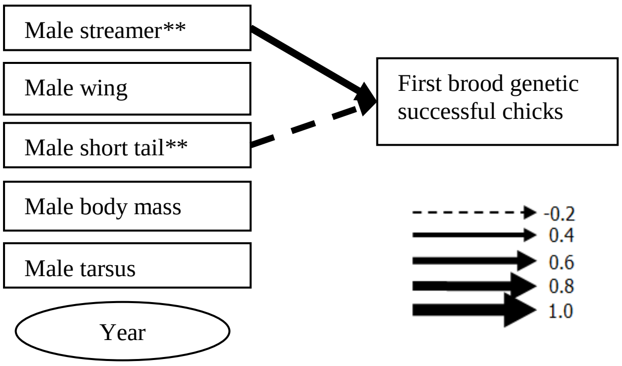

Figure 3.5 Males with longer tail streamers and shorter short tails had more genetic successful chicks in the first brood in a population of barn swallow *H. r. gutturalis* in Qingdao China (plots in **Figure 3.6** and statistic results in **Table 3.4).**

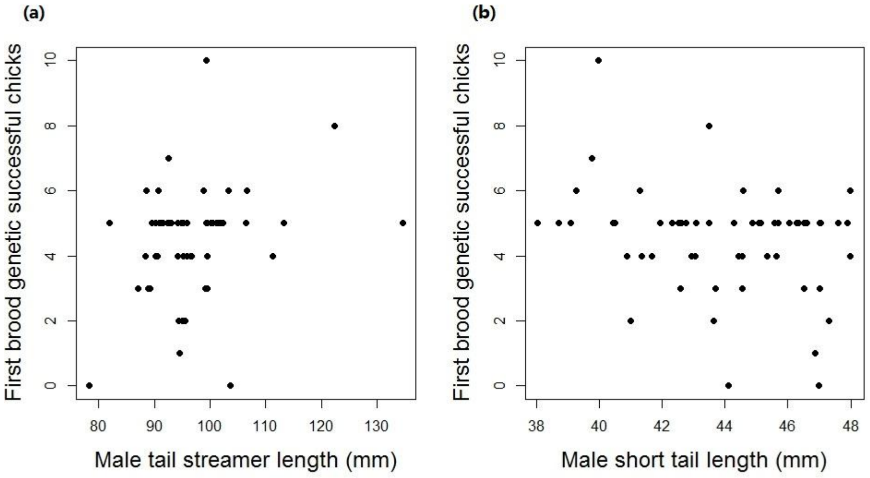

Figure 3.6 Plots of both male tail streamer length (a) and short tail length (b) showing trends with genetic chicks in the first brood in a population of barn swallow *H. r. gutturalis* in Qingdao China.

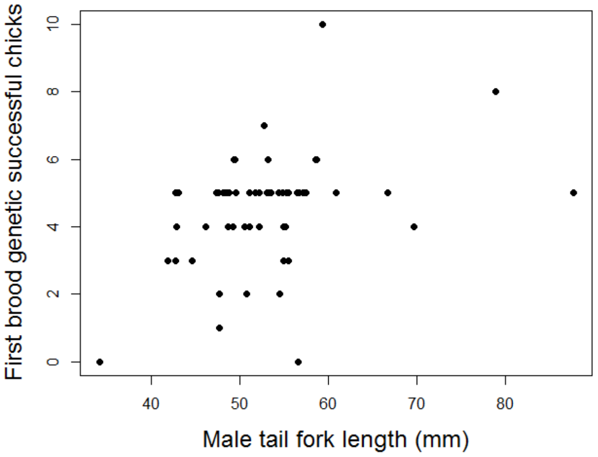

Figure 3.7 Male tail fork depth was related to the number of genetic chicks in the first brood in a population of barn swallow *H. r. gutturalis* in Qingdao China (statistics result in **Table 3.4** within bracket**).**

Table 3.4 Statistics results of linear mixed effects model predicting number of genetic offspring in the first brood using male barn swallow traits. Male tail streamer and tail fork length had a positive correction and male short tail length had a negative correlation with the first brood genetic offspring number in a barn swallow *gutturalis* population in Qingdao, China. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*t*	*p*
Male tail streamer	55	3.263	**0.002**
Male short tail	55	-3.214	**0.002**
(Male tail fork depth)	56	3.098	**0.003**
Random effect	Groups	Variance	SD
Year	2	<0.0001	<0.0001
(Year)	2	<0.0001	<0.0001
Traits excluded	*df*	*t*	*p*
Male tarsus	52	-0.609	0.545
Male body mass	53	-0.733	0.466
Male wing	54	-0.754	0.455

Sired successful chicks In the first brood, males with longer tail streamers and shorter short tails had more genetic fledglings (**Figure 3.5** & **3.6, Table 3.4**). If short tail length is deducted from male tail streamer length, defined as the male tail fork depth, it was positively related to the number of genetic chicks in the first brood (**Figure 3.7**, statistics in bracket in **Table 3.4**). The male tail fork depth was also related to the annual total of genetic successful chicks (**Figure 3.8**, **3.9 & 3.10, Table 3.5**).

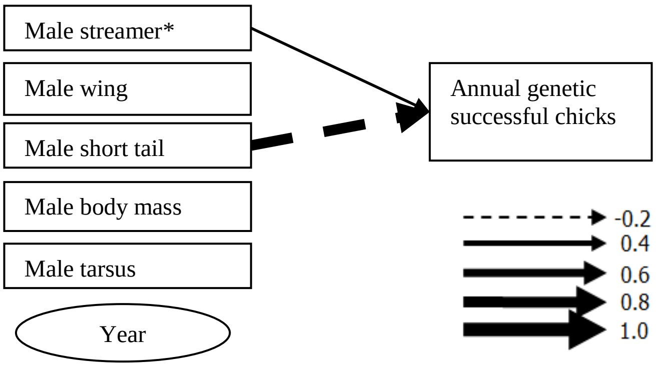

Figure 3.8 Male tail streamer and short tail lengths were related to annual genetic chicks in a population of barn swallow *H. r. gutturalis* in Qingdao China (statistics in **Table 3.5,** plots in **Figure 3.9** & **3.10).**

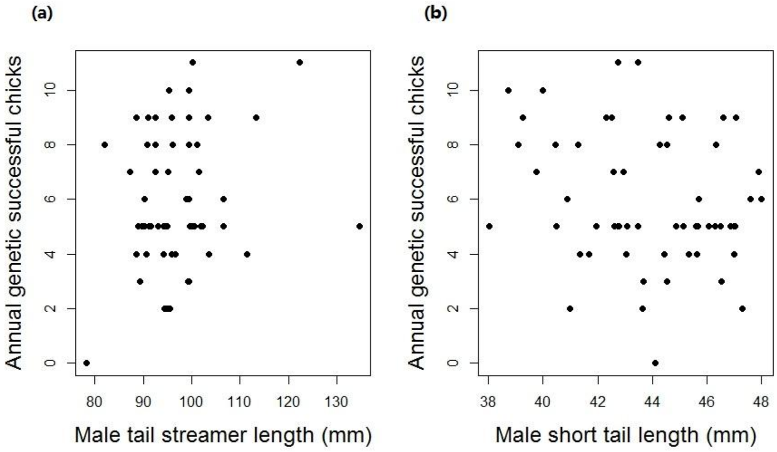

Figure 3.9 Plots showing that male tail streamer and short tail lengths were related to annual genetic chicks in a population of barn swallow *H. r. gutturalis* in Qingdao China.

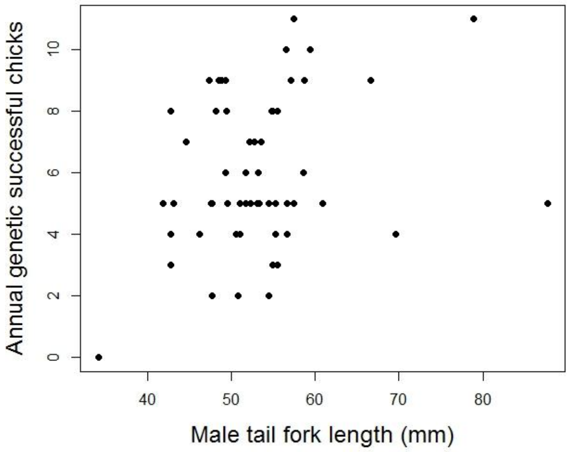

Figure 3.10 Plots showing that male tail fork depth was related to annual genetic chicks in a population of barn swallow *H. r. gutturalis* in Qingdao China (statistics result in **Table 3.5** within bracket**).**

Table 3.5 Statistics results of linear mixed effects model predicting annual genetic chicks using male traits. In the study population of barn swallow *gutturalis* in Qingdao, China, the male tail streamer and short tail lengths had significant relations with the number of annual genetic offspring, and the tail fork depth showed significant relation. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*t*	*p*
Male tail streamer	54	2.169	**0.034**
Male short tail	54	-2.415	**0.019**
(Male tail fork depth)	55	2.060	**0.044**
Random effect	Groups	Variance	SD
Year	2	0	0
(Year)	2	0	0
Traits excluded	*df*	*t*	*p*
Male body mass	51	0.003	0.998
Male tarsus	52	-0.122	0.903
Male wing	53	-0.334	0.740

3.3.3 Parental care

Table 3.6 Female feeding visits percentage on day 10 and 15 after hatching in a population of barn swallow *H. r. gutturalis* in Qingdao China. Values are mean (SD). Paired *t*-test results are also listed from comparing the visiting percentage between females with EPY and without EPY in nests.

Brood number	Day	All female	Females without EPY	Females with EPY	*t*	*df*	*p*
First	10	0.53 (0.14)	0.52 (0.09)	0.56 (0.19)	-0.73	17.79	0.48
	15	0.52 (0.15)	0.54 (0.12)	0.55 (0.14)	-0.34	25.18	0.73
Second	10	0.51 (0.15)	0.52 (0.14)	0.50 (0.19)	0.30	16.65	0.77
	15	0.48 (0.20)	0.51 (0.08)	0.49 (0.25)	0.26	11.21	0.80

Table 3.6 shows the percentage of female feeding visits to chicks on day 10 and 15 after hatching, for the first and the second broods. Among the four days, female visits accounted for 48% - 53%, hence on average, females and males shared the feeding task equally. It also shows females (and also the males) did not differ the feeding contribution percentage when there were EPY in nest or not.

Table 3.7 describes the visits in an hour for both males and females on average. And both males' and females' visiting rates did not differ significantly when they had extra-pair young in nest or not.

Table 3.7 Female and male feeding visits rates (times/hour) and *t*-test between broods that having and not having EPY in a population of barn swallow *H. r. gutturalis* in Qingdao China. Values are mean (SD).

Brood number	Day	All female	Females without EPY	Females with EPY	*t*	*df*	*p*
First	10	18.65 (7.95)	19.65 (6.25)	18.46 (10.02)	0.41	20.41	0.68
	15	19.23 (10.35)	20.88 (9.22)	23.33 (13.72)	-0.61	21.97	0.54
Second	10	16.95 (8.07)	16.22 (6.26)	17.70 (8.89)	-0.42	16.14	0.68
	15	17.09 (10.97)	16.00 (4.69)	18.80 (13.86)	-0.6	11.23	0.56
	Day	All male	Males without EPY	Males with EPY	*t*	*df*	*p*
First	10	16.58 (6.38)	18.19 (5.67)	14.13 (6.80)	1.95	25.21	0.06
	15	16.98 (7.62)	17.67 (7.61)	17.73 (8.03)	-0.02	28.62	0.97
Second	10	15.41 (8.11)	15.44 (8.79)	16.70 (8.50)	-0.32	16.65	0.75
	15	15.86 (6.25)	15.89 (7.62)	15.5 (6.19)	0.12	15.46	0.56

Phenotypic traits and feeding investment

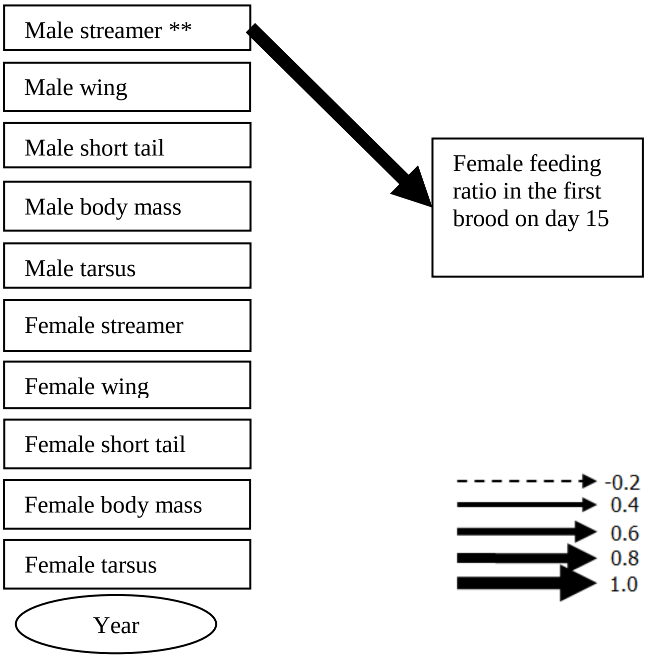

Figure 3.11 On day 15 of the first brood after hatching, females performed a higher proportion of feeding visits when their mates had longer tail streamers in a population of barn swallows *H. r. gutturalis* in Qingdao China (plots in **Figure 3.12** and statistics result in **Table 3.8).**

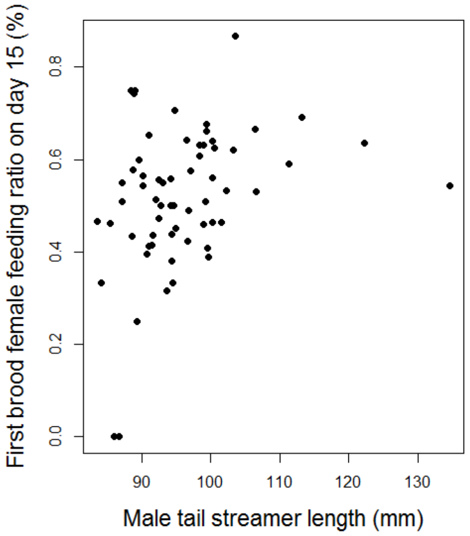

Figure 3.12 Plots of male tail streamer with female parental care ratio in the first brood on day 15 after hatching in a population of barn swallows *H. r. gutturalis* in Qingdao China.

