1 **Ventral colour, not tail streamer length, is associated with seasonal reproductive performance in**

2 **a Chinese population of barn swallows (*Hirundo rustica gutturalis*)**

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22   **Abstract**

23   Phenotypic differentiation is common among populations that have large geographic distributions. One

24   proposed mechanism driving phenotype divergence is sexual selection, which predicts that trait

25   differences among closely related populations are underlain by variation in reproductive performance.

26   Here, we examined a population of Asian barn swallows (*Hirundo rustica gutturalis*) in northeastern

27   China. We ask whether traits shown to be under divergent sexual selection in other barn swallow

28   populations are under sexual selection in this north Asian population by determining whether two

29   features of phenotype variation, tail streamer length and ventral plumage colouration (including both

30   throat and belly regions), are sexually dimorphic, associated with patterns of assortative mating and

31   predictive of reproductive success. In this population, the length of tail streamers did not correlate with

32   ventral plumage colouration in either males or females. The length of tail streamers was sexually

33   dimorphic, but we did not find assortative mating by tail streamer length. By contrast, we found no

34   sexual dichromatism but we did find assortative mating by throat colouration. Our correlational results

35   indicated that the breeding performance of male barn swallows was associated with differences in their

36   ventral plumage colouration, suggesting that ventral plumage colouration is likely a target of sexual

37   selection in this population. Our finding that tail streamer length is unlikely to be under sexual selection

38   is consistent with studies of other *H. r. gutturalis* populations. The result that ventral plumage colour is

39   likely to be under sexual selection is partially consistent with previous studies on Japanese *H. r.*

40   *gutturalis*, in which male throat but not belly plumage colour is the sexually selected trait.

41

Introduction

Sexual selection and natural selection are important forces driving evolutionary changes within and between populations (Coyne and Orr 2004; Panhuis et al. 2001). Recent debates about the role of sexual selection in speciation have moved away from addressing whether sexual selection could be involved in the buildup of population divergence (e.g., Panhuis et al. 2001; Ritchie 2007) to considering the details of how populations evolve in different directions under sexual selection (e.g., Maan and Seehausen 2011; Safran et al. 2013). Indeed, differentiation of secondary sexual ornaments among closely related populations is common (Bro-Jørgensen 2010; Candolin 2003). However, it is necessary to conduct within-population analyses to test whether these phenotypic differences are underlain by sexual selection (e.g., Safran et al. 2005, 2016; Safran and McGraw 2004; Vortman et al. 2011, 2013) or if they are attributable to other processes, such as natural selection or genetic drift.

The barn swallow *Hirundo rustica* is a small passerine that has become a model system in evolutionary and behavioural studies of sexual selection. Barn swallows have a Holarctic distribution and consist of six subspecies that vary in a suite of morphological traits (Scordato and Safran 2014; Turner 2006). In particular, barn swallow populations differ in body size, colour of ventral plumage (ranging from white to dark red-brown) and the length of the outer rectrices (tail streamers) (Scordato and Safran 2014). For instance, the nominate European subspecies *H. r. rustica* is relatively large in size with long tail streamers and pale ventral plumage, while the North American subspecies *H. r. erythrogaster* is smaller, with short tail streamers and dark ventral colouration. In Europe, the tail streamer is the primary sexually selected trait (reviewed by Møller 1994): male barn swallows with long tail streamers arrive at breeding sites earlier, acquire their mates more quickly, and are more likely to raise more nestlings and have second clutches within a single season than males with short tail

66   streamers (Møller 1990). Additionally, male swallows with either naturally long or experimentally

67   elongated tail streamers were cuckolded less frequently and produced more biological offspring over

68   the breeding season than males with naturally short or experimentally shortened tails (Saino et al.

69   1997). Additionally, recent research using lifetime reproductive success to estimate selection on

70   lifespan and male ornaments also found selection on tail length and tail white spots, but not on ventral

71   plumage colouration in European barn swallows (Costanzo et al. 2017). In North American *H. r.*

72   *erythrogaster*, by contrast, there is assortative mating (defined as "the correlation between male and

73   female phenotypes or genotypes across mated pairs", Jiang et al. 2013) by ventral plumage colouration

74   but not tail streamer length, and the number of fledglings per season is associated with male ventral

75   colour, suggesting that dark plumage colouration is the primary sexually selected trait in this

76   subspecies (Neuman et al. 2007; Safran and McGraw 2004; Safran et al. 2016, but for a contrasting

77   view see Kleven et al. 2006). Male *H. r. erythrogaster* individuals with naturally dark ventral plumage

78   colour were cuckolded less frequently than males with pale plumage (Eikenaar et al. 2011), and this

79   result was replicated experimentally by artificially enhancing ventral plumage colouration (Safran et al.

80   2005, 2016). Finally, in Middle Eastern barn swallows (*H. r. transitiva)*, which possess both long tail

81   streamers and dark ventral colouration, both traits appear to be under sexually selection: darker males

82   with longer tail streamers have greater reproductive success than paler males with shorter streamers

83   (Vortman et al. 2011, 2013).

