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

1 **Abstract**

2 Natural and sexual selection on heritable traits arise when individual fitness varies according to

3 focal traits. Sperm competition can determine the intensity of selection by influencing variance in

4 fitness among individuals. Studies of selection require that individual fitness is estimated using

5 proxies of lifetime reproductive success (LRS). However, estimating LRS is difficult in large, open

6 populations where sperm competition causes reallocation of biological paternity. We estimated

7 selection linked to LRS on lifespan and ornamental traits of male barn swallows (*Hirundo rustica*)

8 by sampling all offspring produced during their lives. We found selection linked to LRS on lifespan

9 and tail length mediated by within- but not extra-pair fertilization success. Viability selection

10 operated on tail length favouring long-tailed individuals. Moreover, positive selection on tail white

11 spots operated via extra-pair fertilization success. Finally, we found stabilizing selection on wing

12 length. These results were not confounded by factors that hamper studies of LRS, including non-

13 exhaustive sampling of offspring, biased sampling of males or effects of population size on genetic

14 and demographic parameters. Hence, natural and sexual selection mediated by LRS operates on

15 lifespan and tail length in barn swallows. We discuss the potential evolutionary implications of

16 selection on heritable tail length of males.

17

Introduction

Individuals can vary greatly in their relative contribution to the genetic composition of the next generation (Williams 1992). Large variation in individual fitness, as gauged for example by lifetime reproductive success (LRS), is most often reflected in the distribution of LRS being strongly left-skewed, with most individuals producing few offspring and only a few showing large LRS (Clutton-Brock 1998; Newton 1989). Because variation in individual fitness sets the scope for natural and sexual selection, dissecting the proximate and ultimate sources of variation in LRS is pivotal to our understanding of evolutionary processes (Williams 1992; Webster et al. 1995; Shuster and Wade 2003).

LRS essentially depends on the combination of the lifetime number of reproductive events and the number of viable offspring that are produced on average per event (McGraw and Caswell 1996; Clutton-Brock 1998; Newton 1989). Duration of life can thus be a major source of variation in LRS because it positively affects the lifetime number of reproductive events, implying that natural selection may set a premium on longevity (Gustafsson 1986; Clutton-Brock 1988; Merilä and Sheldon 2000). However, in some instances, duration of adult lifespan does not strongly predict LRS (Mills 1989; Herényi et al. 2012), suggesting that among-individual variance in the number of offspring produced per breeding event has an overwhelming effect on the lifetime number of events in determining LRS.

Variance in male reproductive success can arise in the context of sexual selection processes, whereby males differ in the number and/or the quality of the females that they can monopolize (Andersson 1994). Sexual selection studies have long sought the proximate and ultimate causes of variation in male mating success (Kirkpatrick et al. 1990; Owens and Hartley 1998). Female mate preferences are often non-randomly distributed with respect to the expression of male secondary sexual traits (Jennions and Petrie 1997; Wong and Candolin 2005), as shown both by correlational and experimental studies (Saino et al. 1997; Møller and Ninni 1998; Griffith et al. 2002; Westneat

43 et al. 2003). According to Fisherian/honest indicator mechanisms of sexual selection, such

44 ornamental male traits evolve under the effect of directional inter-sexual selection by females for

45 traits that reliably signal genetic/phenotypic quality of males and/or predict sexual attractiveness of

46 their future offspring (Weatherhead and Robertson 1979; Andersson 1994; Fawcett et al. 2007). In

47 addition, different male ornamental traits (e.g. skin or feather coloration and courtship displays in

48 fish and birds) typically co-occur and are presented to choosy females simultaneously (Møller and

49 Pomiankowski 1993; Iwasa and Pomiankowski 1994). The mechanism of evolution and the

50 function of such 'multiple ornaments' in the mate choice process are still contentious issues of

51 debate (Møller and Pomiankowski 1993; Candolin 2003).

52 Importantly, however, conspecific populations may differ in the strength of selection on individual

53 ornaments, and this may be a mechanism causing population divergence, pre-zygotic isolation and,

54 eventually, speciation (Møller and Cuervo 1998; Panhuis et al. 2001; Van Doorn, Edelaar and

55 Weissing 2009).

56 In species where sperm competition occurs, variance in reproductive output among males can also

57 be affected by the success of males in securing their own ('within-pair') paternity of their social

58 progeny (i.e. the offspring generated by their social mate(s)) and in siring extra-pair offspring by

59 fertilizing females different from their social mate(s) (Lebigre et al. 2012 and references therein).

60 The occurrence of sperm competition is most often believed to boost variance in male realized

61 reproductive output because success in siring extra-pair offspring is not always accompanied by a

62 commensurate reduction in the number of within-pair offspring (Webster et al. 1995; Sheldon and

63 Ellegren 1999; Vedder et al. 2011). However, depending on the magnitude of the positive or the

64 negative covariation between within- and extra-pair reproductive success, sperm competition may

65 both increase or decrease the variance in realized reproductive success among males. Hence,

66 competition for genetic paternity is a potentially ancipital component of sexual selection and can

67 contribute to the evolution of male epigamic traits (Møller and Ninni 1998).

68 Natural and sexual selection, however, act throughout an individual's life, and studies that aim at

69 quantifying selection on 'ordinary' and sexually selected ornamental traits should in principle be

70 able to exhaustively estimate reproductive output, including its components mediated by sperm

71 competition, during the entire life. However, this condition has only rarely been met in studies of

72 free-ranging organisms.

73 In fact, estimating LRS in iteroparous species with sperm competition is difficult, as it requires

74 long-term studies of populations of individually marked organisms where individuals are monitored

75 during their entire life (Clutton-Brock 1988; Shuster and Wade 2003) and where it is possible to

76 identify all the individuals alive during the breeding season. In species with intense sperm

77 competition, frequent extra-pair paternity greatly hinders the scope for LRS studies because they

78 entail collecting exhaustive data on all within- and extra-pair offspring sired by individual males. In

79 addition, 'edge effects', whereby the study sample reproductively interacts via sperm competition

80 with the individuals breeding just outside the study area, can lead to inaccurate LRS estimates due

81 to missed paternity events by the focal males (Webster et al. 1995; Sheldon and Ellegren 1999;

82 Webster et al. 2001). In fact, we are unaware of any study of a large, open vertebrate population

83 with intense sperm competition in the wild where these potentially confounding effects could be

84 assumed to have no or negligible role.