Table 3.8 Statistics results of generalised linear mixed model predicting female feeding ratio on day 15 after hatching in the first brood using male and female barn swallow traits. In the study population of barn swallow *gutturalis* in Qingdao China, longer male tail streamers predicted higher percentage that their mate provisioned on day 15 in the first brood. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*z*	*p*
Male tail streamer	60	2.351	**0.019**
Random effect	Groups	Variance	SD
Year	3	0	0
Traits excluded	*df*	*z*	*p*
Male body mass	51	-0.065	0.948
Male wing	52	-0.081	0.936
Female wing	53	-0.011	0.992
Female short tail	54	-0.303	0.762
Female body mass	55	-0.301	0.763
Male short tail	56	-0.43	0.667
Male tarsus	57	-0.524	0.600
Female tarsus	58	0.503	0.615
Female tail streamer	59	-0.81	0.418

Data from day 15 of the first brood show that females performed a larger proportion of feeding visits when their mates had longer tail streamers (**Figure 3.11** & **3.12, Table 3.8**).

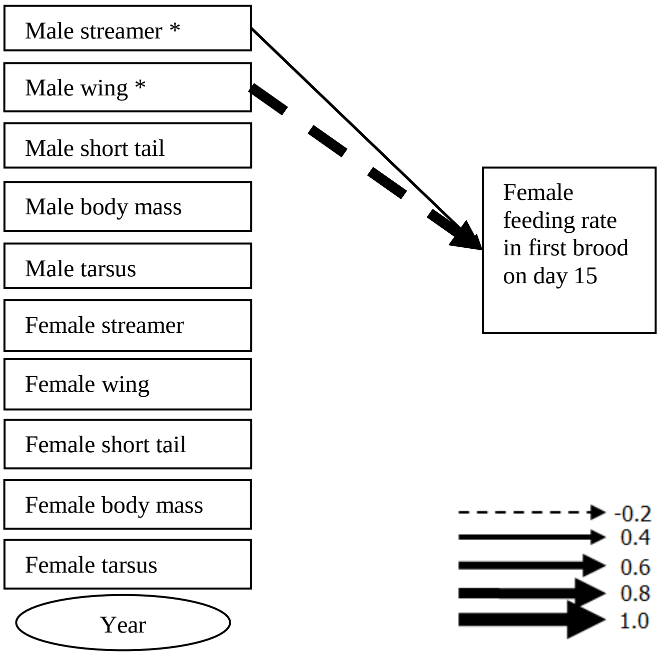

Figure 3.13 On day 15 after hatching in the first brood, female feeding rate was related to their mate traits in a population of barn swallow *H. r. gutturalis* in Qingdao China. Male tail streamer length showed positive correlation and wing length showed negative correlation (plots in **Figure 3.14** and statistics results in **Table 3.9**).

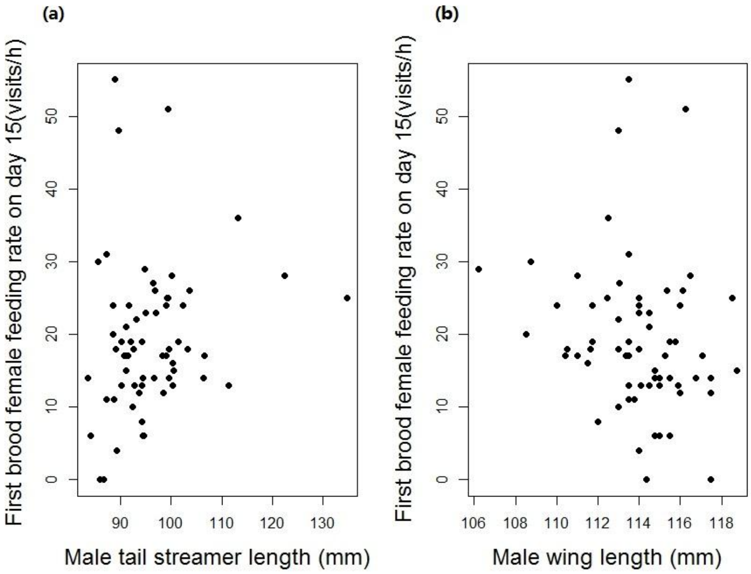

Figure 3.14 Plots of first brood female feeding rate on day 15 after hatching with male tail streamer (a) and wing length (b) in a population of barn swallow *H. r. gutturalis* in Qingdao China.

Table 3.9 Statistics results of linear mixed effects model predicting female feeding rate on day 15 after hatching in the first brood using male and female barn swallow traits. In the study population of barn swallow *gutturalis* in Qingdao China, the male tail streamer had positive relation, and male wing length had negative relation with female feeding rate on day 15 after hatching in the first brood. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in final model			
Fixed effects	*df*	*t*	*p*
Male tail streamer	40.76	2.108	**0.041**
Male wing	49.46	-2.242	**0.030**
Random effect	Groups	Variance	SD
Year	3	1.705	1.306
Traits excluded	*df*	*t*	*p*
Female wing	50.73	0.031	0.975
Male body mass	48.72	0.041	0.967
Male tarsus	43.78	0.523	0.604
Female short tail	54.72	0.672	0.505
Male tarsus	73.00	-0.52	0.603
Female body mass	30.43	-1.523	0.138
Female tail streamer	55.45	-1.559	0.125
Female tarsus	17.07	1.394	0.181
Male short tail	59.00	1.986	0.052

Female feeding rate (visits/h) on day 15 was positively related to male tail length and negatively related to male wing length (**Figure 3.13** & **3.14, Table 3.9**). No significant correlation was found between adult traits and female feeding rate (visits/h) on day 10 after hatching in the first brood (**Table 3.10**).

Table 3.10 Statistics results of linear mixed effects model predicting female feeding rate on day 10 using male and female barn swallow traits. No male or female traits showed significant relation.

Fix effects excluded	*df*	*t*	*p*
Male tail streamer	54.96	0.134	0.894
Female tail streamer	55.27	0.370	0.713
Male short tail	50.39	0.442	0.660
Male tarsus	35.77	0.491	0.627
Female wing	57.81	0.736	0.465
Female body mass	53.35	0.996	0.324
Male wing	45.3	-1.143	0.259
Female short tail	56.34	1.228	0.225
Female tarsus	19.59	1.690	0.107
Male body mass	64	-1.532	0.130
Random effect	Groups	Variance	SD
Year	3	<0.0001	<0.0001

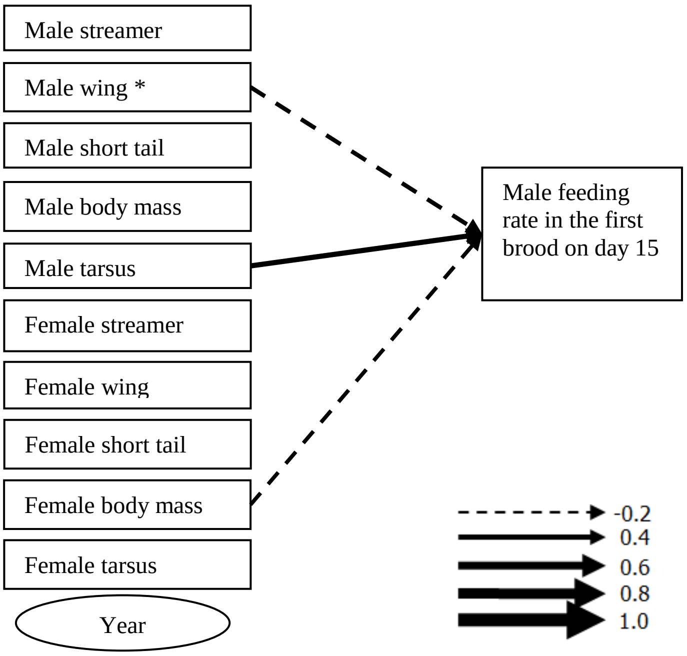

Figure 3.15 Male feeding rate on day 15 after hatching was correlated with male wing length, male tarsus length and female body mass in a population of barn swallows *H. r. gutturalis* in Qingdao China (plots in **Figure 3.16** and statistics result in **Table 3.11).**

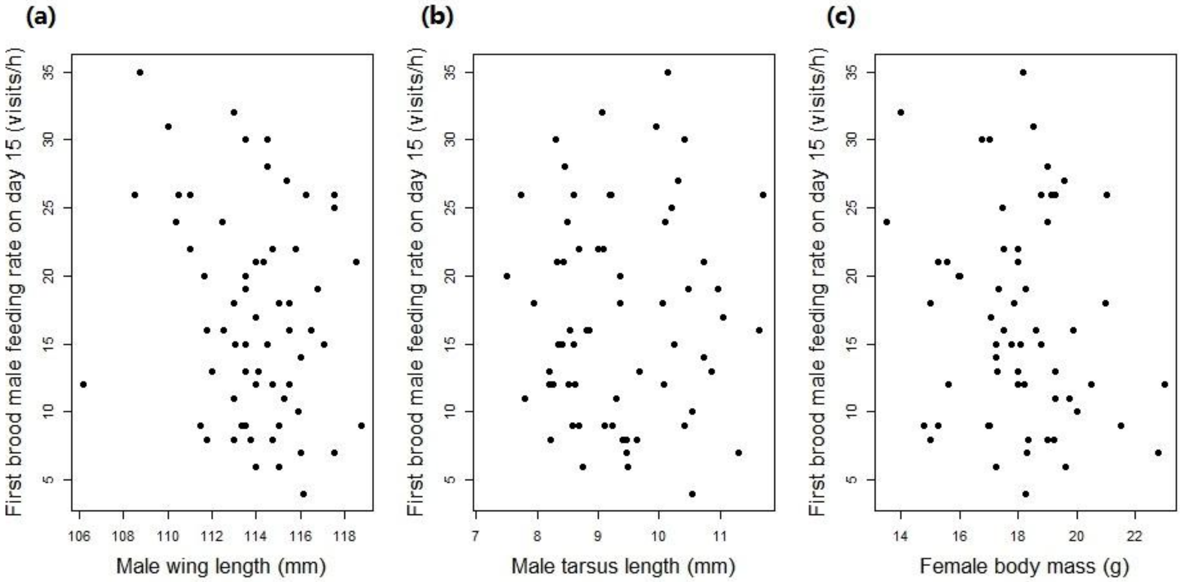

Figure 3.16 Plots of male feeding rate on day 15 after hatching with male wing length (a), male tarsus length (b) and female body mass (c) in a population of barn swallows *H. r. gutturalis* in Qingdao China.

Table 3.11 Statistics results of linear mixed effects model predicting male feeding rate on day 15 after hatching in the first brood. In a barn swallow *gutturalis* population in Qingdao China, male wing length was negatively related to male feeding rate on day 15 after hatching in the first brood with non-significant traits male tarsus length and female body mass in the model. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Traits in the model			
Fixed effects	*df*	*t*	*p*
Male tarsus	10.29	1.04	0.322
Male wing	58.99	-2.097	**0.040**
Female body mass	18.52	-1.481	0.155
Random effect	Groups	Variance	SD
Year	3	0.194	0.441
Traits excluded	*df*	*t*	*p*
Male body mass	52	0.078	0.938
Female tail streamer	53	-0.090	0.928
Male tail streamer	54	-0.321	0.749
Female wing	55	-0.310	0.758
Female short tail	56	0.432	0.667
Male short tail	57	0.636	0.527
Female tarsus	58	-0.863	0.392
Male tarsus	10.29	1.040	0.322
Female body mass	60	-1.249	0.217
Male wing	64	-0.932	0.355

On day 15, longer-winged males provisioned less frequently in one hour than the males that had shorter wings (**Figure 3.15** & **3.16, Table 3.11**). No significant models were found that predicted male feeding rate on day 10 after hatching in the first brood (**Table 3.12**).

