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Extra food provisioning reduces extra-pair paternity in the lesser kestrel (Falco naumanni)

Abstract:	Female promiscuity can function to acquire both direct and indirect benefits from their social mate and extra-pair males. In many raptor species, intense mate-feeding significantly contributes to female energy requirements before and during egg laying. Moreover, females may use mate-feeding effort to assess male quality. In this study of the lesser kestrel (Falco naumanni), we aimed at experimentally manipulating the female's perception of mate quality by providing females with extra food during egg laying, and evaluated the occurrence of extra-pair paternity in food-supplemented and control broods by parentage analyses. No extra-pair offspring (EPO) was found among 19 food-supplemented broods, whereas EPO occurred in five out of 17 control broods. No significant differences in morphological traits, body condition and reproductive success were found between faithful and unfaithful females. However, clutches containing EPO were laid later in the breeding season. Moreover, un-cuckolded males had longer tarsi than cuckolded ones, indicating larger body size. Hence, extra food provisioning and early breeding reduced the occurrence of EPO in lesser kestrels. In addition, we confirmed the occurrence of intraspecific brood parasitism, as five nestlings were not the offspring of the brooding female. The results of our food-provisioning experiment support the idea that mate-feeding ability is a reliable indicator of male quality, and are in accordance with the hypothesis that male mate-feeding behaviour is a sexually selected trait.

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3   and extra-pair males. In many raptor species, intense mate-feeding significantly contributes to

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5   effort to assess male quality. In this study of the lesser kestrel (*Falco naumanni*), we aimed at

6   experimentally manipulating the female's perception of mate quality by providing females with

7   extra food during egg laying, and evaluated the occurrence of extra-pair paternity in food-

8   supplemented and control broods by parentage analyses. No extra-pair offspring (EPO) was found

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18

19 **Keywords** food supplementation, lesser kestrel, paternity, promiscuity, sperm competition,

20 intraspecific brood parasitism.

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22

23   **Introduction**

24

25   In vertebrate taxa showing pair bonds, females may copulate with males other than their social mate

26   (Avise et al. 2002, Griffith et al. 2002, Cohas and Allainé 2009). Extra-pair copulations occur in ca.

27   90% of bird species, despite monogamy is the most common social reproductive strategy (Griffith

28   et al. 2002). The benefits of extra-pair fertilizations are debated and may differ between the sexes.

29   Males should engage in extra-pair copulations whenever the advantages of siring extra-pair

30   offspring exceed the costs of seeking extra-pair mates (Birkhead and Møller 1992). The latter may

31   include losing parentage of own social offspring or increasing the risk of contracting sexually

32   transmitted diseases (Birkhead and Møller 1992). Females, on the other hand, can hardly increase

33   their reproductive success from extra-pair mating, except in cases of low fertility of their social

34   mate. However, whenever females have at least partial control over mating, they may gain various

35   indirect and direct benefits by being promiscuous (Hedrick 1988, Westneat 1990, Gray 1997,

36   Jennions and Petrie 2000, Tregenza and Wedell 2000, Hasson and Stone 2009). Conversely, for

37   males, being cuckolded represents a net fitness cost. Males have evolved two main strategies to

38   increase paternity assurance, i.e. mate guarding and frequent copulations (Møller and Birkhead

39   1991). Mate guarding is performed mainly during the female's fertile period (Birkhead and Møller

40   1992), while high copulation frequency occurs in species where males cannot intensively guard

41   their mates. For instance, the latter can be the case in species where feeding grounds are far from

42   the nesting sites or where males perform mate-feeding (González-Solís et al. 2001).

43   Mate-feeding (or courtship-feeding), whereby males provide food to their mates, has been

44   documented in many species to occur during the pre-laying, laying or egg incubation stages. Males

45   may partly contribute to female food intake in some species (Boulton et al. 2010); while in others,

46   female food intake entirely depends on male provisioning (Poonswad et al. 2004). Different, but not

47   mutually exclusive, hypotheses have been proposed to explain the occurrence of mate-feeding

48   behaviour by males, but the functional evolution of this behaviour is still debated. These can be

2

49   broadly classified as based on natural or sexual selection (Korpimäki et al. 1996, Villarroel et al.

50   1998, Velando 2004, Tryjanowski and Hromada 2005, Galván and Sanz 2011). In the context of

51   natural selection, females may obtain direct benefits from mate-feeding, in terms of improved body

52   condition, for instance during periods of high nutritional need, such as egg laying and incubation

53   (Nager et al. 1997, Ramsay and Houston 1997, Reynolds 2001). As a consequence of a better

54   nutritional status, females can advance laying date, shorten the incubation period, and improve other

55   fitness-related traits such as clutch size, egg mass, and hatching and fledging success; as a

56   consequence, both male and female fitness can be positively affected (Galván and Sanz 2011).

57   Conversely, according to the set of hypotheses related to sexual selection, mate-feeding may

58   have evolved not only under the influence of energetic constraints but also as a signal of male

59   quality or as a reinforcement of pair bonds. Indeed, species where males feed their females are also

60   those where males invest more in feeding their offspring (Møller and Cuervo 2000). Mate-feeding

61   could thus be exploited by females to evaluate their mate parental ability and adjust their

62   reproductive investment accordingly (Nisbet 1973, Simmons 1988, Carlson 1989, Korpimäki

63   1989). In addition, females may trade access to frequent copulation for food (Foote et al. 2011), and

64   mate feeding may therefore foster male's certainty of paternity by diluting ejaculates from potential

65   extra-pair males (Simmons 1990).

66   In many raptors, males perform extensive mate-feeding and contribute to egg incubation and

67   offspring food provisioning (Sarasola et al. 2018). The consequent elevated costs of cuckoldry for

68   male raptors have favoured the evolution of behaviours aimed at promoting paternity assurance

69   (Birkhead and Møller 1992). In particular, raptors perform frequent copulations over extended

70   periods, even during the female non-fertile period (Negro et al. 1992, Villarroel et al. 1998,

71   Mougeot 2000, Martínez et al. 2019), possibly with the function of assessing mate quality and/or to

72   reinforce pair bonds (Villarroel et al. 1998, Mougeot 2000, Mougeot et al. 2002). In addition,

73   copulation frequency and fertilization success positively covary with the intensity of male mate-

74 feeding, which in turn can be exploited by females to assess the quality of the partner and its future

75 investment in offspring provisioning (Donázar et al. 1992; Mougeot et al. 2002).

76   In this study of the lesser kestrel (*Falco naumanni*), a small (ca. 120 g) diurnal raptor with

77 reverse sexual dimorphism and biparental care of the offspring (Cramp 1998), we aimed at

78 investigating whether natural or sexual selection affected the evolution of male mate-feeding

79 behaviour. To this end, we provided extra food to lesser kestrel breeding pairs by placing thawed

80 laboratory mice within the nestboxes during egg laying, and investigated the occurrence of extra-

81 pair offspring (hereafter EPO) in broods of pairs subjected to food-supplementation compared to

82 control broods. Previous studies of this species showed that EPO varied between 3.4 % and 7.3 %

83 (Alcaide et al. 2005), values that are within the typical range observed in raptors (0-11 %)

84 (Rosenfield et al. 2015). Male lesser kestrels start to intensively feed their mates a few days before

85 the onset of egg laying and continue for the whole incubation period (Sarasola et al. 2018). This

86 behaviour may play a pivotal role in determining female condition during the pre-laying and laying

87 period (Donázar et al. 1992). Moreover, males intensively contribute to parental care, incubating the

88 eggs and feeding offspring (Donázar et al. 1992, Cramp 1998). Females may thus assess male future

89 parental investment through their mate-feeding rates, as the intensity of mate-feeding is positively

90 correlated with nestling feeding rates (Donázar et al. 1992). In most cases, females are directly fed

91 by their partner, but males commonly deposit prey items within the nest cavity for later

92 consumption by their mates (Cramp 1998). Hence, providing extra food within the nestboxes

93 mimicked a natural condition of intense male mate-feeding behaviour. We thus expected extra food

94 to directly influence not only female body condition but also female's perception of mate quality

95 (O'Brien and Dawson 2011). This is different from previous studies assessing the effects of extra

96 food provisioning on extra-pair paternity, where extra food was provided in the surrounding of the

97 nest, which rather mimicked favourable environmental conditions for breeding (Kaiser et al. 2015;

98 Václav et al. 2003; Westneat 1994). According to natural selection hypotheses for the evolution of

99 mate-feeding, we expected food-supplementation to improve female body condition and to result in

100 higher reproductive success, without affecting the occurrence of EPO in broods. Conversely, if male

101 mate-feeding behaviour is a sexually selected trait, food-supplementation is expected to foster male

102 certainty of paternity and reduce the risk of being cuckolded, resulting in a higher frequency of EPO

103 in control compared to food-supplemented broods.