85 The European barn swallow (*Hirundo rustica rustica*) that we studied is a small migratory passerine

86 bird. Socially monogamous pairs breed solitarily or in colonies of two to tens of pairs, typically in

87 farms, spatially isolated from other colonies in our study area. Females lay 1-3 clutches of 1-7 eggs

88 per breeding season, from April to July (Møller 1994 b). The frequency of offspring that are sired

89 by a male different from their social father is high, although temporally and spatially variable (see

90 below), as is the frequency of broods where at least one offspring is sired by an extra-pair male

91 (Møller and Tegelström 1997; Saino et al. 1997; Kojima et al. 2009). Barn swallows are short-lived

92 birds, with most adults having only one breeding season in their life (Møller 1994 b). Birds older

93   than 3 years are rare as annual survival of adults is low (0.30-0.40; Møller and de Lope 1999).

94   Importantly, barn swallows have extremely high breeding philopatry (Møller 1994 b). Hence, birds

95   can be followed throughout their reproductive life, and individuals that do not return to the colony

96   where they bred the previous year can confidently be assumed to have died. In addition, males do

97   apparently not fertilize females breeding in other colonies, at least in our study area where colonies

98   are settled in widely spaced farms (Ambrosini et al. 2012). However, since natal dispersal is very

99   high (Balbontín et al. 2009), with the vast majority of yearling recruits immigrating from a colony

100   different from their original one, the frequency of mating between close relatives (parents-offspring;

101   siblings) is extremely low (Kleven et al. 2005), and genetically effective population size is very

102   large, implying that barn swallow populations are not affected by population genetic processes that

103   are typical of small, closed populations.

104   Here we identified parentage of all offspring produced at three colonies over three years and

105   measured LRS (including extra-pair paternities) to estimate selection differentials and partial

106   selection differentials (i.e. selection gradients controlling for the effect of selection on correlated

107   traits) on lifespan and a number of male secondary sexual traits which have been shown to have a

108   role in sexual selection and sperm competition in one or more of the geographical

109   populations/subspecies of this species (Romano et al. 2016 a). Specifically, we estimated selection

110   based on variance in LRS on the length and fluctuating asymmetry of the outermost tail feathers, on

111   the size of the white spots on the tail feathers, and on melanin-based coloration of the white to

112   chestnut ventral plumage region that was objectively quantified in terms of 'visible' and UV-

113   chroma as well as saturation, while taking spectral light sensitivity of birds into account. In

114   addition, we quantified selection on 'ordinary' (i.e. non-sexually-selected) traits including wing

115   length, which is a major trait affecting flight performance, and body size as gauged by tarsus length.

116   Based on previous studies of diverse fitness correlates and estimates of annual reproductive success

117   (review in Romano et al. 2016 a) we expected that individuals with (age-corrected) longer tails,

118 darker ventral plumage coloration, larger white spots on tail feathers, and more symmetric

119 outermost tail feathers had larger lifetime number of 'within-pair' biological offspring in the broods

120 where they were the social parents (LRS_{wpo}) and also sired a larger lifetime number of 'extra-pair'

121 offspring in broods other than their social broods (LRS_{epo}) and that, as a consequence, had larger

122 total lifetime reproductive success (LRS_{tot}, corresponding to $LRS_{wpo} + LRS_{epo}$.). In addition, we

123 expected that all indicators of LRS increased with lifespan because the number of breeding events

124 strongly increases with duration of life.

125

Methods

126 We studied barn swallows breeding at 3 colonies (= farms) located east of Milan (Northern Italy)

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128 over 5 years (2012-2016). The colonies were chosen to represent very small (3-6 breeding pairs),

129 medium (12-19 breeding pairs) or large-sized (22-26 breeding pairs) according to recent large scale

130 censuses (Ambrosini et al., 2012). In all study years, we captured and individually marked with

131 numbered metal and plastic colour rings all the adults breeding in the focal colonies. Thanks to the

132 extremely high breeding philopatry, the individuals that were captured in any year between 2013

133 and 2015 and had not been captured as adults in the previous year could be assumed to be 1-year-

134 old individuals at their first breeding season immigrating from colonies outside our study area,

135 except in rare cases when they were local recruits (i.e. individuals that were ringed as nestlings at

136 the focal colonies allowing us to directly asses age). We could thus *a posteriori* identify a set of 79

137 males that started breeding in 2013-2015 and died before 2016 All males that were captured as

138 adults in 2012 and those that were still alive in 2016 were therefore excluded because their LRS

139 could not be estimated. In 2013-2015 all breeding pairs were identified and breeding activities were

140 monitored. Nestlings from all first, second and third broods were ringed and subjected to blood

141 sampling at the age of 8-12 days for parentage analyses.

142 Standard morphological measurements were taken on all individuals in all capture years, including

143 length of both outermost tail feathers, chord length of both wings, and tarsus length. Tail length and

144 wing length were expressed as the mean of the left and right character. Tail asymmetry was

145 expressed as the unsigned difference between the length of the left and the right outermost tail

146 feathers. Some contour feathers were also collected from the same region of the white to rufous

147 ventral plumage for later spectrometric colour measurements (see below). The 4th (counting

148 outwards) right rectrix (R4) was plucked and stored flat in individual bags for later measurement of

149 the size of the white spot (Saino et al. 2015). A small blood sample was taken for parentage

150 analyses.

151

Spectrometric colour analysis

153   Reflectance of one, randomly chosen ventral feather, was recorded by means of an Avantes DH-

154   2000 spectrometer equipped with a deuterium-tungsten halogen light source in a dark chamber, as

155   described in Saino et al. (2013a, b). As demonstrated in Romano et al. (2015), the reflectance

156   measurement of one ventral feather is highly repeatable and shows high consistency with the same

157   measurement performed on three overlapping feathers and on the bird's body.

158   Coloration was quantified by processing reflectance data according to the tetrahedral colour space

159   model (Goldsmith 1990) using TetraColorSpace program (Version 1a; Stoddard and Prum 2008)

160   implemented in MATLAB 7 (MathWorks, Natick, MA), assuming UVS cone type-retina and

161   adopting the spectral sensitivity of the blue tit (*Cyanistes caeruleus*), the species phylogenetically

162   most closely related to the barn swallow for which spectral sensitivity information is implemented

163   in the TetraColorSpace program. Each colour vector in the tetrahedral colour space was then

164   converted into the spherical coordinates ϑ, ϕ, and rA (Stoddard and Prum 2008). ϑ and ϕ represent

165   the red-green-blue and the ultraviolet components of chroma, while rA reflects colour saturation. In

166   the range of colours of barn swallow ventral feathers increasing ϑ values indicate paler, whitish

167   coloration (Saino et al. 2013 a, b).

