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When mothers make sons sexy: Maternal effects contribute to the increased sexual attractiveness of extra-pair offspring

Barbara Tschirren[1,2*], Erik Postma[2], Alison N. Rutstein[1] & Simon C. Griffith[1]

[1] Department of Biological Sciences, Macquarie University, Sydney, Australia

[2] Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland

*Correspondence: Barbara Tschirren, Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland. Email: barbara.tschirren@ieu.uzh.ch, Phone: +41 44 635 47 77, Fax: +41 44 635 68 18

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Summary

Quality differences between offspring sired by the social and by an extra-pair partner are usually assumed to have a genetic basis, reflecting genetic benefits of female extra-pair mate choice. In the zebra finch (*Taeniopygia guttata*), we identified a colour ornament that is under sexual selection and that appears to have a heritable basis. Hence, by engaging in extra-pair copulations with highly ornamented males, females could, in theory, obtain genes for increased offspring attractiveness. Indeed, sons sired by extra-pair partners had larger ornaments, seemingly supporting the genetic benefit hypothesis. Yet, when comparing ornament size of the social and extra-pair partners, there was no difference. Hence, the observed differences most likely had an environmental basis, mediated, for example, via differential maternal investment of resources into the eggs fertilized by extra-pair and social partners. Such maternal effects may (at least partly) be mediated by egg size, which we found to be associated with mean ornament expression in sons. Our results are consistent with the idea that maternal effects can shape sexual selection by altering the genotype–phenotype relationship for ornamentation. They also caution against automatically attributing greater offspring attractiveness or viability to an extra-pair mate's superior genetic quality, as without controlling for differential maternal investment we may significantly overestimate the role of genetic benefits in the evolution of extra-pair mating behaviour.

Keywords: differential allocation, extra-pair copulations, indirect genetic benefits, maternal investment, parental care, sexual selection

1. INTRODUCTION

Although the large majority of passerines are socially monogamous, extra-pair paternity is commonly observed in most species, with on average more than 10% of offspring being sired by a male other than the social mate [1,2]. Genetic benefits in the form of good or compatible genes for the offspring are still the prominent hypothesis for why females engage in extra-pair matings [3-6] (but see [7-10] for alternative explanations such as sexual conflict). In line with this hypothesis, a growing number of studies have demonstrated that extra-pair offspring (EPO) are superior to their within-pair half-sibs in a number of fitness-related traits [11-16]. Yet, the simple comparison of EPO and within-pair offspring (WPO) does not provide an incontrovertible test of good or compatible gene effects since the possibility that half-sibs within a brood experience different (pre- or post-natal) environmental conditions, in particular due to maternal effects, cannot a-priori be excluded. Although it has been argued that maternal effects are unlikely to cause such quality differences [9,17], this is based on very few empirical studies, and those studies that have measured potential parental investment biases in EPO and WPO focused exclusively on differential post-hatching food allocation [18-20], ignoring potential investment biases before birth.

Notable exceptions are the two recent studies by Magrath *et al.* [21] and Ferree *et al.* [22], who demonstrated that the incidence of extra-pair paternity decreases markedly with laying order in blue tits (*Cyanistes caeruleus*) and Western bluebirds (*Sialia mexicana*), respectively. Consequently, as laying order is closely linked to hatching order in asynchronously hatching passerines [23], EPO will hatch earlier than their WP half-sibs, with associated benefits in terms of increased competitiveness and faster growth [24,25]. Biasing the position of extra-pair paternity

within the laying sequence thus represents one mechanism that allows mothers to favour EPO. Indeed, both Magrath *et al.* [21] and Ferree *et al.* [22] showed that the faster growth and higher survival rates of EPO in those species were explained entirely by the hatching order bias. It shows that subtle, maternally induced differences in the conditions encountered by EPO and WPO can have pronounced effects on their phenotypic quality, thereby mimicking or amplifying good gene effects.

Here we aim at elucidating the relative importance of genetic benefits vs. maternal effects in creating quality differences between WPO and EPO in wild-caught and domesticated zebra finches (*Taeniopygia guttata*). Unlike previous studies, which emphasized differences in size and growth rate between EPO and WPO, we focus on differences in the expression of a sexually selected colour ornament. We ask if females engage in extra-pair copulations with highly ornamented males to obtain genes that produce sexy sons, or if it is the mother herself that makes her extra-pair sons sexy by differentially increasing her reproductive investment in those offspring.