84   Although European and North American barn swallows have been the subject of the most intense

85   study, there are two additional subspecies that breed in eastern Asia. *H. r. gutturalis* breeds throughout

86   eastern China, Japan and eastern Russia and generally have smaller body size and paler ventral colour

87   compared to other subspecies of barn swallows (Scordato and Safran 2014). Previous observational

88   research on Japanese populations of *H. r. gutturalis* showed that throat colour and the size of white

89   spots on the tail feathers may be sexually selected in this subspecies (Hasegawa et al. 2010b), and

90   males with large throat patches acquired older and heavier females with higher viability as their mates

91   (Hasegawa and Arai 2013), while male tail length was not significantly related to either within- or

92   extrapair paternity (Kojima et al. 2009). A second subspecies, *H. r. tytleri*, breeds in central Siberia, has

93   a body size intermediate between *H. r. rustica* and *H. r. gutturalis*, very dark brown ventral plumage

94   colouration, and tail streamers that are intermediate to *H. r. rustica* and *H. r. gutturalis* (Scordato and

95   Safran 2014). *H. r. tytleri* has not been the subject of any studies of sexual selection. A recent meta-

96   analysis of sexual selection in barn swallows utilized these previous studies to show that different

97   plumage ornaments are targets of equally intense sexual selection among different barn swallow

98   populations (Romano et al. 2017).

99   Barn swallows have an unusual biogeographic history: phylogenetic analysis based on mtDNA

100   and nuclear loci shows that about 100,000 years ago, barn swallows from Asia (*H. r. gutturalis*) crossed

101   the Bering Strait and established the North American populations, giving rise to the *H. r. erythrogaster*

102   subspecies. Approximately 27,000 years ago, North American barn swallows re-crossed the Bering

103   Strait and colonized the Baikal region, giving rise to the *H. r. tytleri* subspecies (Dor et al. 2010; Zink et

104   al. 2006). Currently the distributions of *H. r. tytleri* and *H. r. gutturalis* meet in northern Asia. The

105   *tytleri* and *gutturalis* subspecies are most clearly differentiated by their red-brown vs. white ventral

106   colouration, respectively. However, in northeastern China there are barn swallow populations with

107   intermediate ventral colour and short tail steamers (Figure 1). These populations have at times been

108   designated a separate subspecies, *H. r. mandschurica* (Zheng 1987), but more recently they have been

109   considered to be an intergrading form between the northern Asian subspecies *H. r. tytleri* and the

110   southern Asian subspecies *H. r. gutturalis* (Turner 2006). In this study, we refer to this northern Chinese

111   population as *H. r. gutturalis,* consistent with the current designation. The targets of sexual selection

112   have never been studied in Chinese *H. r. gutturalis,* and the purpose of this study is to determine

113   whether and how sexual selection differs in this population compared to other populations of *H. r.*

114   *gutturalis* as well as other subspecies of barn swallow. We test for patterns of sexual selection on tail

115   streamer length and ventral plumage colour (including both throat and belly regions) by analyzing the

116   degree to which these traits are sexually dimorphic, whether there is evidence of assortative mating by

117   these traits, and whether these traits are significantly associated with various measures of seasonal

118   reproductive success.

119

120   **Methods**

121   *Fieldwork methods*

122   We studied a population of barn swallows in Shuangyashan City, Heilongjiang Province, China (46°

123   35' N, 131° 14' E) from May to September 2013 and June to September 2015. In this population, barn

124   swallows usually start breeding in May, during which time each pair establishes and defends a separate

125   territory. We identified active nests and visited them every two days to record the laying date and the

126   number of eggs in the nest. Nests where we knew the exact laying date were visited daily after 12 days

127   into the incubation period to determine the hatching date. We checked nests with unknown laying dates

128   daily to identify and record the hatching date. As it usually takes more than one day for all nestlings to

129   hatch out, we defined the hatching date (day 0) as the date that the first chick(s) hatched for each brood.

130   We considered the number of nestlings present on day 15 to be the total number of fledglings

131   associated with a particular nest (Vortman et al. 2011). The same monitoring procedures were followed

132   for both first and second broods. In total, there were 84 first broods (2013: n = 38; 2015: n = 46) and 20

133   (2013: n = 5; 2015: n = 15) second broods.