168

Tail white spot area measurement

170   The R4 feathers were sellotaped to a cardboard across the shaft and scanned. Using ImageJ 1.46r

171   software (rsbweb.nih.gov), for each feather we measured the area of the white spot (Saino et al.

172   2015). Importantly, in an additional sample of birds from which we also plucked the outermost

173   rectrix (R6) (Saino et al. 2015), there was a positive correlation between the area of the white spot

174 on R4 and on R6 ($r = 0.718$, $n = 17$; $P < 0.05$), indicating that the size of the white spot is correlated

175 within individuals across tail feathers.

176

177 *Genetic parentage analysis*

178 DNA was extracted from blood samples by alkaline lysis according to Saino et al. (2008). Extracted

179 DNA was subsequently diluted to a final concentration of 50 ng/µL (Saino et al. 2008). Genotyping

180 of adults and nestlings was performed on a total of five loci, amplified in a single multiplex

181 reaction. Three of them were highly-polymorphic microsatellite loci previously developed for barn

182 swallows (Hir7, Hir17, Hir20 (Tsyusko et al. 2007), one was a microsatellite in the 3′ untranslated

183 region (UTR) of the Adcyap1 gene (Adenylate Cyclase-Activating Polypeptide 1; original primers

184 Steinmeyer et al. 2009) and one a polymorphic region within the gene for proopiomelanocortin

185 (POMC). POMC primers were designed by the authors using *Hirundo rustica* genomic sequences

186 kindly supplied by Dr. Anne-Lyse Ducrest and Prof. Alexandre Roulin (University of Lausanne,

187 Switzerland; personal communication). Either forward or reverse primers were fluorescently

188 labelled (see Table S1). PCR amplification was performed using a commercial kit (Qiagen,

189 Multiplex PCR Kit) in a final volume of 25 µL with 12.5 µL 2× QIAGEN Multiplex PCR Master

190 Mix, 2.5 µL 10× primer mix (0.5 µL of each primer) (final concentration 0.2 µM), 2 µL RNase-free

191 water (for genomic DNA extracted from blood only), 5 µL 5× Q-Solution and 3 µL of DNA

192 solution (5 µL for DNA extracted from feather samples). PCR amplification profile was: 95° C for

193 15 min, 35 cycles at 94° C for 30 s, 56° C for 90 s, 72° C for 60 s, and a final extension at 60° C for

194 30 min. Polymorphism was determined using a commercial fragment analysis service (Macrogen

195 Inc., Seoul, Republic of Korea) (see Bazzi et al. 2015). Fragment lengths were scored for each

196 individual using GeneMarker® version 2.4.2 software (Softgenetics). The observed (H_{obs}) and

197 expected (H_{exp}) heterozygosity, polymorphic information content (PIC) and frequency of null

198 alleles (F(Null)) were calculated using Cervus version 3.0.3 (Field Genetics Ltd.) (Kalinowski et al.

199 2007; Table S2). The combined non-exclusion probability of the marker set was always above

200 1.06×10^{-3} for the first parent and above 1.07×10^{-4} for the second parent (for a detailed analysis of

201 combined non-exclusion probability, see Table S3).

202 In total, 1046 individuals (235 adults and 811 nestlings) were genotyped at all five loci and 22 (4

203 adults and 18 nestlings) at four loci. Parentage assignment was performed using Cervus version

204 3.0.3 software. Since all social pairs were assigned to their own nest during behavioural

205 observation, and the sex of each parental individual is ascertained, we carried out parent pair

206 analysis (by computing log-likelihood statistics for all possible offspring and candidate parent pairs

207 (hereafter LOC)) in order to distinguish between within- and extra-pair paternity. We

208 conservatively assumed that 99% of breeding females and 95% of males were sampled in each year

209 and colony. Significance of parentage assignment was determined by the observation of Delta

210 statistics value (LOC difference between the most likely and second most likely parental pair).

211 When Delta value was above 95%, indicating full compatibility in the genotype comparison

212 between offspring and parental pair or one mismatch (in most cases due to inconsistences in the

213 alleles of the Adcyap1 gene, see Steinmeyer et al. 2009), the best candidate mother and father were

214 considered the genetic parents of the nestling. The presence of extra-pair paternity was defined

215 when the genetic father identified by parent pair analyses differed from the social father identified

216 during behavioural observations. In no case did we identify instances of brood parasitism, i.e. a

217 nestling that did not genetically match with the social mother.

218

219 *Statistical analyses*

220 The phenotypic value of some traits (e.g. plumage traits that are molted annually) in birds can

221 change during an individual's life due to age effects. To obtain an average estimate of the

222 expression of any *i*-th trait for any *j*-th individual that bred over more than one year, we first

223 computed the difference between the phenotypic value of the *i*-th trait recorded for the *j*-th

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224 individual at age t (x_{ijt}) and the mean population-level phenotypic value of the i-th trait recorded on

225 all individuals at age t. The phenotypic value pertaining to any j-th individual at the i-th trait was

226 then computed as the mean (X_{ij}) of all the x_{ijt} values recorded for that individual during its entire

227 life. This procedure to obtain a synthetic phenotypic value while accounting for age effects was

228 tenable because intra-class correlation coefficients, reflecting repeatability of traits that are renewed

229 annually, as estimated from variance components of linear mixed models including age as a fixed

230 effect, were large for all traits including tail length ($r = 0.743$), size of white spots on tail ($r =$

231 0.533), wing length ($r = 0.863$) and plumage tetrahedral colour components (θ: $r = 0.422$; φ: $r =$

232 0.674; rA: $r = 0.577$) (likelihood-ratio test: all $\chi^2 \geq 4.63$, $P \leq 0.03$). Tail asymmetry also had a

233 relatively large repeatability, although statistically marginally non-significant ($r = 0.610$, $\chi^2 =$

234 3.596, $P = 0.058$) as estimated in a linear mixed model not including the effect of age because tail

235 asymmetry is known not to vary with age (N.S., unpublished data). Hence, individuals were

236 consistent in their expression of the focal phenotypic traits at different ages and their associated

237 phenotypic value could be estimated as the mean of the deviations from the age-specific population

238 means. For tarsus length, no adjustment for age was required because this trait does not vary in

239 adulthood, and its phenotypic value at age 1 was therefore used.