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105

106 **Methods**

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108 **General field procedures**

109 A detailed description of the study area and general field procedures, including data collection and

110 food supplementation experimental design, is reported in Podofillini et al. (2019). Briefly, the study

111 was performed at the Matera lesser kestrel colony (southern Italy, 40°67′N, 16°60′E), during April–

112 July 2016. Here, many pairs breed in specially designed concrete nestboxes (see Podofillini et al.

113 2018 for further details). For the present food-provisioning experiment, we relied on 100 pairs (50

114 food-supplemented, 50 controls) breeding in nestboxes that were checked three times a week to

115 determine the onset of egg laying and clutch size (Podofillini et al. 2019). When the first egg was

116 found, pairs were alternately assigned to the food-supplemented or the control group. Three thawed

117 commercial white laboratory mice (ca. 20 g each) were placed in the nestbox of food-supplemented

118 pairs every two days during egg laying, while a simulation of mice insertion was performed in the

119 nestboxes of control pairs following the same schedule. Mice consumption by the focal females was

120 confirmed by the regular recovery of white fur in regurgitated pellets within the nestboxes. Each

121 nestbox was regularly checked after hatching. Nestling blood samples for genetic analyses were

122 collected at 7–11 days after hatching of the first egg, while brood size was defined as the number of

123 nestlings in the nestbox at 14–18 days after hatching of the first egg. Adults were captured in

124 nestboxes and individually marked. Upon capture, we recorded body mass (to the nearest 0.1 g),

125 wing length (to the nearest 1 mm using a ruler), keel and tarsus length (to the nearest 0.1 mm using

126 a dial calliper) and collected a blood sample for parentage analyses (ca. 500 µl). The scaled mass

127 index (SMI hereafter) was used as an index of body condition (details in Podofillini et al., 2019).

128 SMI standardizes body mass at a linear body measurement (here, keel length) according to the

129 scaling relationship between mass and length (Peig and Green 2009, 2010).

130 For this study we focused our attention on those broods for which: 1) we were able to mark,

131 collect blood sample and successfully obtain microsatellite profiles from both parents; 2) all eggs

132 hatched successfully; 3) at least half of the hatched nestlings were blood sampled and genotyped

133 This selection resulted in a sample of 36 broods, 19 of which were food-supplemented and 17 were

134 controls. Overall, we could genotype 117 nestlings out of the 152 hatched from these 36 broods.

135 Hatched nestlings that were not genotyped mostly disappeared from the nestboxes before blood

136 sampling due to early nestling mortality. Early nestling mortality was not affected by our food-

137 provisioning experiment, as the number of genotyped nestlings did not significantly differ between

138 food-supplemented and control nests (t-test, $t_{34} = 1.16$, $p = 0.25$).

139

140 **Genetic analyses**

141 Blood samples were maintained on ice until centrifugation within 12 hours after collection and

142 subsequently stored at $-20 \degree C$ until molecular sexing and genotyping analyses were performed.

143 DNA was extracted from red blood cells by alkaline lysis using 6 µl of blood in 100 µl of a 50 mM

144 NaOH at 100°C for 20 minutes (Saino et al. 2008) and quantified by a spectrophotometer. DNA

145 was subsequently diluted to a final concentration of 50–100 ng/µl for molecular sexing and 10 ng/µl

146 for genotyping. All genetic analyses were performed blind to the experimental group.

147 Nestlings were molecularly sexed according to Griffiths et al. (1998) after PCR amplification of the

148 sex-specific avian CHD-1 gene (see Saino et al. 2008). A total of 72 adults (36 males and 36

149 females) and 117 nestlings were successfully genotyped across 6 polymorphic microsatellite loci,

150 i.e. Fp5, Fp31, Fp46-1, Fp79-4, Fp89 (Nesje et al. 2000) and Cl347 (Alcaide et al. 2008). Forward

151 primers were labelled with 6-FAM, HEX or NED fluorochromes (Table 1). Each locus was

152   amplified through PCR in a 25 μl reaction (including approximately 10 ng of DNA as template)

153   using puReTaq Ready-To-Go PCR beads (Amersham Bioscience, Freiburg, Germany), according to

154   the manufacturer's instructions. The PCR thermal profile was 3 min of 95°C denaturation followed

155   by 35 cycles of 30 s/95°C, 30 s/55°C and 40 s/72°C, with a 5 min final elongation step. PCR

156   products (1 μl) were mixed with 12 μl of formamide and 0.2 μl of LIZ-500 size standard (Applied

157   Biosystems, Foster City, CA, USA) and then run on an ABI 3130 automated sequencer (Applied

158   Biosystems, Foster City, CA, USA). For each locus allele sizes were identified using Genemapper

159   4.0 (Applied Biosystems, Foster City, CA, USA). Each PCR and sequencing run was conducted

160   including negative controls. The number of alleles, observed and expected heterozygosity,

161   polymorphic information content and frequency of null alleles were assessed using Cervus 3.0.7

162   software (Kalinowski et al. 2007) (Table 1). In addition, Cervus 3.0.7 was used to perform

163   parentage assignment and to calculate the combined non-exclusion probability, that was 7.0×10^{-2}

164   for the first and 1.1×10^{-2} for the second parent. We scored as EPO those individuals for which at

165   least one locus was not inherited from the social father. In addition, nestlings for which at least one

166   locus was not inherited from the social mother were scored as intraspecific brood parasitic offspring

167   (IBPO), a condition rarely found in Falconiformes but previously shown to occur in the lesser

168   kestrel (Yom-Tov 2001).

169

170   **Statistical analyses**

171   We relied on generalized linear models (GLM) to investigate whether individual morphology

172   (wing, tarsus and keel length) and SMI were affected by food supplementation (0 = control, 1 =

173   food-supplemented), while controlling for sex (0 = female, 1 = male). Differences in reproductive

174   success (laying date, clutch size, and brood size) between food-supplemented and control broods

175   were assessed by means of t-test.

176   Because of the small sample size of EPO (see Results), to investigate the association

177   between EPO occurrence in a brood and food supplementation we relied on the Fisher's exact test.

178 The sample of IBPO broods was too low to perform any statistical analysis of association with food

179 supplementation.

180   All subsequent analyses were restricted to control broods because no EPO was detected

181 among food-supplemented broods. We assessed within-sex differences in morphology (wing, tarsus

182 and keel length) and SMI of parents between broods with EPO and those without EPO by means of

183 t-tests; t-tests were also applied to investigate differences in laying date, clutch or brood size

184 between broods with and without EPO. Finally, we tested whether the probability of a nestling

185 being male varied according to EPO (0 = within-pair offspring; 1 = extra-pair offspring) by a

186 binomial generalized linear mixed model (GLMM) with brood identity as a random intercept effect

187 (model fitted by the R lme4 package ver. 1.1-21 (Bates et al. 2014).