134   Adult swallows were captured using mist nets at night during the first five days after their first-

135   brood nestlings hatched, as experience suggested that there was a high risk of them abandoning their

136   nests if they were caught earlier. In total 165 adult swallows (2013: male: n = 36, female: n = 39; 2015:

137   male: n = 44, female: n = 46) were captured; no individuals included in this study were caught in both

138   2013 and 2015. At the time of capture, usually between 21:00 to 24:00, swallows were marked with a

139   unique combination of coloured plastic rings on each of their legs. Sex was determined by checking the

140   presence (females) or absence (males) of a brood patch. For each bird, the length of both wings was

141   measured to the nearest 1 mm using a wing rule and the length of both streamers were measured to the

142   nearest 0.01 mm with digital calipers (Jazooli, UK) following Evans (1998), and body mass was

143   measured to the nearest 0.01g with a digital balance (ChangXie, CX-168, China). All measurements

144   were taken by the same person (Yu Liu) to avoid differences between observers. Feather samples were

145   taken from throat and belly regions for quantification of colour, and we divided feather tract regions

146   following Svensson et al. (2011). For each region, four to ten feathers were plucked and taped to white

147   paper cards following Safran and McGraw (2004). These samples were stored in the dark for future

148   measurement using a spectrophotometer.

149

150   ***Plumage colour measurement***

151   We measured feather samples with a reflectance spectrometer (USB4000, light source: PX-2, Ocean

152   Optics, US) following (Safran et al. 2010). Each feather patch was measured three times with 20 scans

153   per measurement, and the three measures were then averaged for further analysis. We calculated colour

154   variables according to three dimensions of colour vision: hue, brightness and chroma (HSB colour

155   space). Calculation of colour variation is based on spectra with wavelengths between 300 and 700 nm,

156   which is the range of spectral sensitivity in birds (Cuthill et al. 2000). Hue is represented by the

157   wavelength that shows the maximum slope for a red-brown plumage spectrum; red chroma is the

158   proportion of total reflectance occurring in the red region (600 – 700 nm) as a measure of spectral

159   saturation; and brightness is the mean reflectance over the spectra. Ventral plumage in barn swallows

160   does not reflect in the ultraviolet range (Safran and McGraw 2004) so little colour information is lost

161   by using the HSB colour space.

162

163   ***Statistical analyses***

164   ***PCA on colour traits***

165   PCA was used to reduce the dimensionality of plumage colour traits using the function 'prcomp' from

166   the R package 'stats', and variables were scaled to have unit variance before analysis. PCA was

167   conducted on throat and belly regions separately. The first PC explained more than 60% of the

168   variation in colour for each region, and therefore PC1 of throat and belly colour were used for further

169   analysis (Table 1). To facilitate comparisons between patches, we multiplied throat colour PC1 loadings

170   by -1 before the analyses. Swallows with higher PC1 values have darker (lower brightness values),

171   redder (lower hue values, higher chroma values) throat and belly plumage than swallows with lower

172   PC1 values (Table 1).

173   ***Correlations between morphological traits and among potential sexually selected traits***

174   Pearson correlations were used to test whether morphological traits, wing length and body mass were

175   correlated within individuals in males and females, separately. Similarly, we tested whether potential

176   sexually selected traits – the length of tail streamers and ventral plumage colour – were correlated

177   within individuals.

Degree of sexual dimorphism in morphological and potential sexually selected traits

179   Levene's tests were performed to test the equality of variances in potential sexually selected traits (the

180   length of tail streamers and ventral plumage colour) for the two sexes. Due to different sample sizes of

181   males and females, Welch's two sample *t* tests were performed to test the sexual dimorphism of

182   morphological and potential sexually selected traits. Throughout the Results, positive t values

183   correspond to larger values in females.

Assortative mating by morphological or potential sexually selected traits

185   Partial Pearson correlations were used to explore assortative mating based on morphological and

186   potential sexually selected traits in barn swallow breeding pairs. We included year as the third

187   controlled variable in the function 'pcor.test' from the R package 'ppcor'. All statistical tests were two-

188   tailed and were performed in R (R Core Team 2016).

Correlation between reproductive success and morphological/potential sexually selected traits

190   Reproductive success was assessed for each pair of barn swallows by recording the chick hatching

191   date, the number of eggs, and the number of fledglings produced during the entire breeding season.

192   Hatching date is considered to be an important index of reproduction timing of birds (Tomás 2015).

193   Generalized linear mixed models (GLMM) were used to analyse the relationship between reproductive

194   success and different traits, using the functions 'lmer' or 'glmer' from the R package 'lme4' (Bates et

195   al. 2015). A maximal GLMM model (Table S1) was constructed for each measure of reproductive

196   success, with morphological traits (wing length and body mass of both members of the pair) and

197   potential sexually selected traits (streamer length, throat and belly colour PC1 of both sexes) included

198   as fixed effects, and the year (2013 and 2015) as a random effect. We assumed a normal distribution

199   when modeling hatching date and a Poisson distribution when modeling the number of eggs and the

200   number of fledglings. To deal with data overdispersion, nest ID was also included as a random effect in

201   GLMMs when modeling the number of eggs and the number of fledglings. Models were simplified

202   from the maximal model through stepwise dropping of factors non-significantly correlated with the

203   dependent variable until all factors were significant with $\alpha = 0.05$. Analyses of variance (ANOVA)

204   were performed on the minimal models to calculate the proportion of variance explained by each

205   factor. Maximal GLMM models are shown in the appendices (Table S1). All analyses were conducted

206   with R (R Core Team 2016).