240 In linear regression analyses of selection on lifetime reproductive success , X_{ij} vectors (or tarsus

241 length at age 1) were standardized to a mean = 0 and variance = 1. Lifespan was also standardized

242 to mean = 0 and variance = 1. Total number of offspring produced during life by any individual was

243 expressed as the ratio ($rLRS_{tot}$) between the total offspring fathered divided by the mean number of

244 offspring fathered by the 79 males in the sample. Similarly, the number of within or extra-pair

245 offspring sired was expressed as the ratios ($rLRS_{wpo}$ or, respectively, $rLRS_{epo}$) between the within-

246 pair offspring (WPO) or the extra-pair offspring (EPO) sired by an individual and the mean WPO or

247 EPO sired by the males in the sample. Regression coefficients of rLRS variables on standardized

248 phenotypic traits therefore reflect the proportional change in fitness relative to the population mean

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249   caused by a 1 standard deviation change in the phenotypic trait. Tests for stabilizing/disruptive

250   selection were performed in regression analyses of lifetime reproductive success on second-order

251   polynomial terms on standardized phenotypic traits.

252   None of the breeding success variables was found to differ among colonies. Thus, in all analyses

253   males from the three colonies were pooled. However, the results of all the selection analyses

254   remained qualitatively unchanged when in linear models the colony was included as a categorical

255   fixed effect (details not shown).

256   Survival in relation to tail length was analysed in a Cox proportional hazards regression model with

257   time-dependent covariates. To account for tied event times, we adopted the procedures implemented

258   by PROC PHREG (TIES=EXACT), which is based on the probability of the union of the partial

259   likelihoods for all possible orderings of tied events.

260   Statistical parameters are reported with their associated standard error. All analyses were run using

261   the SAS 9.3 statistical package.

262

263

264   **Results**

265

266   We measured lifetime realized reproductive success (LRS_{tot}, LRS_{wpo}, LRS_{epo}) for the 79 males that

267   completed their life-cycle within the study period at the 3 focal colonies. To this aim, we assessed

268   parentage of all the 829 nestlings that were produced and reached sampling age at the study

269   colonies over the study period. The 79 focal males were found to have sired on average 5.38 (0.66

270   SE; range: 0-23) within-pair nestlings (WPO-nestlings) and 0.78 (0.21; range: 0-12) extra-pair

271   nestlings (EPO-nestlings) at the end of their life, yielding a mean total realized reproductive success

272   of 6.16 (0.78; range 0-30) offspring. Thus, out of the 487 nestlings that were found to have been

273   sired by the 79 focal males, 425 (87.3%) were within-pair while 62 (12.7%) were from extra-pair

274   broods. The percentage of broods of the 79 focal males where at least one nestling was found to be

275   extra-pair was 34.72% (58/167 broods).

276

277   *Selection differentials on lifetime reproductive success*

278   Selection on lifespan was large and highly significantly different from 0 for $rLRS_{tot}$ (Fig. 1) and

279   also for $rLRS_{wpo}$ and $rLRS_{epo}$, implying that lifespan is a major determinant of the total number of

280   offspring that male barn swallows sire over their life, and that lifetime reproductive success

281   increases with lifespan both as a result of a larger number of within-pair offspring and of a larger

282   number of offspring sired in extra-pair broods (Table 1).

283   Selection differentials on non-sexually selected morphological traits (wing length and tarsus length)

284   were not significantly different from 0 for all rLRS components (Table 1). Selection on tail length

285   was positive and strong for the total number of offspring sired (Table 1; Fig. 2) and also for the

286   number of within-pair offspring, but not for the number of offspring in extra-pair broods (Table 1).

287   Importantly, tail length was expressed as the within-individual mean of the residuals from the age-

13

288 specific mean phenotypic value (see Methods), implying that this result is unaffected by age-

289 dependent variation in tail length. Tail asymmetry did not significantly predict LRS components

290 (Table 1).

291 Selection differentials on ventral colour plumage components were weak and not significantly

292 different from 0 for all rLRS components (Table 1). Selection differentials on white tail spots were

293 not significantly different from 0 for $rLRS_{tot}$ and $rLRS_{wpo}$. However, selection differential on white

294 tail spots was significantly larger than 0 for $rLRS_{epo}$, meaning that individual with larger white spots

295 on the tail feathers sired a larger lifetime number of offspring in broods from other social pairs

296 (Table 1; Fig. 3).

297 Regression analyses of lifetime reproductive success on second-order polynomial terms of

298 phenotypic traits showed no significant effect of the quadratic term, with the only exception of the

299 $rLRS_{tot}$ selection on wing length (effect of the quadratic term: coefficient: -0.191 (0.095), t = -2.01,

300 P = 0.048; Fig. 4). Polynomial regression analyses for $rLRS_{wpo}$ and $rLRS_{epo}$ disclosed marginally

301 non-significant effects of the quadratic term of wing length ($rLRS_{wpo}$: -0.165 (0.093), t = -1.79, P =

302 0.078; $rLRS_{epo}$: -0.370 (0.208), t = -1.78, P = 0.079). The negative sign of the coefficient associated

303 with the quadratic term and the values of the regression parameters indicate that a maximum in LRS

304 values was attained within the range of variation of standardized wing length. Thus, there was

305 evidence for stabilizing selection on wing length but not on other traits.

306

307 *Selection gradients on total lifetime reproductive success*

308 We first estimated partial selection differentials (i.e. selection gradients) in multiple regression

309 analyses of $rLRS_{tot}$ on lifespan, morphological traits and 'visible' colour chroma. We avoided

310 entering all three tetrahedral colour components in the same model due to multicollinearity. The

311 selection gradient was large and significantly larger than 0 for lifespan, showing that longer-lived

312 individuals accrue larger fitness independent of their phenotypic traits (Table 2). In addition, the

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313 selection gradient was significantly larger than 0 for tail length (Table 2). This implies that long-

314 tailed individuals produce more biological offspring independently of any effect of tail length on

315 viability. In fact, tail length was found to positively predict survival in a Cox proportional hazard

316 regression model (coefficient: -0.045 (0.018), $\chi^2_1 = 685$, $P = 0.009$). Regression gradients were not

317 significantly different from 0 for tarsus and wing length, tail asymmetry and the θ tetrahedral colour

318 component (Table 2). A multiple regression model of $rLRS_{wpo}$ with the same independent variables

319 as in the model in Table 2 confirmed the positive effect of lifespan (coefficient: 0.973 (0.089), t =

320 10.928, $P < 0.001$) and tail length (0.273 (0.079), t = 3.47, $P = 0.001$). A multiple regression model

321 of $rLRS_{epo}$ again with the same independent variables as in Table 2 confirmed (see Table 1) the

322 positive effect of tail white spots area (0.632 (0.241), t = 2.62, $P = 0.011$).