Table 3.12 Statistics results of linear mixed effects model predicting male feeding rate on day 10 after hatching in the first brood in a barn swallow *gutturalis* population in Qingdao China. No traits of males or females was significant in the model.

Fixed traits excluded	*df*	*t*	*p*
Female tail streamer	55	0.190	0.850
Male wing length	56	0.450	0.654
Male body mass	57	-0.773	0.443
Male tail streamer	57	-0.773	0.443
Male tarsus	58	-0.927	0.358
Female wing	59	-1.086	0.282
Male short tail	60	1.339	0.186
Female tarsus	61	1.061	0.293
Female short tail	62	1.899	0.062
Female body mass	63	1.368	0.176
Male body mass	64	-0.932	0.355
Random effect	Groups	Variance	SD
Year	3	<0.0001	<0.0001

3.4 Discussion

Extra-pair mating

In the *gutturalis* population in Qingdao China, almost a quarter of females (23.07%) had at least one EPY in nest, and 8.47 % young are EPY in 17.34% of broods. The ratio of EPY in the population in Qingdao was much higher than that reported in Japan where only under 5% of young were EPY (Hasegawa, Arai et al. 2010b; Hasegawa and Arai 2015). As it is common that monogamous bird species are slightly male biased (Lack 1954), it might be because the sex ratio was more male biased in the Qingdao population than that in the Japanese population, or the population density was higher in Qingdao and the pressure from sexual selection was greater. The data from the two populations of *gutturalis* might also support the view that the extra-pair mating of females varies geographically both inter- and intra-subspecies (Turner 2006), the difference not only occurring in different populations of the same species, but also the EPY percentages of these two populations were both lower than that of other subspecies. In *rustica* one report from Denmark showed that 28% offspring in 33% of broods were EPY, and the distribution was highly skewed so either all of them were EPY or none (Møller and Tegelström 1997). In Italy, 29% of chicks were EPY from 52% of broods while in Spain 18% of offspring were EPY from 32% of broods (Saino, Primmer et al. 1999; Møller, Brohede et al. 2003). The subspecies *erythrogaster* in Canada, 31% offspring in 50% of broods were extra-pair young in one population, and in USA, 23% of offspring were EPY from 49% of broods (Safran, Neuman et al. 2005; Kleven, Jacobsen et al. 2006). In the report for one *transitiva* population in Israel, overall, 43% of 41 first broods hosted one or more extra-pair young (Vortman, Lotem et al. 2011).

Males with longer tail streamers were more successful in extra-pair mating which further supports that this trait might be sexually selected in *gutturalis*. It is believed that females are using extra-pair copulations to obtain indirect benefits for their offspring, either through genes for increased viability, or for a Fisherian mating advantage (Sheldon 1994). Positive associations between male phenotype and success at obtaining extra-pair copulations or extra-pair fertilizations have been found in other subspecies (Møller and Tegelström 1997; Safran, Neuman et al. 2005) and other bird species (Yezerinac and Weatherhead 1997; Wells, Safran et al. 2016), i.e. tree swallow (Whittingham and Dunn 2016).

In this study, the pattern of EPY changed yearly: in 2014, six females only had extra-pair chicks in the first broods, while the other six had EPY only in the second broods; in 2015, only three females had extra-pair chicks and the two of these which had a second brood continued the same strategy. This difference in the pattern of EPY indicates that females can adjust strategies during the same breeding season according to male quality comparison and evidence has been shown in *erythrogaster* (Safran, Neuman et al. 2005), and it also emphasizes the importance of genetic quality of males for females in mate choice.

Genetic reproductive success

In European *rustica* population, it has been found that females adopt quasi-parasitism (QP) to gain extra-pair maternity (EPM) (Petrželková, Michálková et al. 2015), in this *gutturalis* population, no EPM was found. Hence the social breeding success appears to represent the female genetic breeding success in this site. Genetic reproductive success is needed to assess male breeding success.

In the Qingdao population of *gutturalis*, male tail streamer length or tail fork depth predicted male attractiveness and genetic reproductive success, hence male tail streamers are likely to be sexually selected which is in contrast to the Japanese population (Hasegawa, Arai et al. 2010a). There is also the possibility that different populations have multiple sexually selected cues that could drive speciation or result in adaption to local environments. Tail streamer length and short tail length (or tail fork depth) predicted the number of genetic chicks in nests, though other studies did not find tail fork depth to be a useful variable to predict barn swallow behaviour and breeding success (Smith and Montgomerie 1991; Hasegawa and Arai 2013). In the study population, indeed, the short tail lengths were similar in males and females, and the dimorphism of tails thus should exist in tail streamers, but tail fork depth might still be worth consideration in study of tail function, dimorphism degree and sexually selected component on tail streamers among subspecies.

Parental care

In this *gutturalis* population, males and females tended to share feeding equally on average. In other animals, research shows that males might invest less in parental care if they have lower certainty of paternity (Trivers 1972; Andersson 1994), for instance in blue tit *Cyanistes caeruleus*, a socially monogamous passerine whose males decorate their nest with feathers, males fed less when their paternity certainty was decreased by experimentally adding feather ornaments (mimicking presence of an intruder male). In barn swallows, it has been shown that the feeding frequency increased with paternity certainty in *H. r. rustica* and males significantly reduced the feeding frequency with more EPY in nest (Møller 1988b). However, paternity did not significantly affect the feeding rate or feeding proportion for both males and females in study population, which is consistent with the Japanese *gutturalis* population and results found in *erythrogaster* (Maguire and Safran 2010; Hasegawa and Arai 2015).

When the male of a pair had long tail streamers, their mate made more feeding visits in an hour and made a higher proportion of feeding visits, supporting the hypotheses that tail streamer is sexually selected and the “differential allocation theory”. Though reporting on different sexually selected cues, in Japan, female *gutturalis* were found to invest more in incubation when they paired with preferred males (Hasegawa, Arai et al. 2012b), and fed more when they mated with males having better body condition (Kojima, Kitamura et al. 2009).

As for male feeding rate, there was no significant relationship between male tail length and absolute feeding rate, which gives no evidence about more attractive males being a good father. In *rustica, erythrogaster* and other *gutturalis* populations, male ornamental traits did not show positive relation to the share of parental care in feeding, therefore there is overall no support for the “good parent theory” for barn swallows (Møller 1988a; Møller 1989; Møller 1990b; Maguire and Safran 2010).

It is also noted that shorter wings with longer tails together show correlation with female feeding and chance of having EPY. Wing function should be crucial for migratory birds, for instance, the male wing aspect ratio has been demonstrated to determine the arrival date (Matyjasiak, Olejniczak et al. 2013). The wing length in barn swallows has not drawn much attention for mate choice maybe due to little dimorphisms in this trait and it is not displayed during courtship (Turner 2006). However, a study in tree swallow (*Tachycineta bicolor*) suggests that sexual selection dynamics varies depending on environmental contexts like population density: wing length was positively related to the number of EPY, more so at high than at low density while tarsus length variation had a greater effect on reproductive success at low population density than at high density. Therefore there might be multiple male traits affecting female choice in certain environment or time of season.

In general, in the Qingdao population of *gutturalis*, longer-tailed males were sexually preferred in mating and they had higher reproductive success including EPY, and had mates that invested more in parental care. This is the first study to show that male tail streamer length of *gutturalis* is sexually selected providing an explanation for the existence of sexual dimorphism in this trait.

Chapter 4 Chick Growth and the Cross-fostering Experiment

Abstract The choosy sex can expect indirect benefits (good genes) and/or direct benefit (breeding resources) by choosing individuals with particular traits. For altricial bird species, partial cross-fostering has been used as a robust method of separating genetic and environmental effects on offspring growth. As a model animal of sexual selection, the long tail streamers of barn swallow *H. r. rustica* and red belly plumage of another subspecies *erythrogaster* are two typical sexually selected male ornamental traits. There has been a cross-fostering experiment on barn swallow *rustica* showing that the male with longer tail streamers sired offspring with better immune function, but few further reports on the relationship between nestling growth and the traits of both genetic and rearing parents. I observed natural offspring growth and for the first time conducted a partial cross-fostering experiment on *gutturalis* in Qingdao, China. Results show that within original nests, both male and female tail streamers and body mass were related to offspring growth performance. The cross-fostering experiment demonstrates that both nest-of-origin and rearing environment affected nestling growth. Genetic mother's tail streamer length was positively related to the chick peak body mass; rearing mother's body size was correlated to the day seven offspring body mass; growth rate of the chicks was related to rearing male body mass, female wing length and the rearing parents feeding rate. I conclude that male tail length and body size might be sexually selected in male *gutturalis* and their mates tend to obtain indirect benefits by having offspring that grow faster. Also the experiment showed that female body size positively affected offspring growth possibly because they possess genes for larger body size for offspring and they were able to invest more in eggs and provisioning.

4.1 Introduction

Mate choice is believed to increase an individual's fitness via either one or both of direct (parenting ability, resource etc.), and indirect (good genes) benefits (Andersson 1994; Kokko, Brooks et al. 2003). The "runaway theory", "sexy son theory" and the "good genes theory" all explain that by choosing mates with more exaggerated ornamental traits, the choosy sex gains indirect benefits from the heritability of high phenotypic quality for the following generations (Zahavi 1975; Hill 1991; Møller and Gregersen 1994; Mays and Hill 2004; Chandler, Ofria et al. 2013). In biparental care species, natural selection would favour females which obtain direct benefits from choosing a mate when possible, as suggested by the "good parent theory" (Hoelzer 1989). However, for the females that mated with more ornamented males, they might not obtain more direct benefits from their partners because more attractive males commonly consume more time on extra-pair copulations resulting in less parental care than less attractive males (Mitchell, Dunn et al. 2007). As described by the "differential allocation theory", when mating with higher quality males, females allocate more reproductive investment potentially sacrificing part of their future reproductive allocations, to guarantee the best fitness in life (Sheldon 2000).

It can be difficult to separate individual genetic quality from direct investments in breeding such as the provision of care and resources: for example good quality mates can also provide better breeding sites (Alatalo, Lundberg et al. 1986). Cross-fostering experiments in altricial bird species provide a good model to separate the effects of breeding resources and genetic factors (Smith and Wettermark 1995). Various measurements to assess offspring quality including immune function, plumage quality, stress response etc. have been used in experimental designs including nest-of-origin and rearing nest effects on several bird species (Hamilton and Zuk 1982; Hill 1991; Norris 1993; Brinkhof, Heeb et al. 1999; Pickett, Weber et al. 2013; Van Oers, Kohn et al. 2015). Particularly, as a reflection of offspring growth and health, body mass and growth rate have been commonly adopted in the cross-fostering experiments, for instance the experiment in great tits (*Parus major*) (Pickett, Weber et al. 2013; Giordano, Groothuis et al. 2014).

Nest-of-origin effects have been shown to exert an influence on body size in starlings (*Sturnus vulgaris*), yellow-browed leaf warbler (*Phylloscopus inornatus*) and collared flycatcher (*Ficedula albicollis*) (tarsus length) though the correlation can be dependent on environmental conditions (Price 1991; Smith and Wettermark 1995; Meril ä 1997).

Rearing environments including parental care, brood size and predation represented by male traits have also been shown to affect chick growth (Pickett, Weber et al. 2013; Basso and Richner 2015). Blue tit (*Parus caeruleus*) chick growth was positively related to the plumage yellowness of the social male while not being significantly related to any measurements of the genetic parents, which demonstrated that by choosing a brighter yellow mate, the female blue tit appeared to gain direct benefits from better parenting ability (Senar, Figuerola et al. 2002).

There are also cases showing that direct and indirect benefits are not mutually exclusive which means females may obtain both from selected males. Female great tits appear to gain both direct and indirect benefits from choosing bright yellow males (Pickett, Weber et al. 2013). Gular colour of the rearing male brown booby (*Sula leucogaster*) was positively related to parental care and nestling body mass growth, and the gular colour of nest-origin (genetic) male was related to structural growth of offspring (Montoya and Torres 2015). Furthermore, monogamous females often adopt extra-pair mating strategy for genetic benefits from higher quality males beside the direct resources and care from the social mates (Jennions and Petrie 2000), although another idea suggests that females are chosen by males to win mating and sperm competitions (Arnqvist and Kirkpatrick 2005). In many birds, females typically perform EPC with or switch mates for males with more exaggerated ornamental traits than their social or previous mates (Houtman 1992; Wells, Safran et al. 2016). The idea that females select extra-pair mates with superior or complementary genes has been supported by evidence that extra-pair young outperform their maternal half-siblings, e.g. blue tit (*Parus caeruleus*) (Kempenaers, Verheyen et al. 1997) and see review Griffith, Owens et al. 2002.