207

208   **Results**

209   *Correlations between morphological traits and among potential sexually selected traits*

210   The wing length and body mass were not significantly correlated in either males or females (Pearson

211   correlation: male: $r = 0.11$, n = 80, $p = 0.33$; female: $r = 0.03$, n = 84, $p = 0.80$). The length of tail

212   streamers was not significantly correlated with colour traits in either males or females (throat PC1 and

213   belly PC1 values; Pearson correlation: male: $r = -0.12 – -0.08$, n = 80, all $p > 0.30$; female: $r = -0.02 –$

214   0.07, n = 83 – 84, all $p > 0.54$). Throat and belly PC1 values were significantly correlated in both males

215   and females (Pearson correlation: male: $r = 0.43$, n = 81, $p < 0.01$; female: $r = 0.58$, n = 83, $p < 0.01$).

216   *Sexual dimorphism in morphological and potential sexually selected traits*

217   In this population, male and female barn swallows were sexually dimorphic in wing length and body

218   mass (Welch two sample *t* test: wing length: $t_{162.69} = -4.34$, $p < 0.01$; body mass: $t_{131.19} = 3.20$, $p < 0.01$),

219   with males exhibiting longer wings (male: mean ± SD = 112.11 ± 2.64 mm, n = 80; female: mean ± SD

220   = 110.31 ± 2.68 mm, n = 85) and lighter body mass (male: mean ± SD = 15.31 ± 0.89 g, n = 80;

221   female: mean ± SD = 15.96 ± 1.63 g, n = 85) than females. Male tail streamer length was significantly

222   longer than that of females (male: mean ± SD = 100.99 ± 7.72 mm, n = 80; female: mean ± SD = 84.25

223   ± 5.22 mm, n = 85; Welch two sample t test: $t_{137.74}$ = -16.23, $p < 0.01$) and was more variable (Levene's

224   test: F_1 = 16.31, $p < 0.01$). Neither throat nor belly colour PC1 values were significantly different

225   between the two sexes (Welch two sample t test: throat PC1: $t_{163.9}$ = -1.30, $p = 0.19$; belly PC1: $t_{162.86}$ =

226   -1.37, $p = 0.17$). There were also no significant differences in variance of colour traits (throat and belly

227   PC1 values) between males and females (Levene's test: F_1 = 0.065 – 0.072, $p = 0.79 – 0.80$).

228

229   ***Assortative mating by morphological and potential sexually selected traits***

230   We found some evidence for assortative mating by phenotype in our barn swallow population. Male

231   body mass was positively correlated with female mass within the same pair (Partial Pearson

232   correlation: $r = 0.37$, n = 77, $p < 0.01$), but wing length was not significantly correlated between males

233   and females in the same pair (Partial Pearson correlation: $r = -0.12$, n = 77, $p = 0.28$). There was also

234   no evidence for assortative mating by streamer length between males and females in each breeding pair

235   (Partial Pearson correlation: $r = 0.09$, n = 77, $p = 0.46$, Figure 2). Throat colour PC1 was correlated

236   within pairs (Partial Pearson correlation: $r = 0.22$, n = 78, $p = 0.05$, Figure 2) while belly colour PC1

237   was marginally non-significantly correlated within pairs (Partial Pearson correlation: $r = 0.19$, n = 77, p

238     $= 0.09$). Together, these results show assortative mating by body mass and throat colour in barn

239   swallows breeding in northeastern China.

Reproductive success and potential sexually selected traits

Hatching date of first clutches

Hatching date was significantly associated with male and female body mass, female streamer length and male throat colour PC1 (Figure 3, Table 2). Phenotypic variables with the greatest influence on hatching date were: male throat PC1 (explaining 9.71% of the total variance), female body mass (8.45%), male body mass (7.49%) and female streamer length (6.90%). The negative associations between hatching date and male body mass and throat PC1 suggest that heavier male barn swallows with darker, redder throat plumage started breeding earlier than lighter, paler males (Figure 3, Table 2). Heavier females with longer tail streamers also bred earlier than lighter, shorter-tailed females (association between hatching date and female body mass and streamer length, Figure 3, Table 2).

Number of eggs and number of fledglings over the breeding season

Variation in the total number of eggs laid by female barn swallows over the course of the entire breeding season (i.e. first plus second clutches) was explained by female body mass and male belly colour PC1 (Table 2). Similar to hatching date, the variance explained by the minimal GLMM model suggested that the number of eggs was mainly influenced by male belly colour PC1 (explaining 13.00% of total variance) and female body mass (12.39%) (Table 2). Female swallows with heavier body mass and/or longer tail streamers laid more eggs in a breeding season, and so did females mated to males with darker, redder belly plumage (Figure 3).