323 Alternative multiple regression models in which we included the φ or rA components of feather

324 coloration, rather than the θ component, confirmed the significant effects of lifespan and of tail

325 length and the non-significant effects of the other independent variables on both $rLRS_{tot}$ and

326 $rLRS_{wpo}$ (other details not shown) and the significant effect of tail with spot area on $rLRS_{epo}$.

327

Discussion

Estimating the intensity of selection on lifespan and sexually selected traits is a key step in the analysis of the evolution of life-histories and of sexual selection processes. Here, we analysed selection on lifespan based on information on LRS and secondary sexual and non-sexual morphological traits in male barn swallows while accounting for the effects of sperm competition. We found evidence for strong LRS directional selection on lifespan and length of the outermost tail feathers while accounting for age-dependent variation in ornamentation. Selection on tail length was independent of the effect of tail length on annual survival. Selection differentials on other sexually selected traits or body size and wing length did not differ significantly from zero. However, significant selection based on LRS on tail length and lifespan was detectable when the within-pair but not the extra-pair component of LRS was considered. This implies that selection for larger tail ornaments and lifespan only operates via the number of biological offspring that males can secure in the broods where they are the social fathers. The main implications of the present finding are thus that estimated directional selection linked to LRS exists in our study population on lifetime also independently on tail ornament size, and selection is mediated by success in securing paternity of own social offspring rather than by extra-pair fertilization success. However, selection on the size of the white spots on the tail arose via a positive relationship on the lifetime number of extra-pair offspring. Significant estimates of selection on tail length and size of white spots were not the spurious result of the indirect effect of selection on other traits, including body size and wing length, as implied by the significant selection gradients controlling for these potentially confounding effects.

Selection on lifespan was expected based on the fact that in the barn swallow variance in clutch and brood size is small, fledging success is extremely high and broodedness (i.e. the number of broods per breeding season) varies markedly between yearlings and older individuals (Møller 1994 b). Thus, the number of breeding events and, consequently, of offspring produced, markedly increases

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353   with the number of breeding seasons that individuals experience (Saino et al. 2012). Notably,

354   positive selection on lifespan is not necessarily expected even among short-lived small birds, as

355   suggested by the study of an archipelago population of song sparrows *Melospiza melodia*, where

356   lifespan did not strongly predict realized LRS (Lebigre et al. 2012).

357   While sexual selection, including its component mediated by sperm competition, has been

358   intensively studied in the barn swallow, including in the present population, since many years

359   (reviews in Romano et al. 2016 a), this is the first study in which LRS controlling for effects of

360   extra-pair fertilization has been quantified and estimated for sexual ornaments. Previous studies

361   have shown clearly significant geographical variation in current sexual selection including sperm

362   competition on individual male ornaments (i.e. melanin-based coloration and tail length) (Møller et

363   al. 2003; Vortman et al. 2013; Safran et al. 2016; Wilkins et al. 2016; Romano et al. 2016 a).

364   The present results indicate that in Western Palearctic populations, contrary to what apparently

365   occurs in other Eurasian (Vortman et al. 2011; Hasegawa and Arai 2013) and Nearctic populations

366   (Safran and McGraw 2004), tail length is currently under selection linked to directional sexual

367   selection with respect to LRS. This observation is consistent with selection due to variance in

368   annual reproductive success (ARS) on tail length, as observed in several Western Palearctic

369   populations, although without accounting for sperm competition effects (e.g. Møller et al. 1998). In

370   addition, this is consistent with selection on tail length due to variation in ARS mediated by sperm

371   competition recorded in local European populations, including the same population where the

372   present study was conducted (Saino et al. 1997). The area of the white spots on the tail feathers was

373   apparently under positive selection mediated by the success in extra-pair fertilizations. This finding

374   is consistent with the observation that in northern European population white tail spots are targeted

375   by directional sexual selection (Kose et al. 1999), although no previous study has investigated their

376   role in sperm competition. Conversely, plumage melanin-based coloration, which has been

377   suggested to be under inter-sexual selection in other subspecies (Romano et al. 2016 a and

378 references therein) seems not to be currently under directional selection mediated by reproductive

379 success. In fact, selection differentials on 'visible' and UV chroma and on colour saturation were

380 far from being statistically significant, and selection gradients controlling for several traits and for

381 lifespan were also statistically non-significant. These results are therefore consistent with meta-

382 analytic evidence and individual studies indicating that different plumage ornaments are differently

383 selected in distinct barn swallow subspecies (summarized by Romano et al. 2016 a).

384 The relationship between LRS and standardized wing length was curvilinear with a maximum

385 within the range of variation in wing length. This suggests that stabilizing selection is currently

386 ongoing on wing length of male barn swallows.

387 Selection due to variation in LRS on tail length was also accompanied by viability selection, as

388 long-tailed males had larger annual survival, consistent with previous studies (Møller 1994 a; Saino

389 et al. 2011).

390 Selection on tail length may cause micro-evolutionary change in tail length if the prerequisite

391 condition of non-zero additive genetic variation in the trait is met. Previous studies adopting diverse

392 approaches have led to relatively large estimates of heritability in tail length. For example, parent-

393 biological offspring regression analysis has led to narrow sense heritability (h^2) estimates as large as

394 0.394 while regression of extra-pair offspring phenotype on the phenotype of the social (but non-

395 biological) father have disclosed low and statistically non-significant social parent-offspring

396 resemblance ('heritability' estimate: -0.072) (Saino et al., 2003). Such comparison between father

397 and biological versus non-biological offspring resemblance suggests no significant maternal and

398 environmental effects on father-offspring resemblance and, hence, that large additive genetic

399 variation in tail length exists. In addition, genetic correlations between tail length and other

400 morphological traits including wing length and body size are generally low suggesting that genetic

401 correlations are not expected to constrain the evolutionary change of tail length. Moreover,

402 selection on male tail length has been shown to be consistent in time within populations, though

403   variable in strength among populations (Møller et al. 2006). Significant heritability, weak genetic

404   correlations with other traits and consistent selection across generations leads to expectations of

405   evolutionary change in male tail length, although no such change seems to have occurred over 25