The barn swallow is monogamous passerine with biparental care (Turner 2006). A *rustica* chick grows to 22-25 g on average and grows at its fastest rate between 2-10 days, reaching peak mass between day 12-15, after which they grow more slowly as skin and feathers start to lose water (the detailed growth process of *rustica* has been described by Fernaz, Schifferli et al. (2012)). A higher mass before fledging is helpful as they can survive one to two days on the fat stored in their bodies while they learn to feed themselves (Turner 2006).

Many studies have shown that female barn swallows have preferences for male ornamental traits such as longer tail streamers and/or redder ventral plumage (Møller and Gregersen 1994; Møller 1994b; Scordato and Safran 2014). Females get indirect benefits from mating with preferred males (Møller 1994; Kojima, Kitamura et al. 2009; Hasegawa, Arai et al. 2012b): for instance, long-tailed males sire healthier offspring (Møller and Gregersen 1994) and female *rustica* that mated with shorter-tailed males have been found to increase the lutein concentration of their eggs which might be because offspring of short-tailed males were more exposed to parasites once they had hatched (Saino, Bertacche et al. 2002). Females do not seem to receive obvious direct benefits by mating with more attractive males especially in terms of parenting because such males tend to provision less than their mate: studies in *rustica* show that sexually preferred longer-tailed male *rustica* and male *gutturalis* with more colourful throat patches invested less in provisioning, and there is no evidence showing that more successful *erythrogaster* males fed more in North America, but females mating with more preferred males tended to feed offspring more frequently (Møller and Gregersen 1994; Kojima, Kitamura et al. 2009; Maguire and Safran 2010).

Cross-fostering experiments have been done on barn swallow *rustica* for various study purposes for instance, parent-offspring recognition by cross-fostering with other swallows (Beecher, Medvin et al. 1986), heritability of tarsus, tail and wing size and fluctuating asymmetry (Cadée 2000), heritability of variation in glucocorticoid concentrations (Jenkins, Vitousek et al. 2014), relatedness and begging behaviour (Boncoraglio and Saino 2008) and post-fledging care and survival (Martin and Beat 2010). There was only one cross-fostering experiment for sexual selection study in subspecies *rustica* that tested if the longer-tailed males sire offspring with better immune system (Møller 1990a). In the study, cross-fostering treatment was combined with mite (*Macronyssidae Gamasida*) loads manipulation to show that apart from the mite loads, longer tails of original father instead of the rearing environment correlated to the advantages in offspring that they had better immune system and achieved larger body size with longer tarsi and higher maximum body mass before fledging (Møller 1990a). This experiment contributed to the evidence of indirect benefit from mate choice of *rustica*, but no further cross-fostering experiment has distinguished genetic and environment effects on offspring growth in other barn swallow subspecies. I have conducted the first cross-fostering experiment on subspecies *gutturalis* examining the effects of genetic factors and rearing environment including parental care on chick growth which should provide more evidence about sexual selection preference and reproductive strategy of this subspecies.

Objectives

In the last two chapters, I have shown that in the Qingdao population of *gutturalis*, longer-tailed males were sexually preferred and they had higher genetic reproductive success and had mates that invested more in parental care. In this chapter, I further explored that if sexually preferred trait advertise higher genetic quality and/or better parental care by examining offspring growth both under natural conditions and in a cross-fostering experiment. The main objectives of this chapter are as follows:

1) Within the original nest, analyse how parent traits are related to chick growth to explore if sexually selected traits advertise heritable good quality for offspring that shows advantage in growth performance.

2) Within the original nest, also test if extra-pair offspring had advantages in growth compared to other within-pair siblings to determine the genetic benefits that females receive from extra-pair mating.

3) Examine the effects of genes and rearing environment on offspring growth to test the benefits of mate choice for female barn swallow *gutturalis* in a cross-fostering experiment.

4.2 Methods

Data collection

The capture of the adults and their measurements, and the recording of the date when chicks hatched were conducted as described in Chapter 2. When the third egg was laid, the eggs were put on graph paper and photographed (using a Canon EOS 600D, Tokyo) with a label with details of the site and date (**Figure 4.1**). The length and breadth were manually estimated to 0.1 mm from the resulting images, and the mean values of length and breadth were used to calculate the mean egg volume using the formula:

$$\text{Volume} = 0.51 \times \text{Length} \times (\text{Breadth})^2 \text{ (Hoyt 1979).}$$

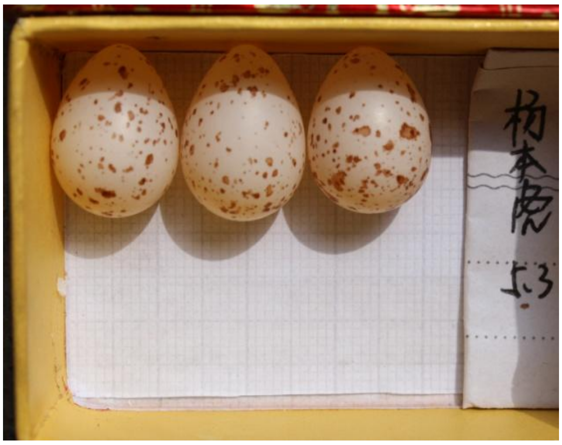

Figure 4.1 A photo taken for the first three eggs of a clutch with graph paper to determine the mean breadth and length of eggs for the *gutturalis* population in Qingdao China.

In 2013 and 2014 nestlings were weighed to 0.25 g accuracy using a spring scale (Pesola, Switzerland) within a fan-shaped plastic bag on day 15 after hatching in 2013 and on each day between day 7-16 in 2014. In 2015 they were weighed on a digital scale (Kubei, Zhejiang, China) to 0.01 g accuracy on each day between 7-16 days old. In 2015, from nests hatched on the same day, on day two after hatching, part of the brood (two chicks out of broods of 4-6 chicks, or one chick out of broods of three) was swapped among two or three nests. They were marked by loosely tying colourful thread to their legs. 41 chicks from 22 nests (18 nests from the first brood, four nests from the second brood) were swapped from a total of 104 offspring.

Feeding behaviour observation methods were conducted as described in Chapter 3, generally feeding frequency is the number of visits of each parent in one hour on day 10 and 15 after hatching. The frequency data used in this chapter was the sum of the feeding rate of both parents over the two days. Extra-pair young were determined by paternity test, methods are described in Chapter 3.

Chick growth analysis within nests

Growth curves were created using Excel (Microsoft 2007), other data processing and figures were conducted using R (version i386 3.2.2) program (R Core Team 2015). Linear mixed effects models were built using the methods described in Chapter 2 and Chapter 3. In 2013 only day 15 body mass of offspring was available, so it was used as peak body mass; in 2014 and 2015, body mass on day 14 was identified as the peak body mass. Total peak body mass (g) was sum of offspring peak body mass in nest. Mean peak body mass (g) or mean day seven body mass (g) was mean value of chick peak body mass or day seven body mass for all the offspring in nest. Growth rate (g/d) was defined as the mean increase per day between day 7-14 per chick. Year was used as a random effect, parent traits as independent variables, data from all three years were used to predict total peak body mass for all offspring in each nest for 61 broods in mixed model. Body mass data from 2014 and 2015 (37 broods) was used as the dependent variable in mixed models to predict mean day seven body mass, mean peak body mass, and growth rate between day 7-14 with fixed effects including five traits of both parents used in previous chapters, adding brood size, mean egg volume (mm^3) and feeding rate (visits/h).

Mean values of growth rate and peak body mass for extra-pair young (EPY) and genetic offspring in nest were compared using paired *t* test for eight nests.

Cross-fostering experiment analysis

To disentangle the rearing environment and genetic effects on chick growth, two sets of models were run for data of 2015 using the lmer function in library lme4 and lmerTest in R (R Core Team 2015) testing their correlations with chick growth rate and body mass on day seven and day 14 for each individual in the nests with cross-fostered chicks. First I ran a model with only random effects which were three nest groups: nest-of-origin, rearing nest in which the chick was raised, and the group that contained the nests between which chicks were swapped (hatched on the same day). *P* values for the random effects were calculated using exactRLRT from RLRsim package. Second, I added 23 fixed effects onto the random model to form mixed models: five phenotypic measurements of the rearing parents and the original parents (20 effects in total), rearing nest brood size, rearing parents feeding rate and egg size to predict each dependent variable which were growth rate, day seven body mass and peak body mass. Statistic results were described by summary command and the non-significant fixed factors with highest *p* value were deleted one by one to leave the simplest model with significant factors. In the figures showing factors and correlations to the dependent variables in the final models, the rectangles on the left in figures with two columns of variables (e.g. **Figure 4.3**), or on both sides in figures with three columns of variables (e.g. **Figure 4.5**) show all the independent variables used in the linear mixed models, year was the random factor in the oval shape and dependent variables were in the square shape where the arrows point to. Factors with arrows were fixed effects in the final model: solid arrows show positive relationship and dash arrows mean negative ones. The weight of coefficient of predictive factors was represented by the relative width of arrows which was calculated using the factor’s coefficient to be divided by the sum of coefficients of all factors in the final model. Significant factors were marked with stars. The fixed effects without arrows were excluded from the model, and all of their statistics are shown in separate result tables.

4.3 Results

4.3.1 Chick growth

In the first brood, nestlings averagely reached a peak body mass of 17.89 ±1.37 g in 2014 and 17.76 ±1.44 g in 2015 on day 14 after hatching. The peak body mass for the second brood was on average 16.05 ±2.04 g and 15.97 ±1.80 g for 2014 and 2015, respectively. On day 15 and 16, body mass decreased by less than 0.5 g a day from the peak body mass on day 14 (**Figure 4.2** and **Table 4.1**). Nestlings fledged between days 19 and 22.

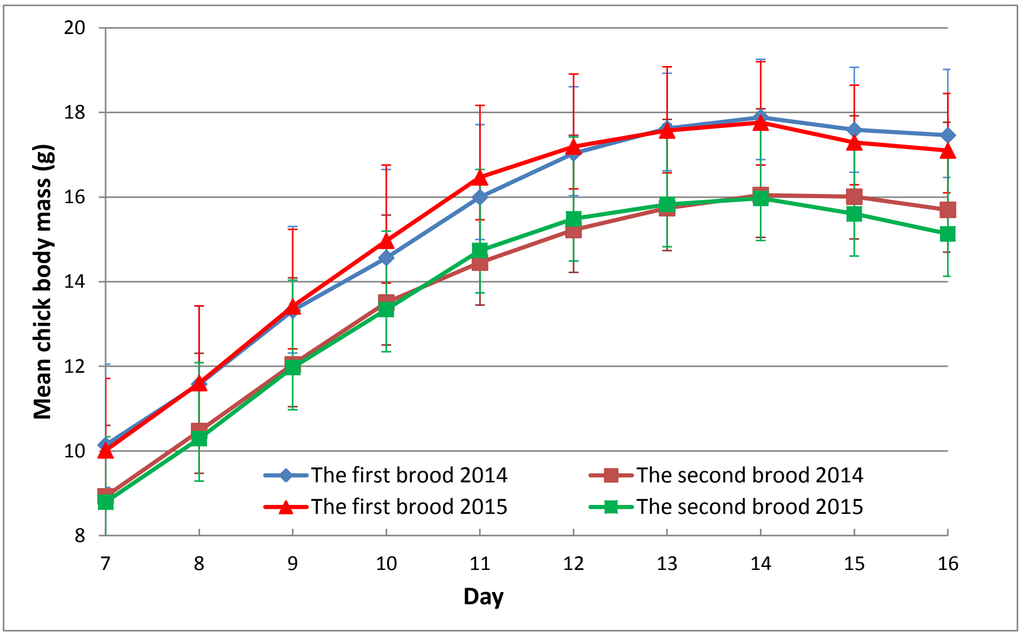

Figure 4.2 Chick growth curves of a barn swallow *gutturalis* population for both the first and the second broods in 2014 and 2015 in Qingdao, China ($N_{1,2014}$=123, $N_{2,2014}$=48, $N_{1,2015}$=153, $N_{2,2015}$=46).

On average *gutturalis* nestlings grew approximately 1 g/d (the first brood 1.11 ±0. 57 g/d, the second brood 1.02 ±0. 52 g/d). Growth curves in the first and the second broods were similar in shape, but the former was on average 1.57 ±0.28 g higher than the latter (**Figure 4.2** & **Table 4.1**).

Table 4.1 Mean body mass (g) of chicks and difference between broods during day 7-16 after hatching in 2014 and 2015 in a barn swallow *gutturalis* population in Qingdao, China.