In addition to examining the total number of eggs produced by females across the entire breeding season, we also considered the total number of fledglings, which can be a more direct measure of reproductive success. In the study population, breeding failures involved unhatched eggs and nestling deaths in the nest (15%, 69 out of 460 eggs/nestlings), which leads to a difference between the total

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262   number of eggs and the total number of fledglings in a nest. In the minimal GLMM model testing the

263   correlation between the total number of fledglings and adult phenotype, 23.25% of the variance in the

264   number of fledglings was explained by female tail streamer length (Table 2), indicating that females

265   with longer tail streamers tended to produce more fledglings than females with shorter streamers. There

266   were no other significant associations between adult traits and number of fledglings (Table 2).

267

268   **Discussion**

269   In this study, we aimed to identify traits under sexual selection in a northeastern Chinese barn swallow

270   population that has been the subject of taxonomic debate based on variation in body size and ventral

271   plumage coloration (Turner 2006). We found that the colour of ventral plumage, including both throat

272   and belly regions, predicted patterns of pairing and reproductive performance of male swallows.

273   Females with longer tail streamers also had higher annual reproductive success, including earlier

274   hatching dates and greater numbers of fledglings. By contrast, the length of male tail streamers (the

275   primary sexually selected trait in European populations) did not significantly correlate with any

276   measure of reproductive success. Based on these results, we find that ventral plumage colour, not the

277   length of tail streamers, is likely to be the primary sexually selected trait in this barn swallow

278   population.

279   Assortative mating can be an important mechanism driving speciation by sexual selection

280   (Servedio 2016). In European barn swallows, it has been shown that the tail length of female barn

281   swallows is positively correlated with the tail length of their mates, after considering age effects; this

282   result supports the suggestion that both male and female tail lengths honestly reflect individual quality

283   (Møller 1993). In contrast, assortative mating based on throat and belly colouration, not tail length, was

284   found in North American barn swallows (Safran and McGraw 2004). In Japanese *H. r. gutturalis*, the

285   height of throat patch did not significantly correlate between paired swallows (Hasegawa and Arai

286   2013), while patterns of assortative mating by other sexual ornaments (white spots in the tail, throat

287   colour) have never been reported. In this study, we found assortative pairing by throat coloration rather

288   than tail length. The mechanism that produces this assortative mating is at present unclear.

289   For northeastern Chinese barn swallows, females with longer tails and heavier body mass bred

290   earlier and produced larger clutches over the breeding season (Figure 3-4). Although male ornaments

291   are usually considered to be the primary targets of sexual selection, female ornaments can also be

292   functional (Clutton-Brock 2009; Tobias et al. 2012). For instance, the length of tail streamers indicates

293   female quality in European barn swallows (Møller 1993), and females with longer tails and more

294   colourful throat patches have a reproductive advantage via earlier clutch initiation in Japanese barn

295   swallows (Hasegawa et al. 2017). Our results further support the view that the relationship between

296   reproductive success and ornaments differs between the sexes, with tail length providing more salient

297   information about individual quality in females, and ventral colouration likely advertising information

298   about quality in males in this study population. We also found a discrepancy between the results for the

299   number of eggs versus the number of fledglings: the ventral colouration of male barn swallows

300   predicted the number of eggs, but failed to predict the number of nestlings. This discrepancy could be

301   due to both sexual and natural selection operating throughout the reproduction process, resulting in

302   female barn swallows investing more in reproduction for darker males by laying more eggs but perhaps

303   greater mortality occurring for individuals in large broods.

304   Although clutch initiation and number of offspring are two major and representative components

305   of sexual selection in the barn swallow (Møller 1992, 1993), extra-pair paternity is also an important

factor which may influence the reproductive fitness of male barn swallows (Møller 1994). Although we

did not test the effect of extra-pair mating on male fitness in this study, the ratio of extra-pair offspring

to all offspring is relatively low in this population (11.6%, 28 EPO out of 241 nestlings, Liu 2017)

compared to *H. r. rustica* or *H. r. erythrogaster* (*H. r. rustica*: 18% - 29%; *H. r. erythrogaster*: 23% -

31%, Turner 2006), so extra-pair mating is not likely to be a major component of sexual selection (but

also see Hasegawa et al. 2010a; Kojima et al. 2009). Even so, in swallow populations in which sexual

selection via extra-pair mating has been examined, the targets of sexual selection are consistent for both

social and extra-pair mating success (e.g., Safran and McGraw 2004, Safran et al. 2005, Vortman et al.

2011, 2013). Another possible confounding factor in our study is that the age of swallows was not

known, and the size of sexually selected traits can change with age, e.g. tail length in European barn

swallows (Møller 1991) and throat colouration in Japanese barn swallows (Hasegawa et al. 2010b).

However, it has also been shown that the intensity of sexual selection in barn swallows does not differ

significantly between age classes (Romano et al. 2017). To test the effect of age on sexual selection in

our barn swallow population, further research is needed with age-identified individual swallows as

samples.