406   years (our unpublished data). This may suggest that selection does not act on the heritable

407   component of phenotypic variation. Alternatively, environmental effects may mask micro-

408   evolutionary change. For example, deterioration of the ecological conditions in the sub-Saharan

409   wintering quarters of our study population may cause both its current marked demographic decline

410   and a reduction in the expression of the tail ornaments, which are produced at the time of the single

411   annual molt during winter in Africa (Saino et al. 2004). Thus, counter-gradient selection may result

412   in apparent evolutionary stasis because phenotypic manifestation of micro-evolutionary change is

413   constrained by negative ecological effects on ornament size. Yet an alternative interpretation is that

414   offspring viability negatively covaries with paternal lifespan or LRS. We deem this explanation

415   unlikely, however, because in a recent study we found no relationship between paternal and

416   offspring longevity (Romano et al. 2016 b; our unpublished data), and in previous studies we could

417   find no evidence for phenotypic differences at traits that are likely to affect post-fledging survival

418   between within- and extra-pair offspring (our unpublished data).

419   Estimating selection based on variation in LRS in the wild is challenging, because it requires that

420   reproductive performance of individuals is monitored throughout their lives (Lebigre et al. 2012).

421   This in turn implies that age of individuals can be assessed, time of death can be established

422   unequivocally, and the entire individual reproductive history can be reconstructed. This was

423   possible in our focal population because adults have extremely high breeding philopatry, and

424   because all breeding events could be monitored and biological parents assigned to all offspring. In

425   addition, one potential pitfall of LRS studies is non-random sampling of individuals, because

426   unmated individuals or individuals that fail their breeding attempt early in the breeding cycle may

427   be more likely to go undetected (Sheldon and Ellegren 1999; Webster et al. 2001). These

428 individuals may not represent a random sample of the population with respect to phenotypic/genetic

429 quality or, in the case of males, with respect to ornamentation because high-quality, ornamented

430 males are expected to be over-represented among the fraction of the population that breeds

431 successfully (Lebigre et al. 2012). In the present study, we avoided this potential source of bias

432 because we paid special attention to not excluding individuals that did not breed successfully either

433 because they failed to acquire a social mate or because their breeding attempt failed.

434 In addition, quantifying selection based on variation in LRS is particularly problematic in species

435 where the occurrence of sperm competition causes a reallocation of paternity among individuals

436 with respect to the apparent, social mating pattern (Webster et al. 1995). Individual males may

437 differ in their success in sperm competition, both because they differ in the likelihood of securing

438 their biological paternity of their own social offspring and because they differ in their chances of

439 fertilizing extra-bond females (e.g. Møller and Ninni 1998). Because variation in success in sperm

440 competition is typically non-random with respect to quality of individual males, as reflected for

441 example by their sexual ornaments (Saino et al. 1997; Safran et al. 2005), neglecting the

442 consequences of sperm competition on realized LRS can result in biased estimates of selection on

443 sexual ornaments. Similarly, because females may differentially allocate paternity to older males

444 (Schmoll et al. 2007; Lifjeld et al. 2011), and the expression of sexual ornaments can covary with

445 age, neglecting the effect of sperm competition may confound the estimates of selection due to

446 variation in LRS on male ornaments. In the present study, we exhaustively sampled all the offspring

447 and the adults that were present in any study colony during the study period, thereby assessing

448 parentage of all the offspring produced in the colonies during the study. Because the genetic father

449 (and mother) could always be identified among the adult males belonging to the focal colonies,

450 extra-pair fertilizations seem not to occur among different colonies, as expected. We can therefore

451 also exclude that our data were confounded by 'edge' effects, i.e. by missed paternity events by

452 individuals from the focal colonies that fertilized females from other colonies, and be confident that

453   all the biological offspring of the focal males that reached blood sampling age were identified,

454   thereby providing unbiased estimates of realized LRS.

455   Admittedly, however, our study did not include four- or more years-old individuals because the

456   study spanned over 5 years (2012-2016), the first year (2012) served to identify the individuals that

457   were 1-year-old recruits in 2013 while the last year (2016) served to identify the individuals that

458   had their last breeding season in 2015. However, relatively old (4 or more years) individuals are

459   relatively rare (Romano et al. 2016 b). While the present results should be considered with this

460   caveat in mind, we are confident that this feature of our data did not markedly bias the results and

461   certainly did not produce spurious evidence of selection for larger lifespan or ornament size because

462   LRS is expected to increase with lifespan and covary positively with tail length. In addition, tail

463   ornament size is consistent within individuals, after discounting the effect of age on ornament size.

464   Notably, the frequency of extra-pair offspring as estimated by the proportion of offspring sired in

465   broods from other pairs relative to the total number of offspring sired was considerably smaller than

466   in previous studies of the same geographical population (Saino et al. 1997; Møller et al. 1998). We

467   speculate that this could result from a combination of factors. First, barn swallow populations have

468   declined by as much as 50% during the last decade (Ambrosini et al. 2012), potentially reducing the

469   scope for sperm competition, if sperm competition increases with breeding density (Westneat and

470   Sherman 1997; Møller and Ninni 1998). It should also be noticed that previous extra-pair paternity

471   estimates were mostly obtained from colonies settled in large cowsheds with a large number of

472   breeding pairs in the same room and typically little physical isolation between nests. This could

473   have boosted sperm competition by increasing promiscuity among breeding pairs. Currently, such

474   large colonies have considerably declined in number and no such type of colony is represented in

475   the present sample. It should be emphasized, however, that the colonies where the present study was

476   carried out do not represent exceptions, but, rather, the rule in terms of size and topographical

477   scatter of breeding pairs among farm rooms (Ambrosini et al. 2012), and we deliberately choose the

478 three colonies to represent the whole spectrum of variation between small and relatively large

479 colonies. Second, in a declining population like our focal one the scope for sexual selection may be

480 reduced if decline in population size also entails erosion of additive genetic variance in male

481 quality, thereby reducing the scope for adaptive differential female preference for particular males.

482 Extensive, long-term analysis of variation in the variance in the size of male tail ornaments will

483 provide a clue as to whether reduction in the frequency of extra-pair paternities is linked to a

484 reduction in the variance in male sexual attractiveness.

485 In conclusion, we showed that selection due to variation in LRS currently exists on lifetime and

486 length of the ornamental tail feathers and size of the white spots on the tail but not on other

487 ornaments including ventral plumage coloration or non-sexual traits in a population of the socially

488 monogamous barn swallow. Extra-pair fertilization analysis showed that selection is mediated by

489 certainty of paternity of own social offspring rather than by success in fertilizing extra-pair females.