188

189

190 **Results**

191

192 Extra food did not significantly affect SMI or morphology ($F_{1,68} < 2.91$, $p > 0.09$); these models

193 also revealed that females had significantly shorter tarsi ($F_{1,68} = 5.97$, $p = 0.02$) and larger SMI

194 ($F_{1,67} = 51.35$, $p < 0.001$) as compared to males (see Podofillini et al. 2019 for further details). Two-

195 way interactions between food supplementation and sex were not significant ($F_{1,66} < 1.78$, $p > 0.19$).

196 Laying date, clutch size, and brood size did not significantly differ between food-supplemented and

197 control broods ($|t_{34}| < 1.03$, $p > 0.31$).

198   Out of the 117 genotyped nestlings from the 36 broods, eight were EPO (6.8 %).

199 Specifically, one brood contained three EPO, one brood contained two EPO, and the remaining

200 three contained a single EPO. No EPO was found among the 19 food-supplemented broods,

201 whereas EPO occurred in five out of 17 control broods, a statistically significant difference

202 (Fisher's exact test, $p = 0.016$). Furthermore, we detected five IBPO in four broods (one control

203   brood contained two IBPOs; two control broods contained one IBPO; one food-supplemented brood

204   contained one IBPO). EPO and IBPO did not co-occur in any brood.

205   Among control broods, faithful and unfaithful females did not significantly differ in SMI or

206   morphology (Table 2). Similarly, SMI and morphology of cuckolded males did not differ from that

207   of un-cuckolded males, except for tarsus length, that was larger among un-cuckolded males (Table

208   2). Clutch and brood size did not significantly differ between broods containing or not EPO (t_{15} =

209   0.44, p = 0.67 and t_{15} = 0.93, p = 0.37, respectively). However, unfaithful females laid their first egg

210   on average 6.28 days later in the season compared to faithful ones (mean laying date of unfaithful

211   females: May 16 (2.4 SD); faithful females: May 10 (8.7 SD); unequal variances t-test, $t_{14.07}$ = 2.30,

212   p = 0.037). The observed difference in laying date between broods with and without EPO

213   corresponds to ca. 1 SD of the mean laying date of the population in the study year (mean laying

214   date = May 13 (8.0 SD), n = 100 clutches). Two out of eight EPO (25.0 %) and 24 out of 44 within-

215   pair control offspring (54.5 %) were males. However, nestling sex was not significantly predicted

216   by EPO status (binomial GLMM, estimate: -1.97 (1.30 s.e.), χ^2_1 = 3.39, p = 0.07).

217

218

219   **Discussion**

220   Our experimental study aimed at exploring alternative explanation for the evolution of mate feeding

221   behaviour in a sexually dimorphic diurnal raptor, the lesser kestrel. To this end, we provided extra-

222   food in nestboxes during egg laying to breeding pairs, therefore manipulating female perception of

223   social mate quality, and analysed the effects of food supplementation on patterns of extra-pair

224   paternity. Overall, EPO were found in 13.9 % of the broods, with 6.8 % of nestlings being EPO,

225   similarly to previous studies of the same species (Negro et al. 1996, Alcaide et al. 2005, 2010). All

226   EPO belonged to the control group. This finding is in accordance with similar previous studies of

227   passerine bird species, in which food supplementation decreased female propensity to seek extra-

228   pair mates, thus increasing social mate reproductive success (Westneat 1994, Václav et al. 2003,

229   O'Brien and Dawson 2011, Kaiser et al. 2015).

230   The absence of EPO in broods belonging to the food-supplemented group provided support

231   to the hypothesis that mate-feeding behaviour has evolved by sexual selection, since females paired

232   to males showing a larger investment in mate-feeding are expected to be more prone to perform

233   frequent copulations with the social mate, providing greater certainty of paternity. In addition, male

234   mate-feeding rate, egg incubation and offspring feeding effort all positively covary in the lesser

235   kestrel (Donázar et al. 1992). Thus, greater female fidelity to males of food-supplemented pairs may

236   be also due to the direct fitness benefits that a female may expect to acquire from a male providing

237   abundant food (i.e. a male of high perceived quality). The difference in the occurrence of EPO in

238   food-supplemented and control broods was not associated to changes in female condition due to

239   extra food, as extra food did not significantly affect body condition (see also Podofillini et al. 2019).

240   In addition, there were no differences in clutch and brood size between food-supplemented and

241   control broods. Hence, our findings are less compatible with the hypothesis that mate-feeding has

242   evolved by natural selection, because in that case we would have expected food supplementation to

243   foster proxies of breeding success. However, it should be emphasized that proper tests of the natural

244   selection hypothesis would require food supplementation experiments to be performed under

245   contrasting ecological conditions (i.e. favourable vs. poor years). Indeed, direct nutritional benefit

246   may become more evident under adverse conditions (Václav et al. 2003), while conditions in the

247   study year were relatively favourable, as breeding success at the population level was relatively

248   high (see Podofillini et al. 2019).

249   No differences in morphology, SMI and reproductive success (in terms of clutch and brood

250   size) between faithful and unfaithful females were detected. In contrast, faithful and unfaithful

251   females showed a statistically significant difference in timing of breeding, as clutches with at least

252   one EPO were laid later in the breeding season. This may be related to the hypothesis that the

253   seasonal decline in male reproductive success, and thus the increase in male paternity loss in the

10

254   own nest, is related to a seasonal decline of individual quality (Williams 2012). In most bird

255   species, older, more experienced, and high-quality individuals are those that breed earlier (Newton

256   and Marquiss 1984, Zabala and Zuberogoitia 2014), while late breeders may be low-quality

257   individuals that miss the optimal time 'window' for reproduction (Drent and Daan 1980). In the

258   lesser kestrel, it is known that high-quality and older males arrive earlier to the breeding grounds,

259   obtain the most suitable nest sites and breed earlier (Serrano et al. 2003, Catry et al. 2016). We may

260   therefore speculate that early-breeding females, mated with high-quality, early-arriving males,

261   avoid seeking extra-pair copulations, whereas late-breeding females, mated with-low quality

262   partners, actively seek extra-pair matings. This speculation is in line with the observation that

263   cuckolded males were smaller (i.e., they had shorter tarsi) than un-cuckolded conspecifics,

264   suggesting that they were of lower quality. Indeed, skeletal size positively covaries with male

265   competitive ability and viability in many bird species (Potti and Merino 1994). By seeking extra-

266   pair fertilizations with larger - possibly early-arriving - males, late-breeding females may therefore

267   obtain genes for high offspring viability (Bouwman et al. 2006).

268   Five out of the 117 genotyped nestlings (4.3 %) were found not to be genetically related to

269   any of their parents, which we interpret as evidence of intraspecific brood parasitism. The

270   occurrence of intraspecific brood parasitism is slightly higher than previously reported for this

271   species (2.2 %; two out of 87 nestlings; Negro et al. 1996). In birds, conditions favouring

272   intraspecific brood parasitism may include limitation of female ability to breed in adverse

273   environmental conditions, clutch loss due to predation during egg laying, the occurrence of

274   alternative reproductive tactics, or simply the occurrence of constraints in obtaining a breeding

275   territory or mate (see Lyon and Eadie 2008 for a detailed review). In our lesser kestrel population,

276   clutch loss due to predation is extremely rare, and in the study year the ecological conditions were

277   generally favourable (see Podofillini et al. 2019). The occurrence of intraspecific brood parasitism

278   may thus be explained by the presence at the colony of prospecting yearling females, which mostly

279   do not breed despite being sexually mature (Hiraldo et al. 1996). For these inexperienced females,

280   the potential difficulties in acquiring a partner may not be compensated by adequate fitness returns.