Secondary contact between divergent populations can lead to either fission or fusion of divergent

taxa, and sexual selection can be an important force driving this process (Coyne and Orr 2004; Panhuis

et al. 2001; Rundle and Nosil 2005). In our population of barn swallows, males with overall darker,

redder plumage colour bred earlier and had higher annual reproductive success compared to males with

pale plumage. We hypothesize that the intermediate ventral colour of northeastern *H. r. gutturalis* may

be the result of introgression of dark colouration from northern *H. r. tytleri* populations into southern

pale-coloured populations, potentially driven by sexual selection. If this is the case, we would predict

328   that darker males in the zone of intergradation would have higher fitness, which could lead to the

329   introgression of alleles for dark plumage colour into the genetic background of the pale-plumage

330   populations.

331   This study confirms that darker males in northeastern China do indeed have better reproductive

332   performance than paler males. However, some questions remain unresolved. For example, the targets of

333   female preferences and male competition in the parental subspecies, *H. r. tytleri* and southern Chinese

334   *H. r. gutturalis,* are unknown. The north Asian subspecies *H. r. tytleri* derives from recolonization of

335   Asia by the North American subspecies, *H. r. erythrogaster*. These two subspecies are similar in

336   morphology, and therefore may have similar behaviour patterns, including behaviour related to sexual

337   selection (Dor et al. 2010; Scordato and Safran 2014; Turner 2006; Zink et al. 2006). Observational

338   analyses in Japan on *H. r. gutturalis* have suggested that the red throat patch and the white spots in the

339   tail are under sexual selection, as males with less saturated throat plumage and larger white spots in the

340   tail bred earlier than other males (Hasegawa et al. 2010b) and males possessing either "less saturated

341   throat colour and shorter tails" or "more saturated throat colour and longer tails" tended to form pairs

342   with females more than other males (Hasegawa and Arai 2017), while pale ventral plumage is unlikely

343   to be a sexual signal (Hasegawa et al. 2017). Our finding that tail length is unlikely to be sexually

344   selected is consistent with previous studies of both North American and Japanese barn swallows. The

345   result that belly colouration is associated with reproductive performance is more similar to observations

346   in North American swallows than Japanese populations; however, we do find assortative mating by

347   throat color, suggesting that this trait may be important in the northeastern Chinese population as well

348   as in Japan. We did not measure the size of white tail spots in this study; this would be an interesting

349   avenue of future research. Our findings also indicate that the targets of sexual selection differ among

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350   closely related but phenotypically differentiated populations, which implies that sexual selection may

351   be an important force driving divergence in this system (Romano et al. 2017; Scordato and Safran

352   2014). In the future, more studies on the behaviour and genetics of the two parental barn swallow

353   populations, *H. r. tytleri* and *H. r. gutturalis,* are required to fully determine if sexual selection drives

354   asymmetric introgression of plumage colour, and whether this trait contributes to erosion of

355   reproductive isolation between these two subspecies.

356

365   **References**

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Figure 1

Figure 1. Morphological variation among three Chinese barn swallow populations.

Left: *H. r. guttrualis* in southern China. Middle: *H. r. tytleri-H. r. gutturalis*

intergrading population in northeastern China. Right: *H. r. rustica* in western China.

Note that the middle swallow has more red-brown ventral plumage colouration. The

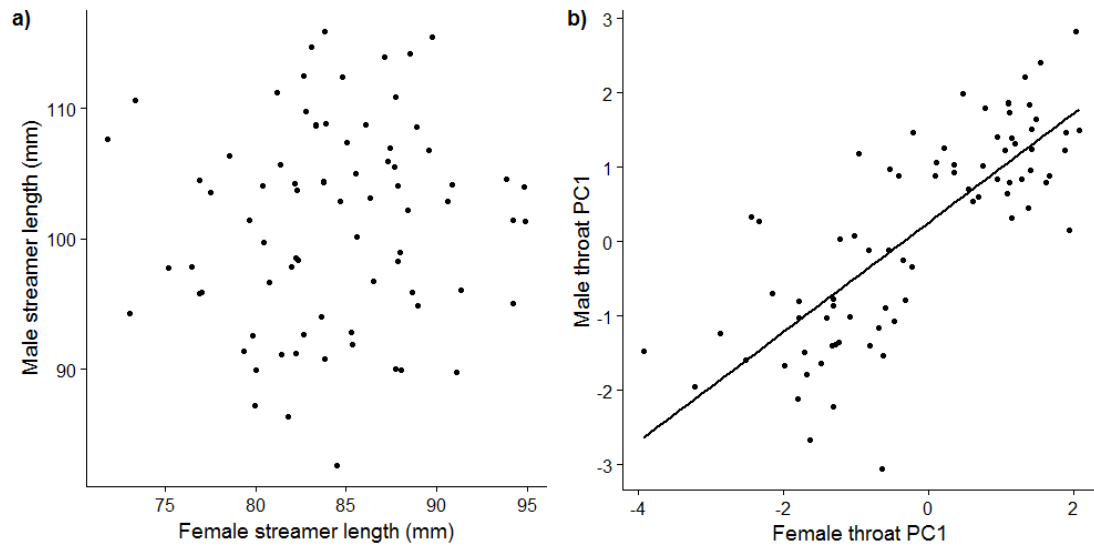brightness of this figure has been adjusted to present the colour clearly.