Year	Brood	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
2014	First	10.14	11.58	13.32	14.56	16.00	17.03	17.62	17.89	17.59	17.46
	SD	1.91	1.84	1.99	2.09	1.72	1.57	1.30	1.37	1.48	1.56
	Second	8.93	10.47	12.04	13.51	14.45	15.22	15.74	16.05	16.01	15.70
	SD	1.68	1.84	2.05	2.07	1.98	2.24	2.10	2.04	1.91	2.07
	Difference	1.21	1.11	1.27	1.06	1.55	1.81	1.88	1.84	1.58	1.76
2015	First	10.01	11.60	13.42	14.97	16.47	17.19	17.57	17.76	17.29	17.10
	SD	1.70	1.83	1.82	1.79	1.70	1.72	1.50	1.44	1.35	1.34
	Second	8.79	10.29	11.97	13.34	14.73	15.49	15.83	15.97	15.60	15.13
	SD	1.54	1.80	2.06	1.85	1.92	1.93	1.90	1.80	1.72	1.97
	Difference	1.22	1.31	1.44	1.62	1.73	1.70	1.74	1.79	1.69	1.97

4.3.2 Chick growth and ornamental traits of parents

Data for three years (2013-2015)

The linear mixed effects model shows that total peak body mass of all chicks in a nest was positively related to male tail streamer length and negatively to male wing length for the first brood (**Table 4.2, Figure 4.3 & 4.4**).

Table 4.2 Statistics results of linear mixed effects model predicting total peak body mass of all offspring in the first brood using male and female traits. In the population of barn swallow *gutturalis* in China, male tail streamer length had a positive relation and male wing length showed a negative relation to the total peak body mass of offspring in the first brood. Significant *p* values are in bold. Year is the random effect. The excluded fixed effects are also shown.

Fixed effects in model	*df*	*t*	*p*
Male tail streamer	58	2.360	**0.022**
Male wing	58	-2.909	**0.005**
Random effect in model	Groups	Variance	SD
Year	3	0	0
Effects excluded	*df*	*t*	*p*
Male tarsus	50	-0.219	0.827
Female tarsus	51	0.224	0.823
Male short tail	52	0.241	0.811
Male body mass	53	-0.482	0.632
Female tail streamer	54	0.746	0.459
Female body mass	55	1.540	0.129
Female wing	56	-1.421	0.161
Female short tail	57	1.975	0.053

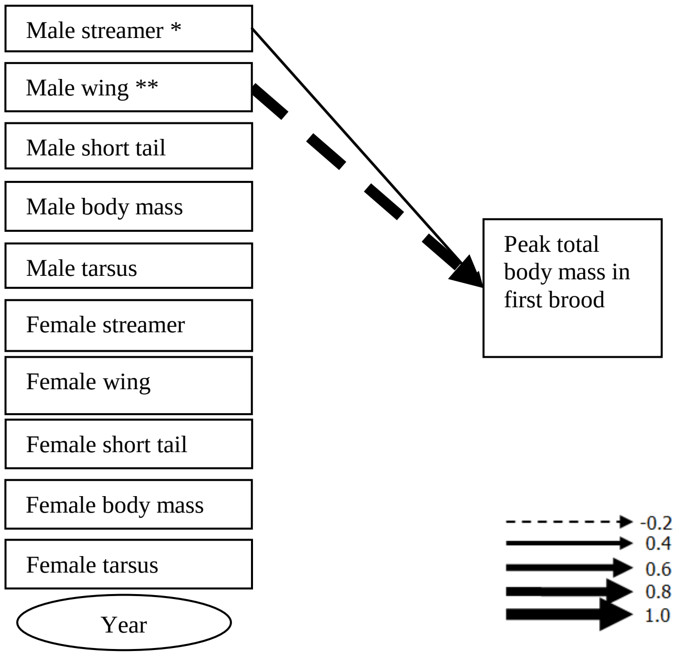

Figure 4.3 Total peak body mass of chicks in the first brood was related to male tail streamer and wing length in the first brood in a barn swallow *gutturalis* population in Qingdao, China (statistics results in **Table 4.2** and plots in **Figure 4.4**).

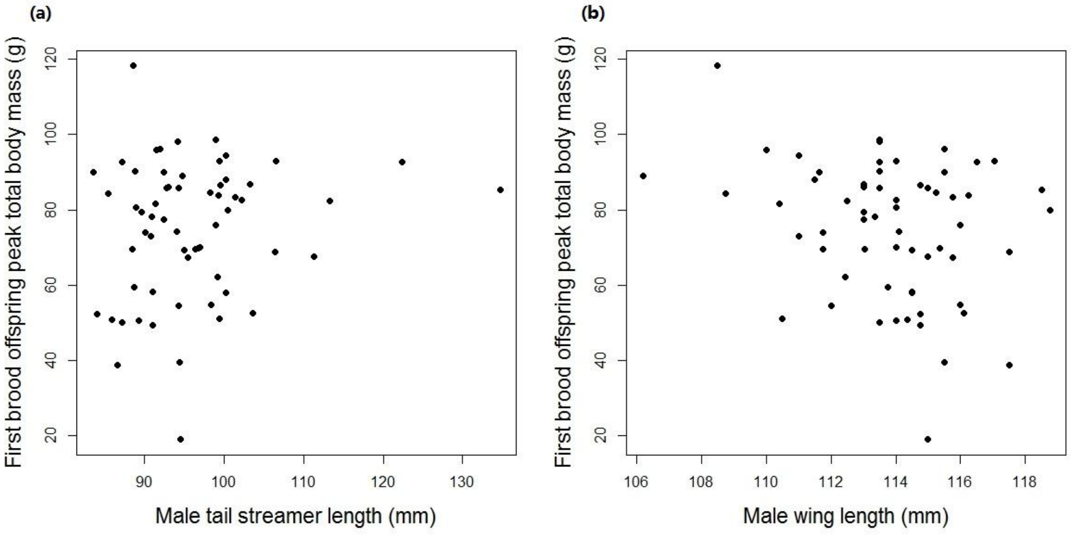

Figure 4.4 Plots of the first brood total peak body mass of chicks in nest against male tail streamer (a) and male wing length (b) in a barn swallow *gutturalis* population in Qingdao, China.

Data from 2014 and 2015

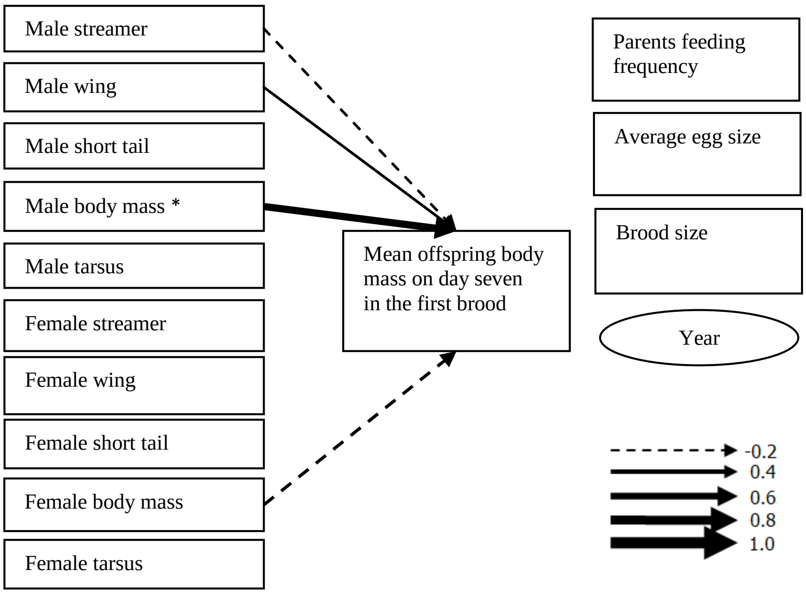

Figure 4.5 Day seven mean body mass of each offspring was positively related to male body mass in the first brood of a barn swallow *gutturalis* population in China (statistics result in **Table 4.3** and plots in **Figure 4.6).**

Table 4.3 Statistics results of linear mixed effects model predicting mean body mass of offspring in the first brood on day seven after hatching in a barn swallow *gutturalis* population in Qingdao, China. Male body mass showed a positive correlation with the mean body mass of offspring in nest with non-significant traits male tail streamer length, male wing length and female body mass in the model. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Fixed effects in model	*df*	*t*	*p*
Male tail streamer	32	-1.979	0.056
Male body mass	32	2.217	**0.034**
Male wing	32	1.874	0.070
Female body mass	32	-1.582	0.124
Random effect	Groups	Variance	SD
Year	2	0	0
Effects excluded	*df*	*t*	*p*
Male tarsus	23	-0.007	0.995
Male short tail	24	-0.019	0.985
Female short tail	25	-0.033	0.973
Brood size	26	0.286	0.777
Parents feeding rate	27	-0.518	0.609
Female tail streamer	28	-0.540	0.594
Female wing	29	-1.034	0.309
Female tarsus	30	1.347	0.188
Egg size	31	1.720	0.095

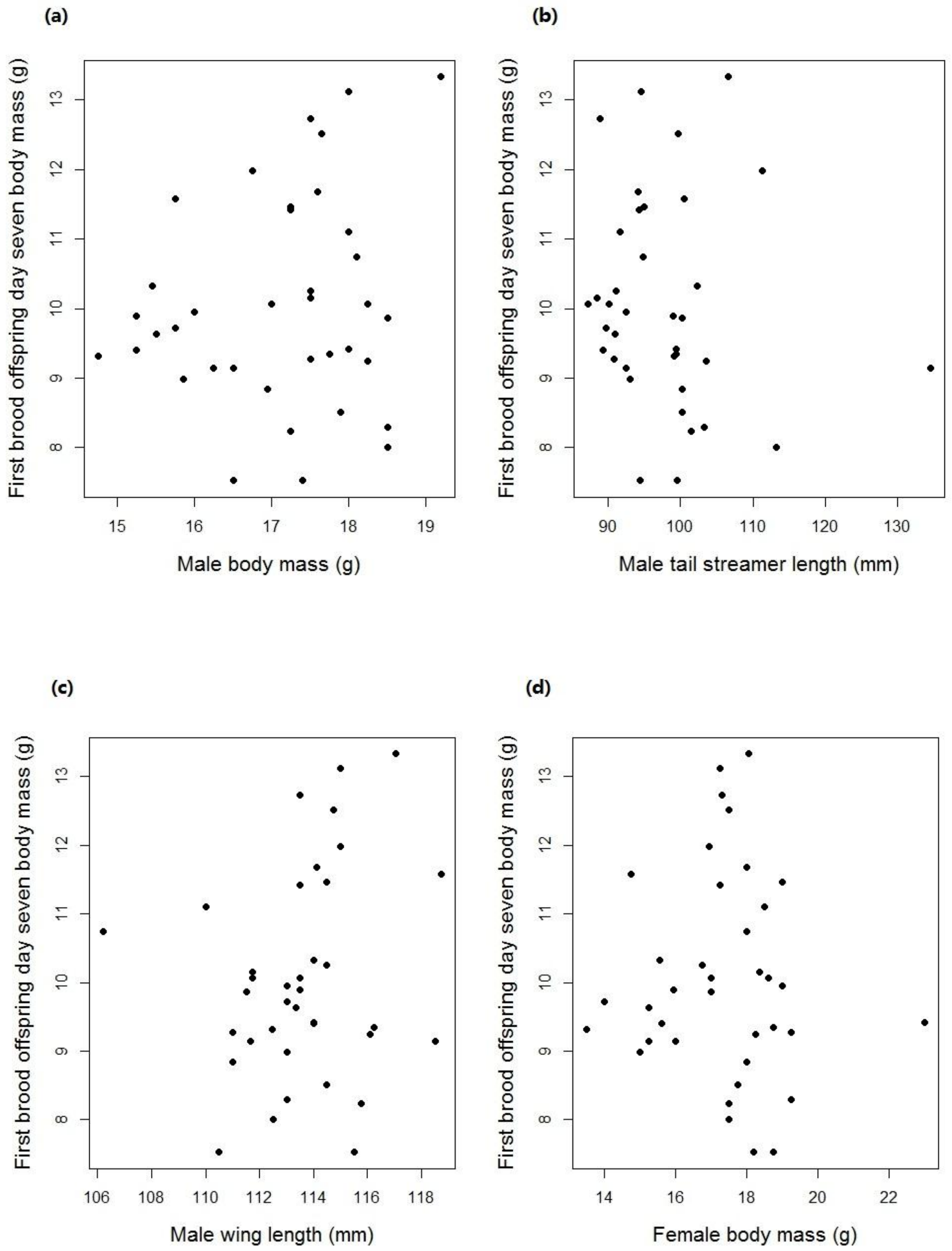

Figure 4.6 Plots of day seven mean body mass of each offspring in the first brood against male body mass (a), tail streamer (b), and wing length (c) and female body mass (d) of a barn swallow *gutturalis* population in China.