281   Hence, rather than skipping reproduction, some of these females may opt for laying eggs in the

282   nests of older females.

283   In conclusion, we demonstrated that experimental food provisioning reduced females'

284   propensity to seek for extra-pair copulations, likely by altering females' perception of male quality.

285   This result provide support to the hypothesis that sexual rather than natural selection is a major

286   driver for the evolution of mate-feeding behaviour in the lesser kestrel. Unfaithful females were not

287   larger or in better body condition than faithful ones and did not show a greater reproductive success,

288   but they started to breed later in the breeding season. In addition, smaller males suffered a reduction

289   of paternity in their social nest. Therefore, our findings support the hypothesis that females assess

290   the quality of their social partner through its mate-feeding ability, and suggest that females may be

291   keener to seek for extra-pair copulations when mated with late-breeding, likely low-quality,

292   partners.

293

304

305   **Data availability** The data on which the study is based will be deposited in DRYAD upon

306   acceptance.

307

308   **Author contributions** AG, MG, JGC, DR conceived and designed the experiments. GCS, MG,

309   JGC, DR collected data in the field. NT, AG, GCS Performed laboratory assay. AC, RA, DR

310   analysed the data. AC, DR wrote the initial draft of the manuscript. All authors read, edited and

311   approved the final manuscript.

312

313   **Conflicts of interest** We declare there is no conflict of interest.

314

315   **Permit** Capture and handling was performed by ISPRA under the authorization of Law 157/1992

316   [Art. 4 (1) and Art. 7 (5)].

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467

468 Table 1. Details and statistics of microsatellite loci used to assess parentage. Size: range of observed

469 alleles (bp); K: number of alleles; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity;

470 PIC: polymorphic information content; F_{null}: frequency of null alleles.

471

Locus	Label	Primer sequences 5′−3′	Size (bp)	K	H_{obs}	H_{exp}	PIC	F_{null}
Fp5	6-FAM	F: CCGTTCTGGAGTCAAAAC R: CATGCAGCACTTTATTCAG	99-109	6	0.79	0.74	0.71	-0.038
Fp31	HEX	F: ATCACCTGCACATAGCTG R: TTTAGCTCCTCTCTCTCAC	111-143	10	0.62	0.70	0.65	0.053
Fp46-1	NED	F: TTAGCCTCGCAGCTTCAG R: GTAATGAAAAGTCTTTGGGG	120-144	10	0.47	0.53	0.51	0.068
Fp79-4	6-FAM	F: TGGCTTCTCTTATCAGTAAC R: GGCTGGGTGGAATTAAAG	126-166	17	0.76	0.90	0.89	0.086
Fp89	HEX	F: CTCTGCCCTGAATACTTAC R: GAATCTTGTTTGCATTGGAG	117-123	4	0.60	0.54	0.48	-0.055
Cl347	6-FAM	F: TGTGTGTGTAAGGTTGCCAAA R: CGTTCTCAACATGCCAGTTT	104-124	11	0.75	0.75	0.72	-0.004

472

473

474 Table 2. Differences (mean and SD) in morphology and SMI of unfaithful (with EPO, n = 5) and

475 faithful (without EPO, n = 12) control females and of cuckolded (with EPO) and un-cuckolded

476 (without EPO) control males. SMI = scaled mass index (see Methods and Podofillini et al. 2019).

477

Trait	With EPO	Without EPO	*t*	*df*	P
Females					
Wing (mm)	236.6 (2.0)	237.9 (5.9)	0.26	15	0.80
Tarsus (mm)	31.0 (1.2)	31.2 (2.0)	0.34	15	0.74
Keel (mm)	31.4 (2.1)	31.7 (2.6)	0.14	15	0.89
SMI (g)	150.2 (6.2)	158.2 (15.3)	0.80	15	0.44
Males					
Wing (mm)	231.2 (7.4)	235.9 (4.9)	1.69	15	0.11
Tarsus (mm)	30.6 (1.7)	32.5 (1.3)	2.40	15	0.030*
Keel (mm)	31.5 (1.1)	31.7 (1.5)	0.14	15	0.89
SMI (g)	132.2 (11.6)	134.8 (12.4)	0.57	15	0.58

478

1 **Abstract**

2 Female promiscuity can function to acquire both direct and indirect benefits from their social mate

3 and extra-pair males. In many raptor species, intense mate-feeding significantly contributes to

4 female energy requirements before and during egg laying. Moreover, females may use mate-feeding

5 effort to assess male quality. In this study of the lesser kestrel (*Falco naumanni*), we aimed at

6 experimentally manipulating the female's perception of mate quality by providing females with

7 extra food during egg laying, and evaluated the occurrence of extra-pair paternity in food-

8 supplemented and control broods by parentage analyses. No extra-pair offspring (EPO) was found

9 among 19 food-supplemented broods, whereas EPO occurred in five out of 17 control broods. No

10 significant differences in morphological traits, body condition and reproductive success were found

11 between faithful and unfaithful females. However, clutches containing EPO were laid later in the

12 breeding season. Moreover, un-cuckolded males had longer tarsi than cuckolded ones, indicating

13 larger body size. Hence, extra food provisioning and early breeding reduced the occurrence of EPO

14 in lesser kestrels. In addition, we confirmed the occurrence of intraspecific brood parasitism, as five

15 nestlings were not the offspring of the brooding female. The results of our food-provisioning

16 experiment support the idea that mate-feeding ability is a reliable indicator of male quality, and are

17 in accordance with the hypothesis that male mate-feeding behaviour is a sexually selected trait.

18

19 **Keywords** food supplementation, lesser kestrel, paternity, promiscuity, sperm competition,

20 intraspecific brood parasitism.

21

22

Introduction

In vertebrate taxa showing pair bonds, females may copulate with males other than their social mate (Avise et al. 2002, Griffith et al. 2002, Cohas and Allainé 2009). Extra-pair copulations occur in ca. 90% of bird species, despite monogamy is the most common social reproductive strategy (Griffith et al. 2002). The benefits of extra-pair fertilizations are debated and may differ between the sexes. Males should engage in extra-pair copulations whenever the advantages of siring extra-pair offspring exceed the costs of seeking extra-pair mates (Birkhead and Møller 1992). The latter may include losing parentage of own social offspring or increasing the risk of contracting sexually transmitted diseases (Birkhead and Møller 1992). Females, on the other hand, can hardly increase their reproductive success from extra-pair mating, except in cases of low fertility of their social mate. However, whenever females have at least partial control over mating, they may gain various indirect and direct benefits by being promiscuous (Hedrick 1988, Westneat 1990, Gray 1997, Jennions and Petrie 2000, Tregenza and Wedell 2000, Hasson and Stone 2009). Conversely, for males, being cuckolded represents a net fitness cost. Males have evolved two main strategies to increase paternity assurance, i.e. mate guarding and frequent copulations (Møller and Birkhead 1991). Mate guarding is performed mainly during the female's fertile period (Birkhead and Møller 1992), while high copulation frequency occurs in species where males cannot intensively guard their mates. For instance, the latter can be the case in species where feeding grounds are far from the nesting sites or where males perform mate-feeding (González-Solís et al. 2001).

Mate-feeding (or courtship-feeding), whereby males provide food to their mates, has been documented in many species to occur during the pre-laying, laying or egg incubation stages. Males may partly contribute to female food intake in some species (Boulton et al. 2010); while in others, female food intake entirely depends on male provisioning (Poonswad et al. 2004). Different, but not mutually exclusive, hypotheses have been proposed to explain the occurrence of mate-feeding behaviour by males, but the functional evolution of this behaviour is still debated. These can be

49   broadly classified as based on natural or sexual selection (Korpimäki et al. 1996, Villarroel et al.