Figure 2

Figure 2. Assortative mating by streamer length (a) and throat (b) plumage colour in northeastern Chinese barn swallows. Note that there is no significant relationship between male and female streamer length, but evidence for assortative mating by throat colour. Simple regression lines are shown in (b).
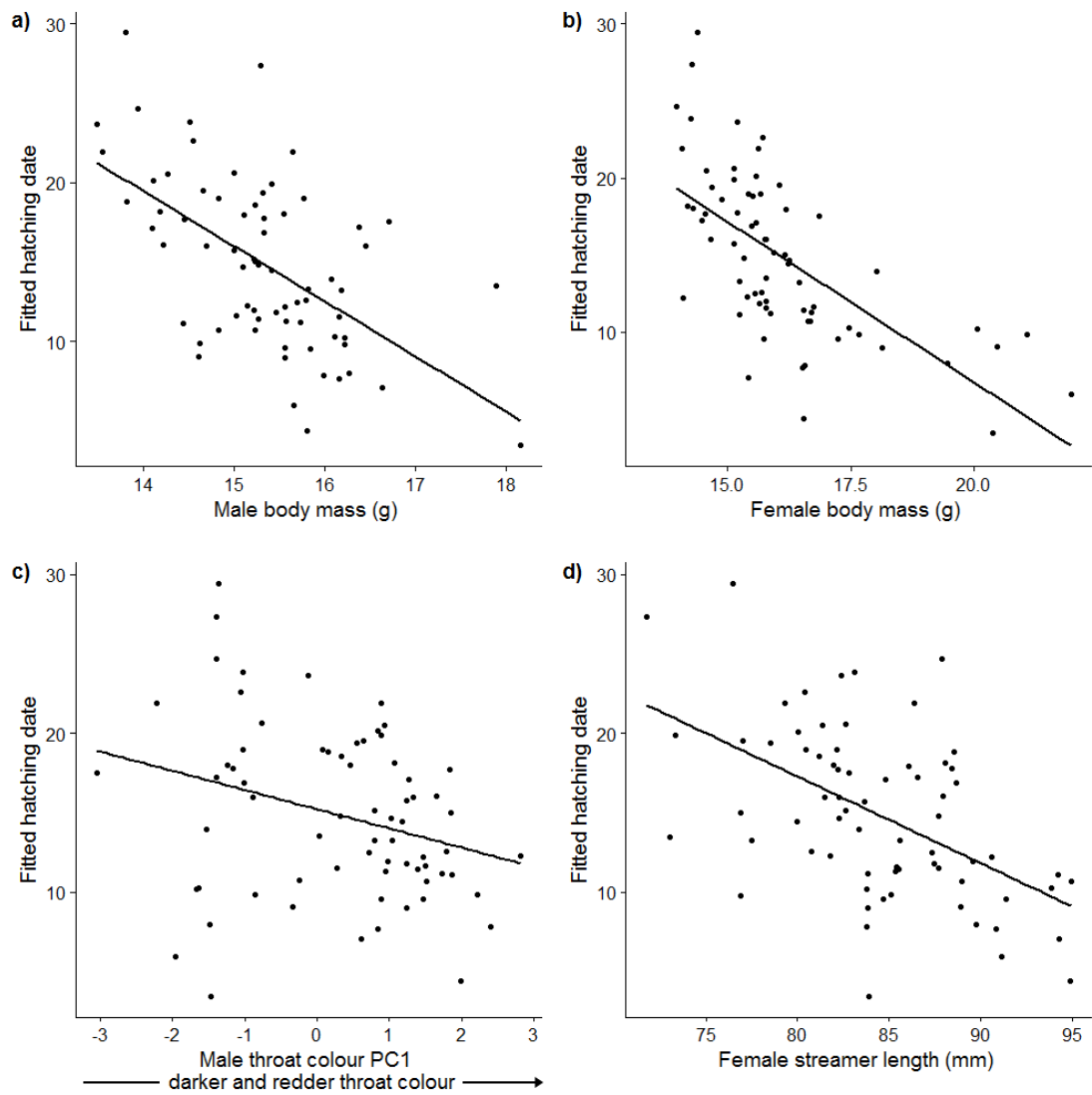
Figure 3