Parent body mass was important in offspring growth performance. Male body mass was positively related to chick body mass on day seven **(Figure 4.5 & 4.6** and **Table 4.3)**, and larger female body mass predicted higher mean peak body mass of offspring in nest (**Figure 4.7 & 4.8** and **Table 4.4**).

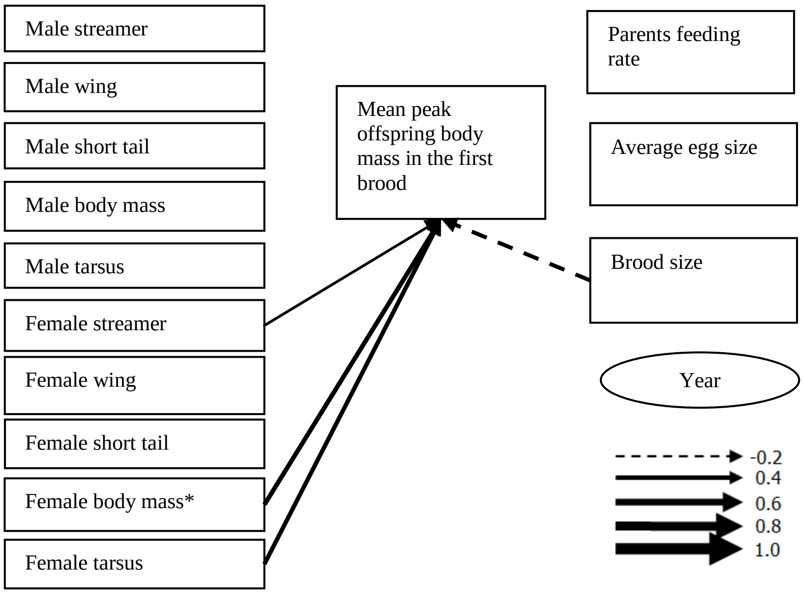

Figure 4.7 Female body mass was positively related to mean peak body mass of each offspring in the first brood of a barn swallow *gutturalis* population in China (statistics result in **Table 4.4** and plots in **Figure 4.8).**

Table 4.4 Statistics results of linear mixed effects model predicting mean peak body mass of offspring in the first brood in a barn swallow *gutturalis* population in Qingdao, China. Female body mass predicted the mean peak body mass of the offspring on average with non-significant traits female tail streamer length, female tarsus length and brood size in the model. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Fixed effects in model	*df*	*t*	*p*
Female tail streamer	31.2	1.387	0.175
Female tarsus	31.67	1.535	0.135
Female body mass	5.84	2.755	**0.034**
Brood size	31.44	-1.787	0.084
Random effect in model	Groups	Variance	SD
Year	2	0.1838	0.4287
Traits excluded	*df*	*t*	*p*
Male tarsus	19.98	0.026	0.980
Male short tail	23.71	-0.201	0.843
Male tail streamer	24.23	-0.343	0.734
Male body mass	25.47	0.323	0.749
Female short tail	26.12	0.675	0.505
Female wing	27.33	-0.494	0.625
Egg size	23.95	0.776	0.446
Parents feeding rate	29.00	1.049	0.303
Male wing	30.36	-1.47	0.152

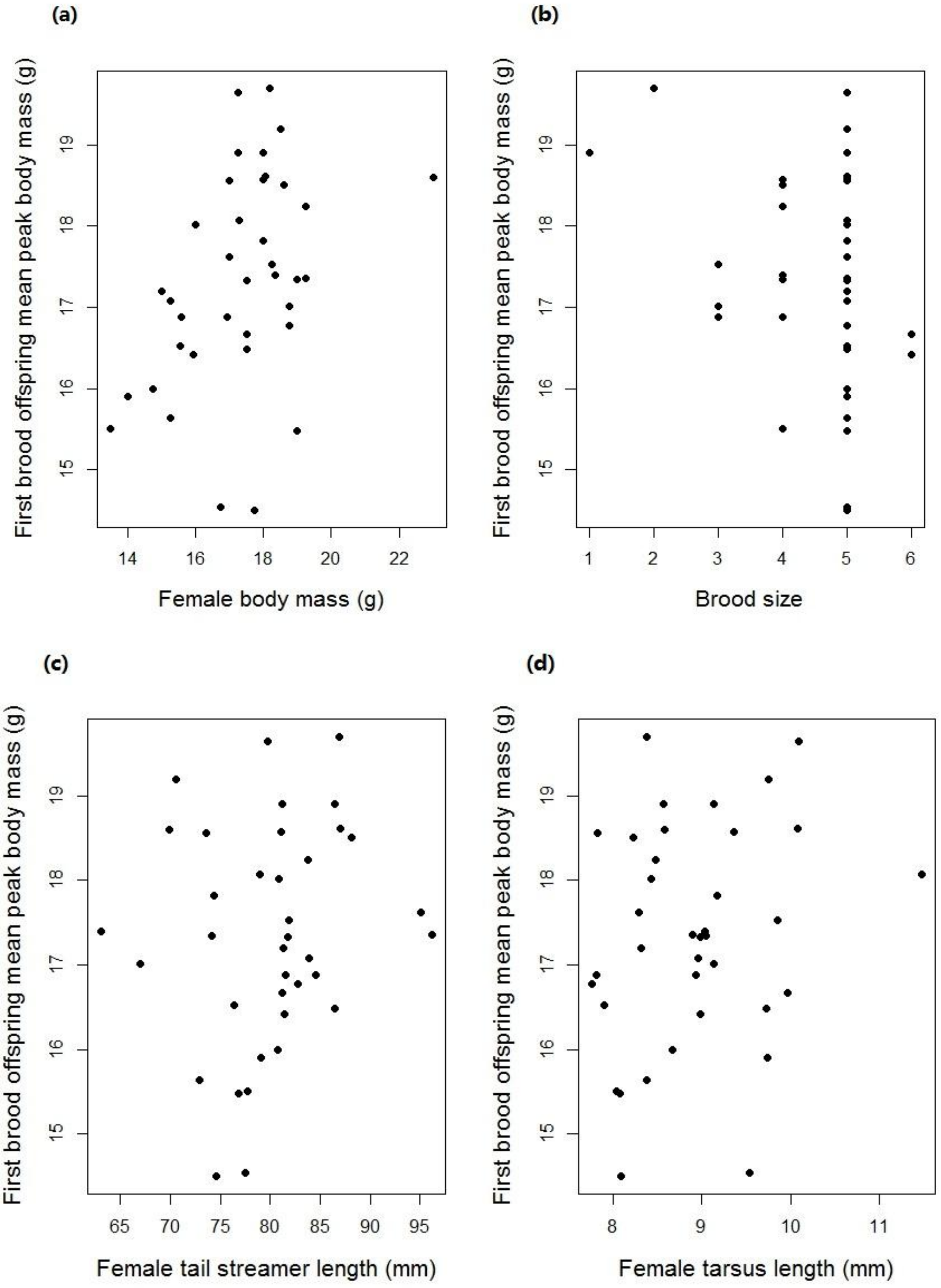

Figure 4.8 Plots of parent traits related to peak body mass of offspring in the first brood of a population of barn swallow *gutturalis* in Qingdao China: female body mass (a), brood size (b), female tail streamer (c) and tarsus lengths (d).

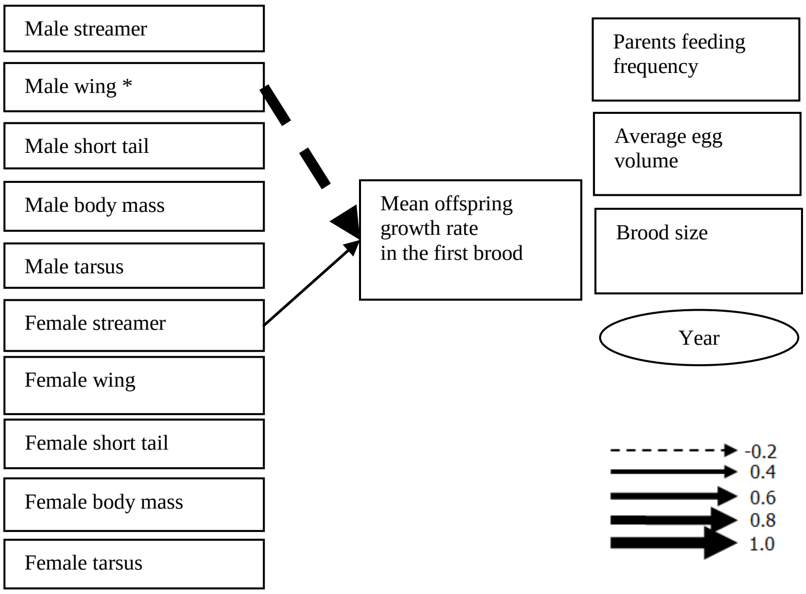

Figure 4.9 The mean growth rate of chicks was negatively related to male wing length in the first brood of barn swallow *gutturalis* in China (statistics result in **Table 4.5** and plots in **Figure 4.10).**

Shorter male wing length interacting with longer female tail streamer length had a positive relationship with mean growth rate of all chicks between day 7-14 in the first brood (**Figure 4.9 & 4.10, Table 4.5**).

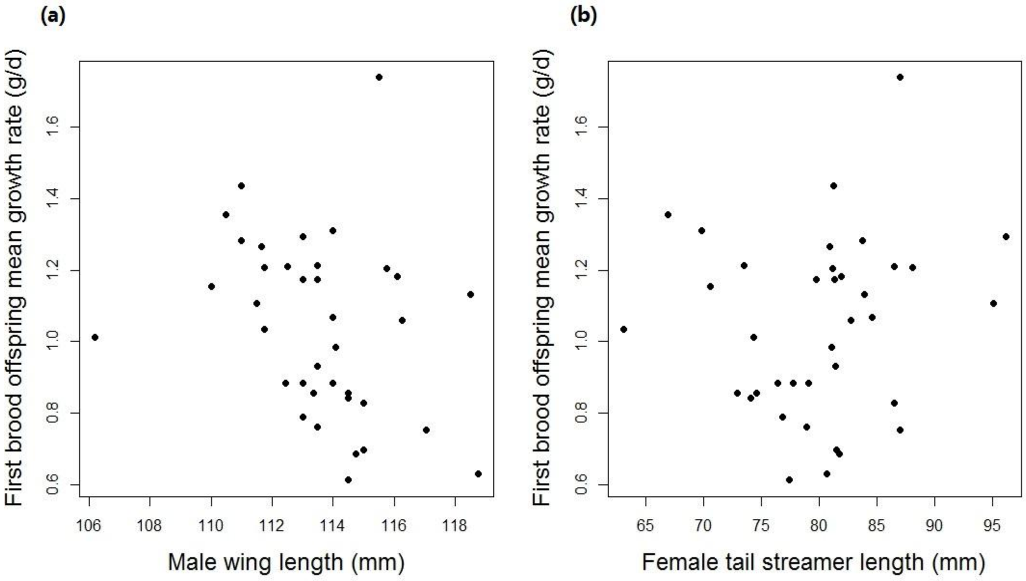

Figure 4.10 Plots of male wing (a) and female tail streamer lengths (b) versus mean growth rate of chicks in nest in the first brood of barn swallow *gutturalis* in Qingdao China.

Table 4.5 Statistics results of linear mixed effects model predicting mean growth rate of offspring in the first brood in a barn swallow *gutturalis* population in Qingdao China. Male wing was significantly related to mean growth rate of offspring on average, with non-significant female tail streamer length in the model. Year is the random effect. The excluded fixed effects are also shown. Significant *p* values are in bold.

Fixed effects in model	*df*	*t*	*p*
Male wing	34	-2.058	**0.047**
Female tail streamer	34	1.313	0.198
Random effect in model	Groups	Variance	SD
Year	2	<0.001	<0.001
Traits excluded	*df*	*t*	*p*
Female wing	23	-0.031	0.976
Male short tail	24	-0.048	0.962
Male tarsus	25	-0.224	0.824
Female tarsus	26	-0.508	0.616
Female short tail	27	0.443	0.662
Egg size	28	-0.791	0.436
Total feeding rate	29	0.952	0.349
Brood size	30	-1.243	0.223
Male tail streamer	31	1.044	0.304
Male body mass	32	-1.628	0.113
Female body mass	33	2.157	0.177

4.3.3 Relatedness and growth

Extra-pair offspring growth

Both for the mean chick growth rate and peak body mass, there was no significant difference between extra-pair young (EPY) and within-pair young (WPY) siblings in the same nest (**Table 4.6**).

Table 4.6 The paired *t* test results for growth between EPY and WPY in the same nest for the barn swallow *gutturalis* population in Qingdao, China. No significant difference was found.