50   1998, Velando 2004, Tryjanowski and Hromada 2005, Galván and Sanz 2011). In the context of

51   natural selection, females may obtain direct benefits from mate-feeding, in terms of improved body

52   condition, for instance during periods of high nutritional need, such as egg laying and incubation

53   (Nager et al. 1997, Ramsay and Houston 1997, Reynolds 2001). As a consequence of a better

54   nutritional status, females can advance laying date, shorten the incubation period, and improve other

55   fitness-related traits such as clutch size, egg mass, and hatching and fledging success; as a

56   consequence, both male and female fitness can be positively affected (Galván and Sanz 2011).

57   Conversely, according to the set of hypotheses related to sexual selection, mate-feeding may

58   have evolved not only under the influence of energetic constraints but also as a signal of male

59   quality or as a reinforcement of pair bonds. Indeed, species where males feed their females are also

60   those where males invest more in feeding their offspring (Møller and Cuervo 2000). Mate-feeding

61   could thus be exploited by females to evaluate their mate parental ability and adjust their

62   reproductive investment accordingly (Nisbet 1973, Simmons 1988, Carlson 1989, Korpimäki

63   1989). In addition, females may trade access to frequent copulation for food (Foote et al. 2011), and

64   mate feeding may therefore foster male's certainty of paternity by diluting ejaculates from potential

65   extra-pair males (Simmons 1990).

66   In many raptors, males perform extensive mate-feeding and contribute to egg incubation and

67   offspring food provisioning (Sarasola et al. 2018). The consequent elevated costs of cuckoldry for

68   male raptors have favoured the evolution of behaviours aimed at promoting paternity assurance

69   (Birkhead and Møller 1992). In particular, raptors perform frequent copulations over extended

70   periods, even during the female non-fertile period (Negro et al. 1992, Villarroel et al. 1998,

71   Mougeot 2000, Martínez et al. 2019), possibly with the function of assessing mate quality and/or to

72   reinforce pair bonds (Villarroel et al. 1998, Mougeot 2000, Mougeot et al. 2002). In addition,

73   copulation frequency and fertilization success positively covary with the intensity of male mate-

74   feeding, which in turn can be exploited by females to assess the quality of the partner and its future

75   investment in offspring provisioning (Donázar et al. 1992; Mougeot et al. 2002).

76   In this study of the lesser kestrel (*Falco naumanni*), a small (ca. 120 g) diurnal raptor with

77   reverse sexual dimorphism and biparental care of the offspring (Cramp 1998), we aimed at

78   investigating whether natural or sexual selection affected the evolution of male mate-feeding

79   behaviour. To this end, we provided extra food to lesser kestrel breeding pairs by placing thawed

80   laboratory mice within the nestboxes during egg laying, and investigated the occurrence of extra-

81   pair offspring (hereafter EPO) in broods of pairs subjected to food-supplementation compared to

82   control broods. Previous studies of this species showed that EPO varied between 3.4 % and 7.3 %

83   (Alcaide et al. 2005), values that are within the typical range observed in raptors (0-11 %)

84   (Rosenfield et al. 2015). Male lesser kestrels start to intensively feed their mates a few days before

85   the onset of egg laying and continue for the whole incubation period (Sarasola et al. 2018). This

86   behaviour may play a pivotal role in determining female condition during the pre-laying and laying

87   period (Donázar et al. 1992). Moreover, males intensively contribute to parental care, incubating the

88   eggs and feeding offspring (Donázar et al. 1992, Cramp 1998). Females may thus assess male future

89   parental investment through their mate-feeding rates, as the intensity of mate-feeding is positively

90   correlated with nestling feeding rates (Donázar et al. 1992). In most cases, females are directly fed

91   by their partner, but males commonly deposit prey items within the nest cavity for later

92   consumption by their mates (Cramp 1998). Hence, providing extra food within the nestboxes

93   mimicked a natural condition of intense male mate-feeding behaviour. We thus expected extra food

94   to directly influence not only female body condition but also female's perception of mate quality

95   (O'Brien and Dawson 2011). This is different from previous studies assessing the effects of extra

96   food provisioning on extra-pair paternity, where extra food was provided in the surrounding of the

97   nest, which rather mimicked favourable environmental conditions for breeding (Kaiser et al. 2015;

98   Václav et al. 2003; Westneat 1994). According to natural selection hypotheses for the evolution of

99   mate-feeding, we expected food-supplementation to improve female body condition and to result in

4

100 higher reproductive success, without affecting the occurrence of EPO in broods. Conversely, if male

101 mate-feeding behaviour is a sexually selected trait, food-supplementation is expected to foster male

102 certainty of paternity and reduce the risk of being cuckolded, resulting in a higher frequency of EPO

103 in control compared to food-supplemented broods.

104

105

106 **Methods**

107

108 **General field procedures**

109 A detailed description of the study area and general field procedures, including data collection and

110 food supplementation experimental design, is reported in Podofillini et al. (2019). Briefly, the study

111 was performed at the Matera lesser kestrel colony (southern Italy, 40°67′N, 16°60′E), during April–

112 July 2016. Here, many pairs breed in specially designed concrete nestboxes (see Podofillini et al.

113 2018 for further details). For the present food-provisioning experiment, we relied on 100 pairs (50

114 food-supplemented, 50 controls) breeding in nestboxes that were checked three times a week to

115 determine the onset of egg laying and clutch size (Podofillini et al. 2019). When the first egg was

116 found, pairs were alternately assigned to the food-supplemented or the control group. Three thawed

117 commercial white laboratory mice (ca. 20 g each) were placed in the nestbox of food-supplemented

118 pairs every two days during egg laying, while a simulation of mice insertion was performed in the

119 nestboxes of control pairs following the same schedule. Mice consumption by the focal females was

120 confirmed by the regular recovery of white fur in regurgitated pellets within the nestboxes. Each

121 nestbox was regularly checked after hatching. Nestling blood samples for genetic analyses were

122 collected at 7–11 days after hatching of the first egg, while brood size was defined as the number of

123 nestlings in the nestbox at 14–18 days after hatching of the first egg. Adults were captured in

124 nestboxes and individually marked. Upon capture, we recorded body mass (to the nearest 0.1 g),

125 wing length (to the nearest 1 mm using a ruler), keel and tarsus length (to the nearest 0.1 mm using

126   a dial calliper) and collected a blood sample for parentage analyses (ca. 500 µl). The scaled mass

127   index (SMI hereafter) was used as an index of body condition (details in Podofillini et al., 2019).

128   SMI standardizes body mass at a linear body measurement (here, keel length) according to the

129   scaling relationship between mass and length (Peig and Green 2009, 2010).

130   For this study we focused our attention on those broods for which: 1) we were able to mark,

131   collect blood sample and successfully obtain microsatellite profiles from both parents; 2) all eggs

132   hatched successfully; 3) at least half of the hatched nestlings were blood sampled and genotyped

133   This selection resulted in a sample of 36 broods, 19 of which were food-supplemented and 17 were

134   controls. Overall, we could genotype 117 nestlings out of the 152 hatched from these 36 broods.

135   Hatched nestlings that were not genotyped mostly disappeared from the nestboxes before blood

136   sampling due to early nestling mortality. Early nestling mortality was not affected by our food-

137   provisioning experiment, as the number of genotyped nestlings did not significantly differ between

138   food-supplemented and control nests (t-test, $t_{34} = 1.16$, p = 0.25).

139

140   **Genetic analyses**

141   Blood samples were maintained on ice until centrifugation within 12 hours after collection and

142   subsequently stored at – 20 °C until molecular sexing and genotyping analyses were performed.