Figure 3. Relationship between hatching date and male (left) and female (right) morphology. Hatching date was negatively correlated with (a) male body mass, (b) female body mass, (c) male throat colour PC1 and (d) female streamer length in the minimal GLMM model. Fitted values of the hatching date from the minimal GLMM model and simple regression lines are shown.
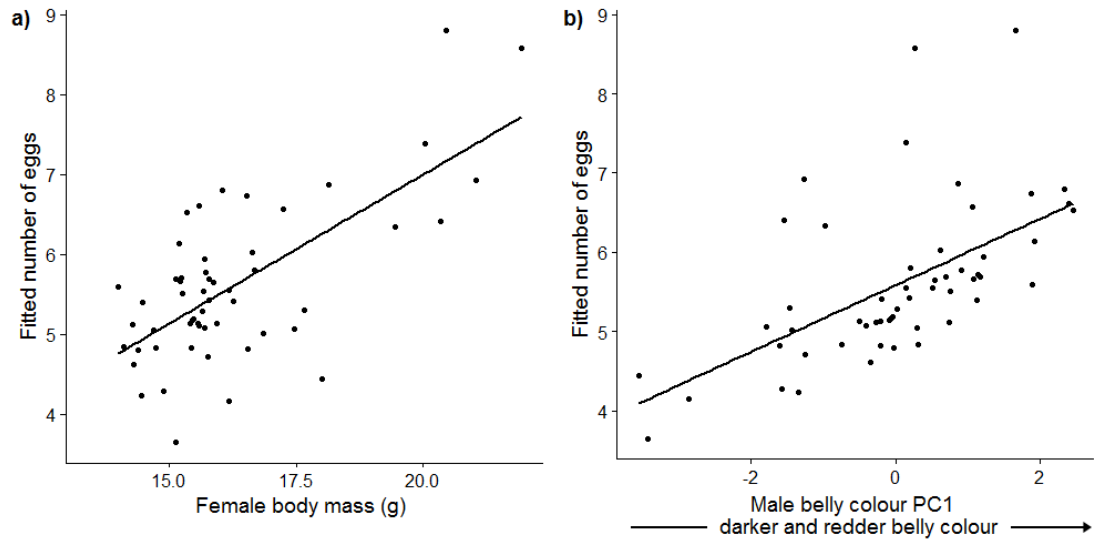
Figure 4

Figure 4. **Relationship between number of eggs and adult morphology.** Number of eggs was positively associated with (a) female body mass and (b) male belly colour PC1 in the minimal GLMM model. Fitted values of the number of eggs from the minimal GLMM model and simple regression lines are shown.

Table 1

Table 1. Principal component loadings for colour traits. Correlation values (opposite numbers for throat PC1) and cumulative proportion of variance for color traits are shown. Traits strongly correlated with PC values (loading > 0.5) are in bold.

	Throat PC1	Belly PC1
brightness	**-0.58**	**-0.66**
hue	**-0.52**	-0.31
chroma	**0.62**	**0.69**
Standard deviation	1.39	1.35
Cumulative proportion of variance	63.97%	60.82%

Table 2

Table 2. Morphological and potential sexually selected traits predicting reproductive success in this barn swallow population. The year

(2013 and 2015) and the IDs of nests were included as random effects when modeling number of eggs and number of fledglings, and the year

was included as a random effect when modeling hatching date. Final models including all significant variables ($p < 0.05$) are shown in boldface.

Values of non-significant terms are from the model immediately prior to the elimination of that factor. Variance explained by each fixed

significant factor is shown (% Var). Sample size (number of swallow pairs): hatching date: n = 69; number of eggs: n = 54; number of fledglings:

n = 55.

	Hatching date				Number of eggs				Number of fledglings			
	coefficient	T	*p*	% Var	coefficient	Z	*p*	% Var	coefficient	Z	*p*	% Var
Morphological Traits												
male												
body mass	**-2.96**	**-2.67**	**<0.01**	**7.49%**	-0.06	-0.73	0.47	-	-0.06	-0.60	0.54	-
wing length	0.52	1.52	0.13	-	-0.02	-0.86	0.39	-	-0.04	-1.17	0.24	-
female												
body mass	**-1.68**	**-2.83**	**0.01**	**8.45%**	**0.07**	**2.47**	**0.01**	**12.39%**	0.04	1.04	0.30	-
wing length	0.57	1.60	0.12	-	-0.003	-0.11	0.91	-	0.002	0.06	0.96	-

Streamer Length												
male	-0.14	-1.11	0.27	-	0.003	0.37	0.71	-	0.008	0.80	0.42	-
female	**-0.45**	**-2.56**	**0.01**	**6.90%**	0.02	1.77	0.08	**-**	**0.04**	**3.11**	**<0.01**	**23.25%**
Colour Traits												
male												
throat PC1	**-2.18**	**-3.04**	**<0.01**	**9.71%**	0.06	0.75	0.45	-	0.05	1.17	0.24	-
belly PC1	0.61	0.81	0.42	-	**0.10**	**2.16**	**0.03**	**13.00%**	0.06	1.15	0.25	-
female												
throat PC1	0.06	0.06	0.95	-	0.06	1.31	0.19	-	0.04	0.53	0.60	-
belly PC1	1.04	1.26	0.21	-	-0.07	-1.24	0.22	-	-0.08	-1.32	0.19	-
R-squared			0.35				0.15				0.17	