	WPY	EPY	*t*	*df*	*p*
Growth rate (g/d)	1.04	1.09	0.796	8	0.45
Peak body mass (g)	16.73	17.21	0.833	8	0.43

Cross-fostering chicks growth analysis

Random effects models (rearing environment group analysis) show that the original nest, rearing nest and the fostering groups all had significant impacts on chick growth suggesting that genes, rearing environment and hatching time have all played roles in determining offspring body mass. These three factors were all related to body mass on day seven after hatching. The cross-fostering group (chicks hatched on the same day) had a significant effect on peak body mass. Growth rate was significantly influenced by nest-of-origin effect (**Table 4.7**).

Table 4.7 Result table of random effects model testing of cross-fostering effects on chick growth in a population of barn swallow *gutturalis* in China. Significant *p* values are in bold.

Dependent variable	Source	Groups	Variance	*P*
Day seven body mass	Nest of origin	21	0.542	**0.016**
	Rearing nest	21	0.360	**0.046**
	Cross-foster group	10	0.540	**0.005**
	Residual		2.111	
Peak body mass	Nest of origin	21	<0.001	1
	Rearing nest	21	0.054	0.314
	Cross-foster group	10	0.217	**0.026**
	Residual		1.651	
Growth rate	Nest of origin	21	0.011	**0.017**
	Rearing nest	21	0.002	0.319
	Cross-foster group	10	0.001	0.298
	Residual		0.050	

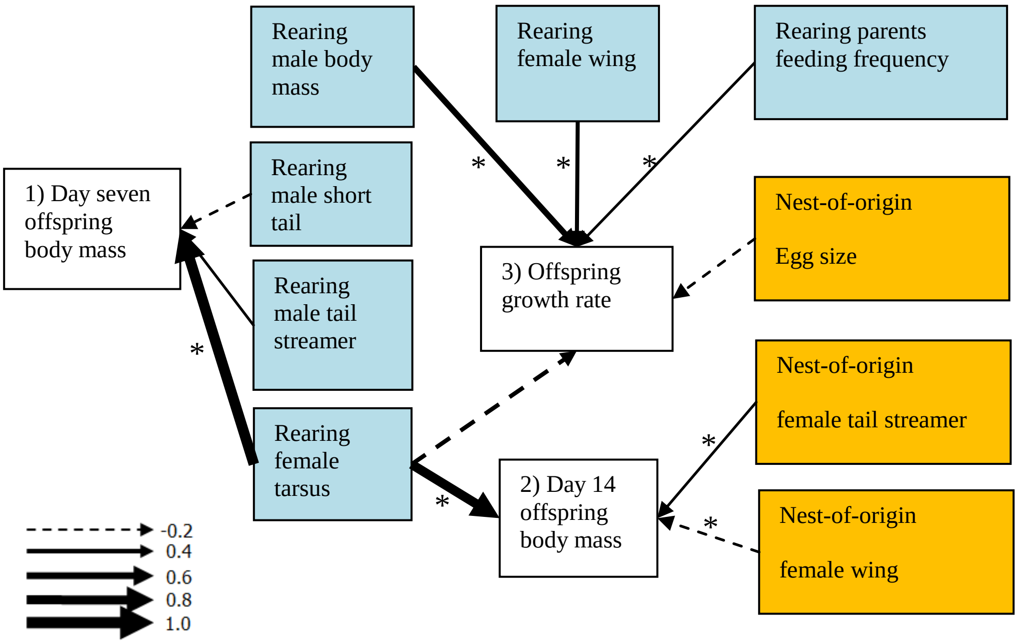

Figure 4.11 Summary figure from a cross-fostering experiment on barn swallow *gutturalis* in Qingdao China. The figure is showing only the fixed independent variables that remained in the simplest LMER model from both nest-of-origin and rearing nests to predict: 1) Body mass on day seven, 2) Peak body mass on day 14, 3) Growth rate. Nest-of-origin effects are in orange, rearing environment effects are in blue. Year was the random effect. The arrow width shows their coefficient weight in the final model, solid lines indicate positive correlations and the dash lines indicate negative correlations, with stars showing their significance (statistics result in **Table 4.8, 4.9** and **4.10**).

The LMER models further show that both original nest and the rearing environment influenced chick growth (**Figure 4.11** and **Table 4.8-4.10**). 1) On day seven after hatching, body mass of chicks was positively related to rearing female tarsus length with rearing male tail streamer and short tail in the model but lacking significance. 2) Both biological and rearing parent traits were related to the peak body mass of offspring in the first brood. Genetic female tail streamer and wing lengths showed a positive effect and a negative effect on chick peak body mass, respectively, and the rearing female tarsus length was also positively related to it. 3) Rearing parents feeding rate, rearing male body mass and rearing female wing length were all positively related to the offspring average growth rate between day 7-14. The egg size from original nest and rearing female tarsus length were also in the model with negative correlation but not significant.

Table 4.8 Result table of linear mixed effects model predicting chick body mass (g) on day seven after hatching in a cross-fostering experiment of barn swallow *gutturalis* in China. Rearing female tarsus predicted nestling body mass when they were seven days old. The rearing male tail streamer length and rearing male short tail length were both in the model but not significant. Significant *p* values are in bold. Random effects and the excluded fixed effects are also shown.

Fixed effects in model	***df***	***t***	***p***
Rearing male tail streamer	9.55	1.335	0.213
Rearing male short tail	12.54	-1.608	0.133
Rearing female tarsus	13.24	2.489	**0.027**
Random effects in model	**Groups**	**Variance**	**SD**
Nest of origin	21	0.512	0.716
Rearing nest	21	0.152	0.389
Cross-foster group	10	0.583	0.764
Effects excluded	***df***	***t***	***p***
Original male body mass	27.43	0.046	0.963
Original male wing	12.66	-0.173	0.866
Original female tarsus	8.81	-0.557	0.591
Egg size	9.48	0.444	0.667
Original male tarsus	9.64	0.528	0.609
Rearing female wing	71.01	0.484	0.630
Original female body mass	20.88	-0.503	0.620
Original male short tail	12.69	0.780	0.450
Original female wing	10.17	-1.015	0.334
Original male tail streamer	13.3	-0.942	0.363
Original female short tail	22.96	-0.885	0.385
Rearing male wing	46.05	0.813	0.420
Rearing female short tail	47.13	0.662	0.511
Rearing female body mass	15.68	1.117	0.281
Original female tail streamer	21.11	1.502	0.148
Rearing brood size	12.32	1.563	0.144
Rearing male tarsus	89.09	-1.412	0.162
Rearing parents feeding	73.67	-1.45	0.151
Rearing female tail streamer	40.11	-1.843	0.073
Rearing male body mass	10.93	-1.380	0.195

Table 4.9 Result table of linear mixed effects model predicting chick peak body mass (g) in a cross-fostering experiment of a barn swallow *gutturalis* population in China. Original female tail streamer length and rearing female tarsus length had positive relations, and original female wing length had a negative relation to the peak body mass of offspring. Significant *p* values are in bold. Random effects and the excluded fixed effects are also shown.

Fixed effects in model	*df*	*t*	*p*
Original female tail streamer	62.91	2.191	**0.032**
Original female wing	98.19	-2.070	**0.041**
Rearing female tarsus	95.94	2.368	**0.020**
Random effects in model	Groups	Variance	SD
Nest of origin	21	0	0
Rearing nest	21	0	0
Cross-foster group	10	0.342	0.585
Effects excluded	*df*	*t*	*p*
Egg size	9.98	0.115	0.910
Rearing female body mass	80.24	-0.17	0.866
Original female body mass	4.85	0.261	0.805
Rearing male body mass	82.65	0.578	0.564
Original male wing	78.3	-0.278	0.782
Original male body mass	53.91	-0.404	0.688
Rearing parents feeding rate	28.29	-0.531	0.600
Rearing female short tail	86.13	0.523	0.602
Rearing male tarsus	87.66	-0.753	0.453
Original female short tail	18.95	-0.788	0.441
Rearing female wing	5.79	0.052	0.960
Rearing brood size	16.77	0.443	0.663
Original male tarsus	91.61	0.556	0.580
Rearing female tail streamer	85.19	0.442	0.660
Original female short tail	53.19	-0.502	0.618
Original male short tail	82.01	0.625	0.534
Rearing male wing	77.37	0.883	0.380
Original female tarsus	96.98	-1.863	0.065
Original male tail streamer	78.68	-1.581	0.118
Rearing male tail streamer	88.74	1.447	0.151

Table 4.10 Result table of linear mixed effects model predicting chick growth rate (g/d) in a cross-fostering experiment in a population of barn swallow *gutturalis* in China. Rearing parents feeding rate, rearing male body mass and rearing female wing length all showed positive relations with the offspring growth rate, with egg size and rearing female tarsus length in the model but not significant. Significant *p* values are in bold. Random effects and the excluded fixed effects are also shown.

Fixed effects in model	*df*	*t*	*p*
Rearing parents feeding rate	17.18	2.185	**0.043**
Egg size	16.5	-1.373	0.188
Rearing male body mass	30.14	2.013	**0.053**
Rearing female tarsus	83.25	-1.084	0.282
Rearing female wing	21.27	2.621	**0.016**
Random effects	Groups	Variance	SD
Nest of origin	21	0.009	0.097
Rearing nest	21	0	0
Cross-foster group	10	0.0003	0.018
Effects excluded	*df*	*t*	*p*
Original male short tail	17.99	0.276	0.786
Original female wing	9.91	-0.264	0.797
Rearing male wing	65.95	0.199	0.843
Original male tarsus	11.65	0.145	0.887
Original male wing	13.15	-0.263	0.797
Original female short tail	21.51	0.429	0.672
Original male tail streamer	14.75	0.436	0.669
Original female tarsus	13.43	-0.892	0.388
Rearing male tarsus	85.11	0.929	0.356
Rearing female short tail	57.89	-1.087	0.282
Original female body mass	32.58	0.984	0.332
Original male body mass	18.14	-0.632	0.535
Rearing female body mass	9.64	-0.74	0.477
Rearing male tail streamer	7.35	-0.808	0.444
Rearing female tail streamer	72.74	0.981	0.330
Rearing male short tail	10.94	0.821	0.429
Original female tail streamer	17.88	-1.056	0.305
Rearing brood size	56.79	-0.979	0.332

4.4 Discussion

In the study *gutturalis* population, chicks on average reached a peak body mass of just under 18 g at 14 days old with body mass decreasing after this before fledging between day 19-22. Therefore *gutturalis* has a similar nestling period with the subspecies *rustica* but reaches a lower body mass (*rustica* reaches 22-25 g peak mass (Møller and Gregersen 1994)), which is in line with the fact that *gutturalis* adult ornamental traits and body size are smaller than *rustica* (Scordato and Safran 2014).

Ornamental traits and chick growth in natural nests

1) Male ornamental traits and chick growth performance

Consistent with the results in Chapter 2 and 3 that males with longer tails and larger body mass had reproductive success advantage, an analysis of chick growth in natural nests showed that longer-tailed males had higher total peak body mass of chicks in their nest, and males with larger body mass had offspring with higher body mass on average at seven days old. This strengthens the conclusion that the tail steamer and body size show male attractiveness and good quality in *gutturalis* and thus mating with such males, females obtain indirect benefit from having better performed offspring.

2) Female phenotype predicts parenting success

In terms of females, in contrast to the studies on *rustica* showing that female body mass was not directly related to chicks growth (Thompson 1992), in *gutturalis* population, female body mass was significantly related to the peak body mass of offspring. Female body condition can be important not only for laying eggs and passing the genes for larger body size, but also in determining the outcome of female competition as shown in other bird species. For instance, a recent study in female mallards (*Anas platyrhynchos*) has shown that the body mass and immune system are the factors related to female dominance rather than ornamental trait bill colouration (Ligon and Butler 2016).

Additionally, female tail streamer length was related to the growth rate of the chicks. In Chapter 2, it was associated with number of eggs in the first brood. These results together confirm that tail streamers could be a signal in sexual selection though no significant relationship of the tail length within a pair was demonstrated in this population. Research in *rustica* has demonstrated that tail streamer is a heritable trait and the length of it shows positive correlation within a pair and longer-tailed *rustica* females enjoyed higher reproductive success (Møller 1993a).

Chick growth in the cross-fostering experiment

In the cross-fostering experiment, rearing environment, original and fostering parents all influenced offspring growth performance.

The rearing environment of the same cross-fostering group was similar because they hatched on the same day and the nests were relatively close to each other, therefore it indicates that hatching time of the season and rearing site could influence the peak body mass reached by offspring. This could also be supported by the fact that the mean body mass of offspring was larger in the first brood than in the second brood, hence it is crucial to initiate breeding earlier in the season to guarantee successful fledglings before the weather becomes too harsh. Larger males have shown they started brood earlier which might explain that they had better growing offspring than smaller males. Brood size (and mean egg size) did not impact significantly on individual growth. That might be because there was little variation in brood size, which was almost always 4 or 5. In *rustica*, adding a small number of chicks to a brood did not significantly decrease the peak body mass or survival rate, which indicates that within a range, the parents are able to adjust their input by the total number of chicks (Thompson 1992).