2. MATERIAL AND METHODS

Study subjects and housing

The study population consisted of wild zebra finches (*Taeniopygia guttata*), caught at East Mandelman on the Fowlers Gap Arid Zone Research Station in Western New South Wales, Australia (31°05'S, 142°43'E), and domesticated zebra finches obtained from three different finch breeders around Sydney, NSW, Australia. The birds were kept in single-sex groups in large outdoor aviaries under identical

conditions for seven months before the start of the study. The sexes were physically, but not visually or acoustically isolated, and males and females were thus familiar with each other. For the preference tests (see below), the birds were moved to single-sex cages, measuring 75 x 40 x 30 cm, and kept under full-spectrum light (light regime: 14 h light : 10 h dark) at a temperature between 20 – 23º C [26].

Mate preference trials

We performed mate choice tests to determine what makes a zebra finch male attractive to females. We placed a male and a female together in a cage and recorded the response of the female to the courting male during a five-minute period [27]. For each male (*n* = 67 wild-caught males and 65 domesticated males), this was repeated with 10 different, randomly chosen (wild and domestic) females on separate days. The trials were carried out under full-spectrum light in a cage similar to the housing cages. For each male, we calculated the proportion of females showing a positive response during the trials, and used this proportion as a measure of 'male attractiveness' (see [27] for a detailed description of the protocol). Males to which more females responded positively during the choice trials were considered to be more attractive. Choices made by zebra finch females in this set-up have been shown to reflect mate choice situations [27].

Morphology and ornamentation

We measured each bird's body mass to the nearest 0.5 g using a Pesola balance. Metatarsus, wing, bill and tail length were measured to the nearest 0.1 mm using a digital calliper. We performed a principle component (PC) analysis to obtain an overall measure of body size. The first principle component (PC1) of this analysis,

118 henceforth termed body size PC1, explained 48% of the variation and correlated
119 positively with all size measures (eigenvector: metatarsus length: 0.567, bill length:
120 0.522, wing length: 0.600, tail length: 0.216).
121 Male zebra finches display a number of colour ornaments, including a red bill
122 and several plumage colour traits (ESM 1), all of which have been suggested to be
123 sexually selected [28-31]. We measured several aspects of these colour ornaments,
124 as well as song rate [32], and established their association with male attractiveness.
125 Objective measures of the colour of the red bill and the rufous cheek patch
126 were made using a USB2000 spectrophotometer (Ocean Optics, Dunedin, USA) and
127 a fiber-optic reflectance probe coupled to a xenon light source (PX-2, Ocean Optics,
128 Dunedin, USA) [26]. Reflectance spectra were processed using the *R* package SPEC
129 [33] following [34]. Using this method, we obtained four quantal cone catches for bill
130 and cheek patch colour, which were transformed into three independent log contrasts
131 using the long wavelength catch as a denominator [35]. These three log-contrasts
132 (c_{1-3}) were then used in principle component analyses. For bill colour, PC1 explained
133 90.5% of the colour variation (eigenvector: c_1: 0.578, c_2: 0.586, c_3: 0.568), PC2
134 explained 6.3% of the variation (eigenvector: c_1: -0.573, c_2: -0.205, c_3: 0.217), and
135 PC3 explained 3.2% of the variation (eigenvector: c_1: 0.582, c_2: -0.784, c_3: 0.217).
136 For cheek patch colour, PC1 explained 88.6% of the colour variation (eigenvector: c_1:
137 0.551, c_2: 0.602, c_3: 0.578), PC2 explained 9.9% of the variation (eigenvector: c_1:
138 0.802, c_2: -0.190, c_3: -0.566), and PC3 explained 1.5% of the variation (eigenvector:
139 c_1: 0.231, c_2: -0.776, c_3: 0.587).
140 To measure the area of the rufous cheek patch and the black band on the
141 breast, we photographed each male in a standardized setting. A digital camera
142 (Canon PowerShot A80 4MP) was mounted on a tripod next to a table, pointing