143   DNA was extracted from red blood cells by alkaline lysis using 6 µl of blood in 100 µl of a 50 mM

144   NaOH at 100°C for 20 minutes (Saino et al. 2008) and quantified by a spectrophotometer. DNA

145   was subsequently diluted to a final concentration of 50–100 ng/µl for molecular sexing and 10 ng/µl

146   for genotyping. All genetic analyses were performed blind to the experimental group.

147   Nestlings were molecularly sexed according to Griffiths et al. (1998) after PCR amplification of the

148   sex-specific avian CHD-1 gene (see Saino et al. 2008). A total of 72 adults (36 males and 36

149   females) and 117 nestlings were successfully genotyped across 6 polymorphic microsatellite loci,

150   i.e. Fp5, Fp31, Fp46-1, Fp79-4, Fp89 (Nesje et al. 2000) and Cl347 (Alcaide et al. 2008). Forward

151   primers were labelled with 6-FAM, HEX or NED fluorochromes (Table 1). Each locus was

152 amplified through PCR in a 25 μl reaction (including approximately 10 ng of DNA as template)

153 using puReTaq Ready-To-Go PCR beads (Amersham Bioscience, Freiburg, Germany), according to

154 the manufacturer's instructions. The PCR thermal profile was 3 min of 95°C denaturation followed

155 by 35 cycles of 30 s/95°C, 30 s/55°C and 40 s/72°C, with a 5 min final elongation step. PCR

156 products (1 μl) were mixed with 12 μl of formamide and 0.2 μl of LIZ-500 size standard (Applied

157 Biosystems, Foster City, CA, USA) and then run on an ABI 3130 automated sequencer (Applied

158 Biosystems, Foster City, CA, USA). For each locus allele sizes were identified using Genemapper

159 4.0 (Applied Biosystems, Foster City, CA, USA). Each PCR and sequencing run was conducted

160 including negative controls. The number of alleles, observed and expected heterozygosity,

161 polymorphic information content and frequency of null alleles were assessed using Cervus 3.0.7

162 software (Kalinowski et al. 2007) (Table 1). In addition, Cervus 3.0.7 was used to perform

163 parentage assignment and to calculate the combined non-exclusion probability, that was 7.0×10^{-2}

164 for the first and 1.1×10^{-2} for the second parent. We scored as EPO those individuals for which at

165 least one locus was not inherited from the social father. In addition, nestlings for which at least one

166 locus was not inherited from the social mother were scored as intraspecific brood parasitic offspring

167 (IBPO), a condition rarely found in Falconiformes but previously shown to occur in the lesser

168 kestrel (Yom-Tov 2001).

169

170 **Statistical analyses**

171 We relied on generalized linear models (GLM) to investigate whether individual morphology

172 (wing, tarsus and keel length) and SMI were affected by food supplementation (0 = control, 1 =

173 food-supplemented), while controlling for sex (0 = female, 1 = male). Differences in reproductive

174 success (laying date, clutch size, and brood size) between food-supplemented and control broods

175 were assessed by means of t-test.

176   Because of the small sample size of EPO (see Results), to investigate the association

177 between EPO occurrence in a brood and food supplementation we relied on the Fisher's exact test.

178   The sample of IBPO broods was too low to perform any statistical analysis of association with food

179   supplementation.

180   All subsequent analyses were restricted to control broods because no EPO was detected

181   among food-supplemented broods. We assessed within-sex differences in morphology (wing, tarsus

182   and keel length) and SMI of parents between broods with EPO and those without EPO by means of

183   t-tests; t-tests were also applied to investigate differences in laying date, clutch or brood size

184   between broods with and without EPO. Finally, we tested whether the probability of a nestling

185   being male varied according to EPO (0 = within-pair offspring; 1 = extra-pair offspring) by a

186   binomial generalized linear mixed model (GLMM) with brood identity as a random intercept effect

187   (model fitted by the R lme4 package ver. 1.1-21 (Bates et al. 2014).

188

189

190   **Results**

191

192   Extra food did not significantly affect SMI or morphology ($F_{1,68} < 2.91$, $p > 0.09$); these models

193   also revealed that females had significantly shorter tarsi ($F_{1,68} = 5.97$, $p = 0.02$) and larger SMI

194   ($F_{1,67} = 51.35$, $p < 0.001$) as compared to males (see Podofillini et al. 2019 for further details). Two-

195   way interactions between food supplementation and sex were not significant ($F_{1,66} < 1.78$, $p > 0.19$).

196   Laying date, clutch size, and brood size did not significantly differ between food-supplemented and

197   control broods ($|t_{34}| < 1.03$, $p > 0.31$).

198   Out of the 117 genotyped nestlings from the 36 broods, eight were EPO (6.8 %).

199   Specifically, one brood contained three EPO, one brood contained two EPO, and the remaining

200   three contained a single EPO. No EPO was found among the 19 food-supplemented broods,

201   whereas EPO occurred in five out of 17 control broods, a statistically significant difference

202   (Fisher's exact test, $p = 0.016$). Furthermore, we detected five IBPO in four broods (one control

203   brood contained two IBPOs; two control broods contained one IBPO; one food-supplemented brood

204   contained one IBPO). EPO and IBPO did not co-occur in any brood.

205   Among control broods, faithful and unfaithful females did not significantly differ in SMI or

206   morphology (Table 2). Similarly, SMI and morphology of cuckolded males did not differ from that

207   of un-cuckolded males, except for tarsus length, that was larger among un-cuckolded males (Table

208   2). Clutch and brood size did not significantly differ between broods containing or not EPO (t_{15} =

209   0.44, p = 0.67 and t_{15} = 0.93, p = 0.37, respectively). However, unfaithful females laid their first egg

210   on average 6.28 days later in the season compared to faithful ones (mean laying date of unfaithful

211   females: May 16 (2.4 SD); faithful females: May 10 (8.7 SD); unequal variances t-test, $t_{14.07}$ = 2.30,

212   p = 0.037). The observed difference in laying date between broods with and without EPO

213   corresponds to ca. 1 SD of the mean laying date of the population in the study year (mean laying

214   date = May 13 (8.0 SD), n = 100 clutches). Two out of eight EPO (25.0 %) and 24 out of 44 within-

215   pair control offspring (54.5 %) were males. However, nestling sex was not significantly predicted

216   by EPO status (binomial GLMM, estimate: -1.97 (1.30 s.e.), χ^2_1 = 3.39, p = 0.07).

217

218

219   **Discussion**

220   Our experimental study aimed at exploring alternative explanation for the evolution of mate feeding

221   behaviour in a sexually dimorphic diurnal raptor, the lesser kestrel. To this end, we provided extra-

222   food in nestboxes during egg laying to breeding pairs, therefore manipulating female perception of

223   social mate quality, and analysed the effects of food supplementation on patterns of extra-pair

224   paternity. Overall, EPO were found in 13.9 % of the broods, with 6.8 % of nestlings being EPO,

225   similarly to previous studies of the same species (Negro et al. 1996, Alcaide et al. 2005, 2010). All

226   EPO belonged to the control group. This finding is in accordance with similar previous studies of

227   passerine bird species, in which food supplementation decreased female propensity to seek extra-

228   pair mates, thus increasing social mate reproductive success (Westneat 1994, Václav et al. 2003,

229   O'Brien and Dawson 2011, Kaiser et al. 2015).

230   The absence of EPO in broods belonging to the food-supplemented group provided support

231   to the hypothesis that mate-feeding behaviour has evolved by sexual selection, since females paired

232   to males showing a larger investment in mate-feeding are expected to be more prone to perform

233   frequent copulations with the social mate, providing greater certainty of paternity. In addition, male

234   mate-feeding rate, egg incubation and offspring feeding effort all positively covary in the lesser

235   kestrel (Donázar et al. 1992). Thus, greater female fidelity to males of food-supplemented pairs may

236   be also due to the direct fitness benefits that a female may expect to acquire from a male providing

237   abundant food (i.e. a male of high perceived quality). The difference in the occurrence of EPO in

238   food-supplemented and control broods was not associated to changes in female condition due to

239   extra food, as extra food did not significantly affect body condition (see also Podofillini et al. 2019).