490 These results on selection due to variance in LRS were obtained while controlling for several

491 potentially confounding factors, including 'edge effects' and non-random sampling of the study

492 individuals.

493

494   **Conflicts of interest**

495   The authors declare no conflicts of interest.

496

497 **Data archiving**

498 The data on which the study is based will be deposited in DRYAD.

499

500   **References**

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662

Table 1. Selection differentials on lifespan, morphological and colour traits of male barn swallows for total lifetime reproductive success ($rLRS_{tot}$), and number of within- ($rLRS_{wpo}$) or extra-pair ($rLRS_{epo}$) offspring. Effect sizes (Pearson's r) and sample size for number of males are reported. For 2 individuals tail asymmetry could not be measured due to breakage of either outermost tail feather.

	N	$rLRS_{tot}$				$rLRS_{wpo}$				$rLRS_{epo}$			
		Coefficient (SE)	r	t	P	Coefficient (SE)	r	t	P	Coefficient (SE)	r	t	P
Lifespan	79	1.112 (0.090)	0.816	12.39	<0.001	1.049 (0.092)	0.793	11.44	<0.001	1.547 (0.285)	0.526	5.42	<0.001
Morphological traits													
Tarsus length	79	0.131 (0.118)	0.126	1.17	0.268	0.091 (0.114)	0.091	0.80	0.427	0.403 (0.252)	0.180	1.60	0.113
Wing length	79	0.118 (0.127)	0.105	0.92	0.358	0.125 (0.123)	0.115	1.02	0.313	0.066 (0.277)	0.027	0.24	0.812
Tail length	79	0.350 (0.122)	0.311	2.87	0.005	0.387 (0.116)	0.355	3.33	0.001	0.095 (0.277)	0.039	0.34	0.733
Tail asymmetry	77	-0.148 (0.130)	-0.131	-1.14	0.258	-0.150 (0.126)	-0.136	-1.19	0.237	-0.135 (0.283)	-0.055	-0.48	0.634
Tail white spots	79	0.126 (0.127)	0.112	0.99	0.325	0.048 (0.124)	0.044	0.39	0.701	0.662 (0.266)	0.273	2.49	0.015
Tetrahedral feather colour traits													
θ	79	-0.097 (0.128)	-0.086	-0.76	0.449	-0.105 (0.124)	-0.085	-0.85	0.397	-0.041 (0.277)	-0.017	-0.15	0.882
φ	79	0.016 (0.128)	0.014	0.12	0.904	0.013 (0.124)	0.012	0.11	0.916	0.032 (0.277)	0.013	0.12	0.909
rA	79	0.144 (0.127)	0.128	1.14	0.260	0.156 (0.123)	0.143	1.27	0.208	0.063 (0.277)	0.026	0.23	0.821

Table 2. Selection gradients on lifespan, morphological and 'visible' plumage chroma for total lifetime reproductive success (rLRS$_{tot}$). The sample included 77 males for which complete phenotypic information was available.

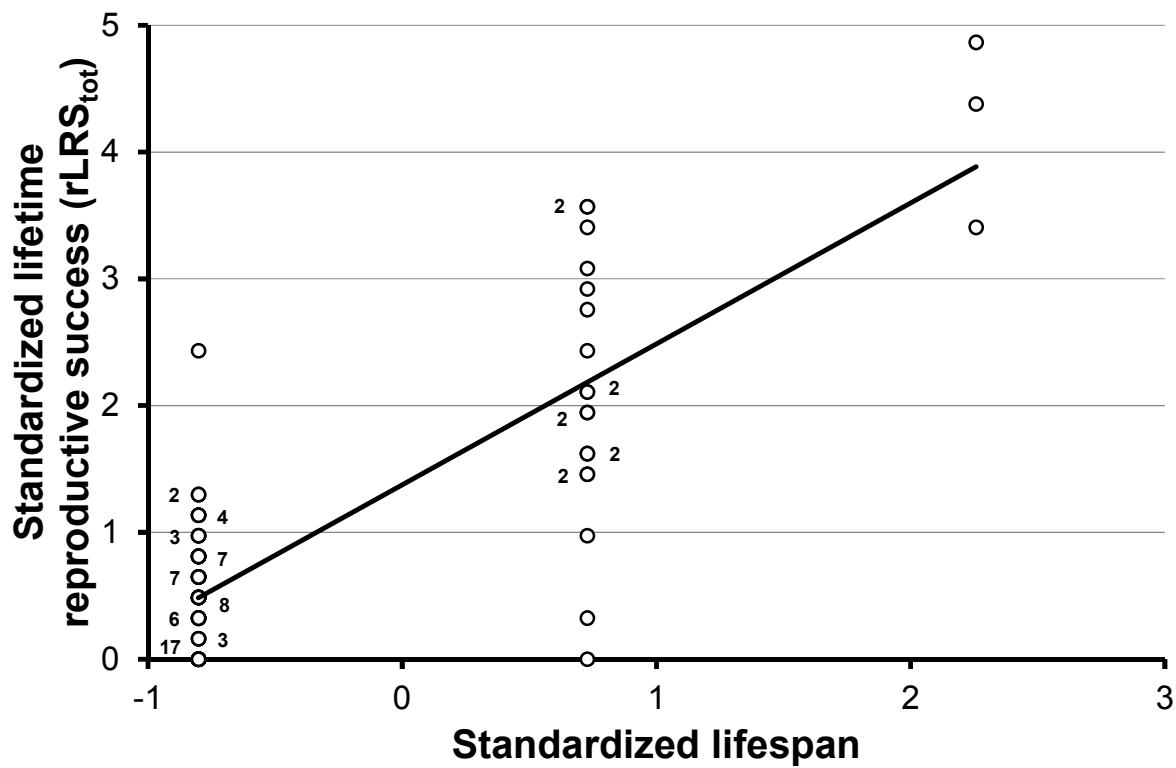|	Coefficient (SE)	t	P
Lifespan	1.050 (0.090)	11.89	<0.001
Tarsus length	-0.049 (0.074)	-0.46	0.513
Wing length	0.002 (0.079)	0.02	0.983
Tail length	0.225 (0.078)	2.88	0.005
Tail asymmetry	-0.136 (0.077)	-1.77	0.082
Tail white spots	0.140 (0.073)	1.93	0.058
θ	-0.126 (0.073)	-1.74	0.086