The nest-of-origin factor was related to average growth rate but no further evidence was found that the genetic father's ornamental traits were directly related to offspring growth performance. However, genetic mother tail streamer had a positive relationship with offspring peak body mass which enhanced the indication that tail streamer can advertise good quality.

Rearing father's body mass was positively related to the offspring growth rate. Though not significant itself, the rearing male tail fork interacting with rearing female tarsus length (significant) predicted day seven chick body mass. Based on the results in Chapter 2 and Chapter 3, the offspring growth advantage in males with larger body mass might be because they started breeding earlier to have a better breeding environment and that in longer-tailed males might be because they had mates that invested more in feeding, rather than them being a good father. Rearing female body size (tarsus length) was also positively related to the body mass of chicks both on day seven and 14, rearing female wing length was related to offspring growth rate which together shows that higher quality females are able to pass good genes of larger body size and provide high quality maternal care to achieve higher reproductive success.

General conclusion

From the offspring growth analysis in both natural nests and the partial cross-fostering experiment, larger males and longer-tailed males tended to have offspring that better performed in growth. This strengthens the conclusion from Chapter 2 and

Chapter 3 that male body mass and tail streamers are sexually selected by female *gutturalis* in the Qingdao population, and females obtain indirect benefits from mating with such males to have higher quality offspring. This contrasts with findings from Japanese populations in which the tail white spot size and throat patch are reported to be sexually selected traits (Hasegawa, Arai et al. 2010a).

Females did not tend to obtain obvious direct benefits mating with more attractive males. In Japan, female *gutturalis* were found to feed more when they mated with males having a better body condition (Kojima, Kitamura et al. 2009) and invest more in incubation when they paired with preferred males which have larger tail spots and more colourful and larger throat patch (Hasegawa, Arai et al. 2012b). In other subspecies, there is similarly no support for attractive males being good parents (De Lope and Møller 1993; Maguire and Safran 2010; Hasegawa and Arai 2015), although females might obtain direct benefit from other aspects for example high quality breeding site with better food resources that are less exposed to parasites and predators (Møller and Gregersen 1994; Kojima, Kitamura et al. 2009; Maguire and Safran 2010). Instead, combined with the results from previous chapters that females invested more in offspring provisioning when their mates had longer tails, breeding strategy of female *gutturalis* could be explained by the “differential allocation theory”, the same as that have been reported in subspecies *rustica* and *erythrogaster* (De Lope and Møller 1993; Maguire and Safran 2010) and other birds (Horváthová, Nakagawa et al. 2012).

Females might also obtain indirect benefits by extra-pair mating with more preferred males which should result in better survival of extra-pair offspring, but in this population, extra-pair young did not appear to have a growth advantage. This might be because there was only a small sample of EPY, although research on tree swallows also reported that EPY did not show an advantage compared to the within-pair chicks (Whittingham and Dunn 2001). Other research has also reasoned the better growth of EPY was due to their earlier hatching order than WPY, rather than genetically based, and when the hatching order was controlled, the growth advantage disappeared, e.g. western bluebirds (*Sialia mexicana*) (Ferree, Dickinson et al. 2010) and blue tits (*Cyanistes caeruleus*) (Magrath, Vedder et al. 2009).

Chapter 5 General Discussion

Tail streamer length and ventral plumage colouration are two classical sexual selection ornamental traits found in various subspecies of barn swallow *Hirundo rustica* (Møller 1994a; Scordato and Safran 2014; Safran, Vortman et al. 2016). In Chapter 2, 3 and 4, the results of a sexual selection study were presented on a population of *H. r. gutturalis*, which has pale belly feathers, shorter tail streamers and smaller body size than other subspecies (Scordato and Safran 2014). Studies of Japanese populations of this subspecies have generally excluded the possibility of tail streamer and ventral plumage being sexually selected traits but instead indicated the attractive traits are male throat patch and tail white spot (Hasegawa, Arai et al. 2010a). In this chapter, the main findings will be summarised followed with suggested future work on potential sexual selection traits of this population.

5.1 Dimorphism

In the population of *gutturalis* in Qingdao China, adults show sexual dimorphism in tails as males have longer tail streamers (deeper tail fork depth) with larger white spot on them than that of females. Males have slightly longer wings, similar body mass, tarsus length with females which indicates that male *gutturalis* have similar body size to females but a deeper tail fork with longer tail streamers. Japanese populations have shown similar sex differences as well as another subspecies *rustica* though on average tail streamers of *gutturalis* are approximately 10 mm shorter than *rustica* in each sex (Møller 1994a; Hasegawa, Arai et al. 2010a).

5.2 Phenotypic traits and reproductive success

5.2.1 Plumage colouration

The plumage regions that have been tested for an effect of colouration are throat, breast, belly and vent regions, in which the latter three are areas of the ventral plumage and are mainly pale, and all the four parts do not appear colourful. In our population of *gutturalis*, plumage colourations are unlikely to be sexually dimorphic or sexually selected. This is consistent with the other studies in the subspecies *gutturalis* and confirms that the plumage colouration of *gutturalis* is close to that of *rustica* which has pale ventral plumage and some of them have a chestnut ventral side (4% in a Denmark population), and unlike other four subspecies that have dark red ventral plumage (Møller 1994a; Scordato and Safran 2014). In Japanese populations, consistent with our conclusion, the ventral side plumage colouration has not been considered to be dimorphic or to predict breeding success of male barn swallows but red throat colourations are suggested to be sexually dimorphic (Hasegawa, Arai et al. 2010a).

5.2.2 Ornamental trait size

Wing Though male wings are slightly longer than that of females, longer wings were not related to any advantage in breeding success. Males with shorter wings were less likely to be cuckolded and their mate invested more in feeding, also had a higher total peak body mass of offspring in the first brood. This might be because males with shorter wings are less good parents but the longer tails (which interacts with shorter wings often) resulted in their partner having to feed more frequently.

Wings in *rustica* (males 126.83 mm, females 124.77 mm, in Kraghede study area), were not considered as a significant sexually dimorphic trait and thus no report has been shown on sexual selection study on this trait in this or other subspecies (Møller 1994a; Scordato and Safran 2014).

Tail white spot The size of the tail white spot was dimorphic and it was positively related to the length of tail feather, which is also shown in the Japanese population (Hasegawa, Arai et al. 2010a). The tail white spot length of both males and females did not relate to any breeding advantage, but showed a negative relationship to number of offspring in a season. And because the large white spot is related to the length of the streamers, in parental care and cross-fostering analysis, I did not use the white spot as an independent variable.

However, larger white spots in tails have been found to relate to attractiveness presumably because they could show low parasite loading of the male, and the shape seems important rather than length (Saino, Romano et al. 2015). In the *rustica*, white spot in tails was shown to correlate to earlier initiation and number of nestlings in a season (the ones with part of the white spot covered delayed laying date and less likely to have a second brood); they are preferred by feather-eating Mallophaga, so by showing the white spot without parasite, males show their high quality (Kose and Møller 1999; Kose, Mänd et al. 1999). In Japanese populations of *gutturalis*, white spots in male tails were found to predict earlier initiation date in breeding (Hasegawa, Arai et al. 2010a).

Tails Males with longer tail streamers were less likely to be cuckolded thus had sired more successful genetic fledglings in the first brood and achieved higher annual reproductive success. Also the mates of these males invested a larger portion of feeding and there was a larger total peak body mass of offspring in their first broods. Additionally, males with deeper tail forks had more genetic fledglings in the first brood. These findings suggest that male *gutturalis* tail streamer or potentially tail fork depth could be sexually preferred and this might explain the sexual dimorphism in tail streamers. This is consistent with the studies in subspecies *rustica* which has dimorphism in tail streamers and they are shown to be sexually selected (Møller 1988a). In Japanese populations of *gutturalis*, tail streamer length was not correlated to male reproductive success, but males with longer tails were more likely to return in the next year (Kojima, Kitamura et al. 2009; Hasegawa, Arai et al. 2010a; Hasegawa, Arai et al. 2014b).

Females with longer tail streamers also show better quality in breeding, they laid more eggs, and their offspring reached a higher peak body mass in a cross-fostering experiment. This further supports the importance of male tail streamer in mating though the correlation of tail length in a pair was not found. In a manipulation study in *rustica*, original female tail streamer length was not found to be related to reproductive success but longer-tailed females arrived at the breeding site earlier, and after manipulation, longer female tail streamers were positively related to length of tail streamers of their mates (Cuervo, de Lope et al. 1996).

5.2.3 Body size

Two measurements were used to represent individual body size: body mass and tarsus length (describes the skeletal size of the bird). They were positively correlated within a pair, which means that males with larger body size mated with larger females.

Males with a larger body mass started the first brood earlier, which shows an advantage in attracting females and having more successful offspring because the early ones often survive better. Also male body mass shows a positive effect on offspring growth. These confirm that males with larger body size were more preferred by females. In a Japanese population, it was shown that males with better body condition sired more within pair young and more extra-pair young (Kojima, Kitamura et al. 2009).

Generally, body mass also played an important role in female breeding success showing that females with a larger body mass had first brood offspring that grew faster and their offspring achieved a larger peak body mass on average. Females with shorter tarsus length had a greater chance to have a second brood though not a higher breeding success. This might indicate that the actual fat storage (body condition) is more important than skeletal size for females.

Body mass is important during migratory and so it can indicate the body condition when they arrived at the breeding site, this might have affected the female choice (Pattenden and Boag 1989; Lovvorn, Richman et al. 2003). Body mass is important especially for females during laying eggs and incubation (Blums, Mednis et al. 1997). The result confirms the importance of body mass for both males and females, and the correlation of body mass in a pair might show that barn swallows prefer larger individuals in mating choice for both males and females. However, body mass is rather more variable than other fixed secondary sex traits like tails, it is more often examined as a parameter of body condition, but not indicated as a trait in mate choice (Møller 1993b; Galeotti, Saino et al. 1997).

5.3 Parental care and cross-fostering experiment

Though rearing male body mass indicates better chick growth, it might be because females invested more in feeding when they mated with higher quality males, therefore we suggest that females tend to obtain indirect benefit rather than direct benefit from mating with more attractive males in our population of *gutturalis*.

Female invested more in feeding when they mated with longer-tailed males, which again suggests that long tail streamers are attractive and also supports the “differential allocation theory” (Burley 1988). In Japan, female *gutturalis* were found to invest more in incubation when they paired with preferred males (Hasegawa, Arai et al. 2012b), and feed more when they mated with males having a better body condition (Kojima, Kitamura et al. 2009). And evidence of differential allocation was also found in the other sex of *gutturalis* that males increased their feeding rate when their throat patch area was manually painted to appear smaller (Hasegawa and Arai 2015). Concluding studies on different subspecies, there is no support for attractive males being good parents of barn swallows (De Lope and Møller 1993; Maguire and Safran 2010; Hasegawa and Arai 2015).

The cross-fostering experiment has demonstrated that rearing males with a higher body mass had nestlings that grew faster. This lends more support to the conclusion that male tail length and body size might be sexually selected and further shows that female *gutturalis* invested more in parental care when they mated with more attractive males. Though more attractive males did not tend to feed more, females might obtain direct benefits from mating with these males in other aspects for example a good breeding site with more food resources which help offspring growth (Hasegawa, Arai et al. 2012a).

5.4 General conclusion and future work

In the population of barn swallow *Hirundo rustica gutturalis* in Qingdao China, sexual dimorphism occurs in tail streamer length, also the white spot on them. Various pieces of evidence suggest that male tail streamer (or tail fork depth) is sexually selected and that larger male body size also has an advantage in reproductive success. Males with longer tail streamers (deeper tail fork) were less likely to be cuckolded, achieved higher reproductive success, and had mates that invested more in provisioning and had a higher total peak body mass of offspring in nest. Longer female tail streamers also have shown advantage in breeding with a larger first clutch and both rearing and genetic mother tail streamer lengths have been related to chick growth. And it seems that females obtain indirect benefits rather than direct benefit from mating with more attractive males. Body size has shown positive mutual association in a pair and an advantage in breeding: larger males initiated breeding earlier and their offspring grew faster. Female body condition also played an important role in offspring growth.

Future work could be done on manipulation experiment of male tail streamers length to make further conclusions. More work could also be done on the indirect benefits that female *gutturalis* might obtain from mating with preferred males (including extra-pair males) in respect of immune function in offspring, survival rate and the attractiveness of offspring etc.

Measurements of attractiveness of the throat patch size can also be done in *gutturalis* populations in China as a comparison sexual selection study to Japanese population (Hasegawa, Arai et al. 2010a; Hasegawa and Arai 2015). Moreover, study in *gutturalis* could add evidence on divergent sexually selected traits in association with speciation of barn swallow (Safran, Vortman et al. 2016).

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