240   In addition, there were no differences in clutch and brood size between food-supplemented and

241   control broods. Hence, our findings are less compatible with the hypothesis that mate-feeding has

242   evolved by natural selection, because in that case we would have expected food supplementation to

243   foster proxies of breeding success. However, it should be emphasized that proper tests of the natural

244   selection hypothesis would require food supplementation experiments to be performed under

245   contrasting ecological conditions (i.e. favourable vs. poor years). Indeed, direct nutritional benefit

246   may become more evident under adverse conditions (Václav et al. 2003), while conditions in the

247   study year were relatively favourable, as breeding success at the population level was relatively

248   high (see Podofillini et al. 2019).

249   No differences in morphology, SMI and reproductive success (in terms of clutch and brood

250   size) between faithful and unfaithful females were detected. In contrast, faithful and unfaithful

251   females showed a statistically significant difference in timing of breeding, as clutches with at least

252   one EPO were laid later in the breeding season. This may be related to the hypothesis that the

253   seasonal decline in male reproductive success, and thus the increase in male paternity loss in the

10

254 own nest, is related to a seasonal decline of individual quality (Williams 2012). In most bird

255 species, older, more experienced, and high-quality individuals are those that breed earlier (Newton

256 and Marquiss 1984, Zabala and Zuberogoitia 2014), while late breeders may be low-quality

257 individuals that miss the optimal time 'window' for reproduction (Drent and Daan 1980). In the

258 lesser kestrel, it is known that high-quality and older males arrive earlier to the breeding grounds,

259 obtain the most suitable nest sites and breed earlier (Serrano et al. 2003, Catry et al. 2016). We may

260 therefore speculate that early-breeding females, mated with high-quality, early-arriving males,

261 avoid seeking extra-pair copulations, whereas late-breeding females, mated with-low quality

262 partners, actively seek extra-pair matings. This speculation is in line with the observation that

263 cuckolded males were smaller (i.e., they had shorter tarsi) than un-cuckolded conspecifics,

264 suggesting that they were of lower quality. Indeed, skeletal size positively covaries with male

265 competitive ability and viability in many bird species (Potti and Merino 1994). By seeking extra-

266 pair fertilizations with larger - possibly early-arriving - males, late-breeding females may therefore

267 obtain genes for high offspring viability (Bouwman et al. 2006).

268   Five out of the 117 genotyped nestlings (4.3 %) were found not to be genetically related to

269 any of their parents, which we interpret as evidence of intraspecific brood parasitism. The

270 occurrence of intraspecific brood parasitism is slightly higher than previously reported for this

271 species (2.2 %; two out of 87 nestlings; Negro et al. 1996). In birds, conditions favouring

272 intraspecific brood parasitism may include limitation of female ability to breed in adverse

273 environmental conditions, clutch loss due to predation during egg laying, the occurrence of

274 alternative reproductive tactics, or simply the occurrence of constraints in obtaining a breeding

275 territory or mate (see Lyon and Eadie 2008 for a detailed review). In our lesser kestrel population,

276 clutch loss due to predation is extremely rare, and in the study year the ecological conditions were

277 generally favourable (see Podofillini et al. 2019). The occurrence of intraspecific brood parasitism

278 may thus be explained by the presence at the colony of prospecting yearling females, which mostly

279 do not breed despite being sexually mature (Hiraldo et al. 1996). For these inexperienced females,

280   the potential difficulties in acquiring a partner may not be compensated by adequate fitness returns.

281   Hence, rather than skipping reproduction, some of these females may opt for laying eggs in the

282   nests of older females.

283   In conclusion, we demonstrated that experimental food provisioning reduced females'

284   propensity to seek for extra-pair copulations, likely by altering females' perception of male quality.

285   This result provide support to the hypothesis that sexual rather than natural selection is a major

286   driver for the evolution of mate-feeding behaviour in the lesser kestrel. Unfaithful females were not

287   larger or in better body condition than faithful ones and did not show a greater reproductive success,

288   but they started to breed later in the breeding season. In addition, smaller males suffered a reduction

289   of paternity in their social nest. Therefore, our findings support the hypothesis that females assess

290   the quality of their social partner through its mate-feeding ability, and suggest that females may be

291   keener to seek for extra-pair copulations when mated with late-breeding, likely low-quality,

292   partners.

293

304

305   **Data availability** The data on which the study is based will be deposited in DRYAD upon
306   acceptance.
307

308   **Author contributions** AG, MG, JGC, DR conceived and designed the experiments. GCS, MG,

309   JGC, DR collected data in the field. NT, AG, GCS Performed laboratory assay. AC, RA, DR

310   analysed the data. AC, DR wrote the initial draft of the manuscript. All authors read, edited and

311   approved the final manuscript.

312

313   **Conflicts of interest** We declare there is no conflict of interest.

314

315   **Permit** Capture and handling was performed by ISPRA under the authorization of Law 157/1992

316   [Art. 4 (1) and Art. 7 (5)].

317

318

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467

468　Table 1. Details and statistics of microsatellite loci used to assess parentage. Size: range of observed

469　alleles (bp); K: number of alleles; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity;

470　PIC: polymorphic information content; F_{null}: frequency of null alleles.

471

Locus	Label	Primer sequences 5′−3′	Size (bp)	K	H_{obs}	H_{exp}	PIC	F_{null}
Fp5	6-FAM	F: CCGTTCTGGAGTCAAAAC R: CATGCAGCACTTTATTCAG	99-109	6	0.79	0.74	0.71	-0.038
Fp31	HEX	F: ATCACCTGCACATAGCTG R: TTTAGCTCCTCTCTCTCAC	111-143	10	0.62	0.70	0.65	0.053
Fp46-1	NED	F: TTAGCCTCGCAGCTTCAG R: GTAATGAAAAGTCTTTGGGG	120-144	10	0.47	0.53	0.51	0.068
Fp79-4	6-FAM	F: TGGCTTCTCTTATCAGTAAC R: GGCTGGGTGGAATTAAAG	126-166	17	0.76	0.90	0.89	0.086
Fp89	HEX	F: CTCTGCCCTGAATACTTAC R: GAATCTTGTTTGCATTGGAG	117-123	4	0.60	0.54	0.48	-0.055
Cl347	6-FAM	F: TGTGTGTGTAAGGTTGCCAAA R: CGTTCTCAACATGCCAGTTT	104-124	11	0.75	0.75	0.72	-0.004

472

473

474 Table 2. Differences (mean and SD) in morphology and SMI of unfaithful (with EPO, n = 5) and

475 faithful (without EPO, n = 12) control females and of cuckolded (with EPO) and un-cuckolded

476 (without EPO) control males. SMI = scaled mass index (see Methods and Podofillini et al. 2019).