Legends to figures:

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

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

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

Figure 4. Lifetime reproductive success ($rLRS_{tot}$) expressed as the ratio between the total number of offspring produced by individual males (n = 79) throughout their life and the population mean in relation to wing length corrected for age (see Methods) and standardized to mean = 0 and a variance = 1. The quadratic regression line shows stabilizing selection. Larger dots indicate two or three overlaying data points.
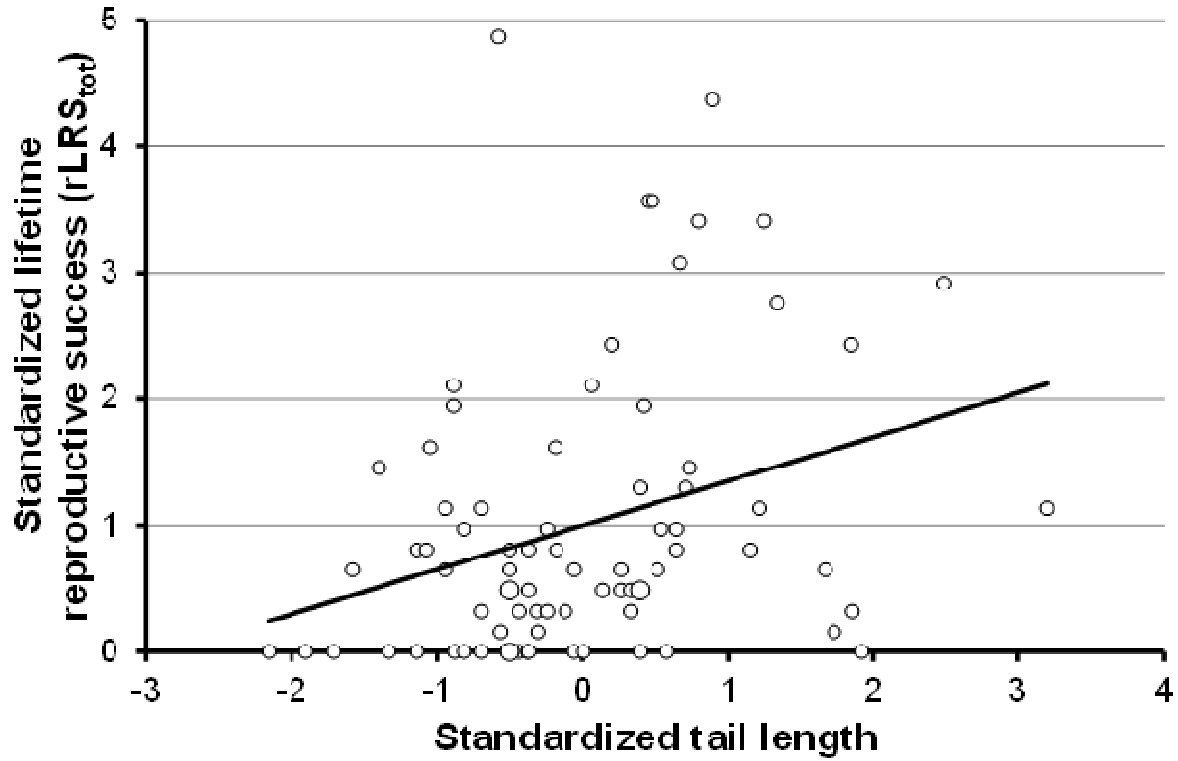
Figure 1.

Figure 2.

Figure 3

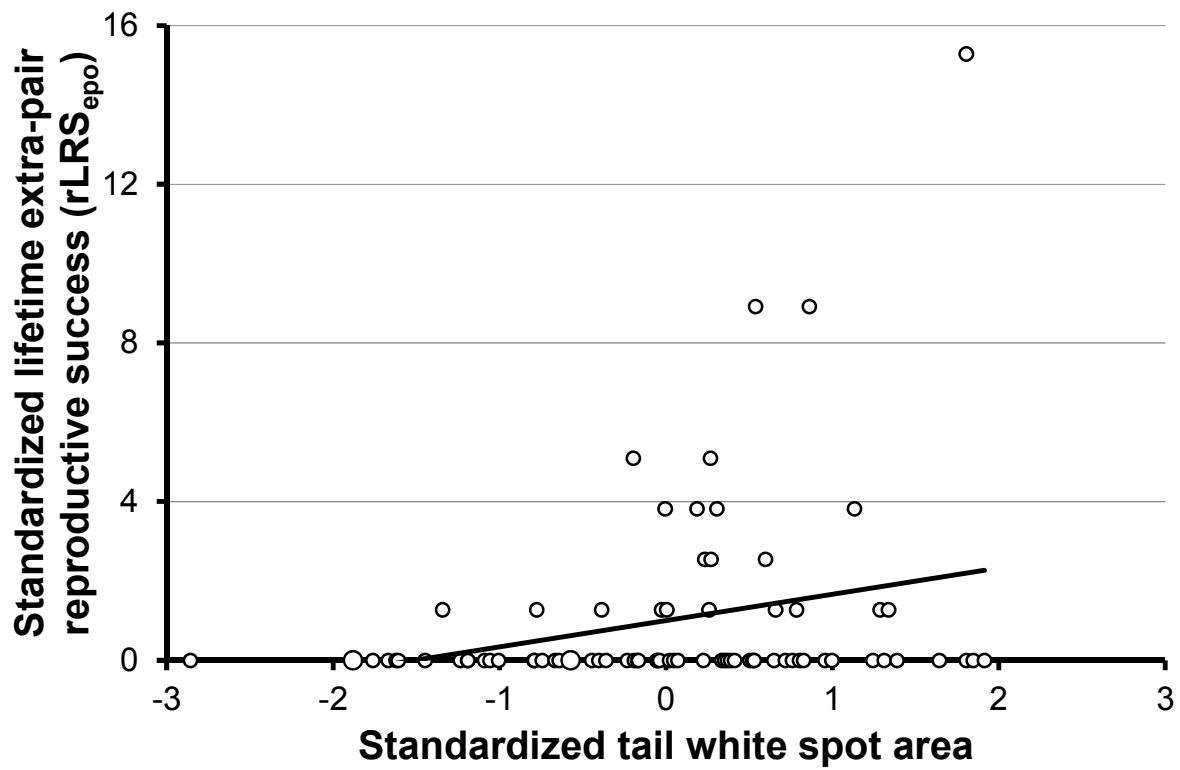Figure 4