477

Trait	With EPO	Without EPO	*t*	*df*	P
Females					
Wing (mm)	236.6 (2.0)	237.9 (5.9)	0.26	15	0.80
Tarsus (mm)	31.0 (1.2)	31.2 (2.0)	0.34	15	0.74
Keel (mm)	31.4 (2.1)	31.7 (2.6)	0.14	15	0.89
SMI (g)	150.2 (6.2)	158.2 (15.3)	0.80	15	0.44
Males					
Wing (mm)	231.2 (7.4)	235.9 (4.9)	1.69	15	0.11
Tarsus (mm)	30.6 (1.7)	32.5 (1.3)	2.40	15	0.030*
Keel (mm)	31.5 (1.1)	31.7 (1.5)	0.14	15	0.89
SMI (g)	132.2 (11.6)	134.8 (12.4)	0.57	15	0.58

478

05-Jun-2020

Dear Dr. Costanzo:

Manuscript ID JAV-02535 entitled "Extra food provisioning reduces extra-pair paternity in a raptor species, the lesser kestrel (Falco naumanni)" which you submitted to Journal of Avian Biology, has been reviewed. The comments of the reviewer(s) and the recommendation by the Subject Editor are included below.

The reviewer(s) have suggested some minor revisions to your manuscript. Based on the recommendation by the SE, I invite you to respond to the comments by the Subject Editor and reviewer(s)' and to revise your manuscript accordingly.

Recommendation by the Subject Editor:

We have positive comments from two reviewers. They have provided good feedback. Please address their comments and revise the manuscript accordingly. Note one of the reviewers attached a file with additional comments.

Dear Editor, thank you very much for your response and the positive evaluation of our work. We have now revised our manuscript and included most of the suggested changes. Please note that we have also decided to make a slight change to the title of the article, to be more in line with the journal's focus. Please find below our point by point reply to the reviewer comments.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

COMMENT: In their paper, AAs design an experiment to address the role and meaning of EPO. Their attempt is well conceived and worked. I have not many concerns about the paper, some minor points have been addressed in the commented file.

REPLY: we thank the reviewer for the positive evaluation of our study. We have carefully considered her/his comments and incorporated several of the suggested changes. Please find below the details of how we have dealt with each comments.

C: I wonder why AAs have used independent univariate tests. To support the design and results just by t-tests is perhaps too simplistic. Independency of tests can be misleading. A generalized linear model with binomial response (0 = control, 1 = food suppl) and logit link on parent body sizes could be done, controlling for sex, or sex by sex (see result rows 179-183). Standardization will solve different scales of measure, if AAs want include also SMI (perhaps collinear with other measures).

R: We thank the reviewer for pointing this out. We agree that we could test sex and treatment effects on some phenotypic traits with a GLM instead of independent univariate sex. We have thus performed separate GLMs where the response variable is the morphological trait and the predictors were sex, treatment and their interaction. Due to collinearity among different phenotypic traits, we preferred not to include all of them in a single model. We hope that these changes are in line with the referee's suggestions.

C: Again on table 2 data, one anova per sex with EPO (yes, no) as categorical predictor should be attempted to test the main effect, if any of body sizes.

R: we apologize but we are unclear with this comment. We do not think that it is very informative to ran ANOVAs (or rather MANOVAs with multiple dependent variables) per sex with EPO as a categorical predictor. In the case of MANOVAs, however, the global tests (e.g. Pillai's trace or the like) are generally poorly informative, and then one has to look back at trait-specific ANOVAs, which is exactly the same approach we have done now (the only difference being that we have used t-tests instead of F-test for checking differences, but the two give exactly the same outcome for univariate tests). For the sake of simplicity, we would therefore prefer to leave the analyses as they are now.

C: In some parts rewording and more explicit structure of paragraphs would help an easier reading.

R: We thank the reviewer for the suggestion. We have now tried to improve the readability of the text, by also including the comments that he/she have made directly in the commented file.

Response to the comments provided directly on the text:

C: Line 2: social environment? please reword this unclear sentence.

R:we have now reworded the sentence, and specified that we intended 'social mate' (as opposed to the extra-pair mate).

C: Line 14: you corroborated intrasp brood parasitism, since is not the first study on the LK.

R: the sentence has been changed accordingly.

C: Line 25. do you mean that copulation with extra pair-mates is common in social vertebrates? Be more explicit at the beginning of the paper, their own mate (=social mate) should be defined here for readers not accustomed to terminology of behavioural ecology

R: the sentence has been reworded in order to be clarified.

C: Line 32. I miss here what are the benefits of promiscuous males, that implicitly (according to the sense of row 29), should have most of control over extra-pair mating. In addition, in raptors with reverse sexual dimorphism, female control should be high.

R: We thank the reviewer for the comment. We have now added information regarding the benefits of extra-pair copulation for males.

C: Line 41. ; while in others,

R: The text has been changed as suggested

C: Line 43. also here reword, evolution of mate-feeding behaviour from the males.

R:the sentence has been reworded

C: Line 47. , for instance during ....

R: The text has been changed as suggested

C: Line 65. with the function to please reword "reciprocally assess mates by pair members", what do you mean and what is the difference with reinforcing pair-bonds

R: The sentence has been modified as suggested

C: Line 67. do you mean here success in terms of being accepted, success in access to female, or in terms of future fertilization?
R: The sentence has been clarified as suggested

C: Line 114. here and below, is preferable you add mean +/- SD without range, or simply write 'collected at 7-11 days.....'
R: changed as suggested

C: Line 174. avoid double brackets here.
R: Deleted as suggested

C: Line 184. Replace with EPOs whenever the case.
R: We thank the reviewer for this comment. We checked this out, and it seems that 'offspring' is a so-called 'mass noun', whose plural form is rarely used. In the ecological literature, it is only used in its singular form. We therefore kept it as such.

C: Line 195. I guess you're testing only the control sample, but what happens in the food suppl. sample? what is the mean laying date and to what SD it corresponds? According to your inference we may expect a difference (earlier laying) from the population average due to food suppl.
R: We thank the referee for this comment. We wish to point out that our experimental protocol implied starting food supplementation upon laying of the first egg in a nest. Nests were assigned to treatments alternately. Hence, by design, there is no significant difference in laying date because of food supplementation. A test of this (lack of) difference is reported in Podofillini et al. (2019).

C: Line 200. I guess, hopefully, the studied clutches are not within this sample
R: As mentioned in the previous comment, this is the laying date of the population (all clutches laid in that year), and it is completely independent of food supplementation treatment. Hence, this sample includes the study clutches (n = 36). With this comparison, we only want to check how late the clutches containing extra pair nestlings were compared to the population average.

C: Line 210. the only correct, replace accordingly whenever it matters
R: Please see our reply to comment at line 184

Reviewer: 2

Comments to the Author

C:The paper deals with the impact of supplementary feeding on extra-pair paternity and shows that fed females perceive the feeding as sign of mate quality and thus reduce the occurrence of extra-pair paternity. Overall the paper is really well written and the study design is well planned and sounding. The sample size is small but the results show a clear pattern.

R: we are glad the reviewer appreciated our work. We have now included his/her comments in the text

C: My only concern is that from the paper is a bit unclear what novelty this study brings. In the introduction it is mentioned that the methodology adopted is designed to specifically investigate supplementary feeding as mate quality, compared to previous studies, but it would be beneficial to clearly highlight the missing link in the current knowledge. And especially in discussion, I think the manuscript would benefit from a partial rephrasing to highlight why is it important to investigate this question in this system.

R: we have now highlighted that the aim of the work was to better understand the functional role of mate-feeding behaviour and to determine whether, in the lesser kestrel, it evolved (mainly) under the influence of natural or sexual selection.

Minor comments:

C: Line 125 the point 4) is unclear, please rephrase? It is difficult to understand how 4) is different from the previous points, it seems to be a combination of 1) having both parents genotype and 3) at least 2 nestlings per brood genotyped?

R: we agree with the reviewer. Indeed, the information provided by point 4 is redundant and we have now removed it from the text.

C: Lines 135-136: could the authors please provide more details on the protocol for DNA extraction and a reference to the extraction method? At what temperature and in which substrate were the samples preserved until DNA extraction?

R: additional details have now been added to the text.