downwards. Illumination was provided by a single 20W halogen spot from above. The distance between the camera and the table was approximately 0.4 meters. The birds were immobilized on top of a millimetre grid in a standardized manner, always by the same person. To photograph the left and the right cheek patch, the bird was placed on its side, holding the bill with one hand and the rest of the body with the other. To photograph the breast band, the bird was placed on its back, holding the bill and the legs. Cheek patch size was measured in mm^2 by tracing its outline on the photograph in the program ImageJ [36], using the millimetre grid as a size reference. Breast bands are more irregular and instead of tracing them by hand, we first converted the photograph to a grey scale image and subsequently used the threshold tool to select the breast band. Again, its size was measured in mm^2.

In addition to the colour ornaments, we recorded the total amount of song (in seconds) that a male produced during the preference trials, and calculated for each male an average song duration over all trials [27].

Repeatabilities of measurements were high (see [26] and ESM 2). Differences in morphology and ornamentation between wild-caught and domesticated zebra finches are accounted for statistically in all analyses and discussed in detail in [26].

Breeding

We performed a total of three breeding rounds (in March 2006, October 2006, February 2007) during which females were free to mate with their social and extra-pair partners. During each breeding round, groups of 12 – 14 birds (6 - 7 females and 6 - 7 males) were colour-ringed for visual identification and released in each of 12 aviaries, measuring 4 x 2.3 x 2.4 m each. The composition of the groups was different in each round. Wild and domesticated birds were kept in separate aviaries,

168 visually isolated from one another. The aviaries were alternated, with wild-caught
169 birds in the first aviary, domesticated birds in the second aviary, and so on. Each
170 aviary contained 12 nest boxes and nesting material. All birds had access to *ad*
171 *libitum* food (finch mix Golden Cob® Premium Finch Mix, Masterfoods), water and
172 cuttlebone. Spinach was provided once per week.
173 We checked the nest boxes twice weekly for eggs, which were marked and
174 measured (length and width) to the nearest 0.1 mm with a digital calliper. Egg volume
175 (mm^3) was calculated as volume = length * $width^2$ * 0.51 [37]. After hatching,
176 nestlings were uniquely marked by removing down feathers on the back and head,
177 and when old enough, they received an individually numbered plastic ring. A subset
178 of the nestlings of all broods was cross-fostered 0 – 2 days after hatching (34% of all
179 nestlings across all breeding rounds). They were partially (and randomly with respect
180 to hatching order) exchanged between two or more nests, depending on the number
181 of broods available with similarly aged nestlings. Cross-fostering was performed
182 within type only, i.e. wild nestlings were only exchanged with wild nestlings, and
183 domestic nestlings with domestic nestlings. The social parents of a brood were
184 determined by observing colour-ringed parents feeding their nestlings. A small blood
185 sample was taken from the brachial vein of all adults and offspring for the assignment
186 of genetic parenthood. At adulthood, offspring morphology and ornamentation was
187 measured as described above.
188
189 *Genetic parentage assignment*
190 DNA was extracted from a subsample of blood using magnetic beads (MagneSil
191 BLUE, Promega, Switzerland). We genotyped the birds using 8 highly polymorphic
192 microsatellite markers: Tgu1, Tgu3, Tgu4, Tgu8, Tgu10, Tgu12 [38], INDIGO41 [39]

193 and Ase 50 (Z-linked) [40]. DNA was amplified using a polymerase chain reaction
194 (PCR) run in a 10 µl volume using Multiplex PCR Kit (QIAGEN AG, Basel,
195 Switzerland) with fluorescent-labelled forward primers and non-labelled reverse
196 primers on a GeneAmp 9700 thermal cycler (Applied Biosystems, Rotkreuz,
197 Switzerland). PCR started with an initial denaturation step at 95°C for 15 min,
198 followed by 8 cycles of 30 sec at 94°C, 90 sec at 60°C minus 1°C per cycle, 60 sec
199 at 72°C, and 20 cycles of 30 sec at 94°C, 90 sec at 56°C, 60 sec at 72°C followed by
200 a final extension step of 15 min at 70°C. PCR fragments were separated by capillary
201 electrophoresis on an ABI Prism 3100 Sequencer and analyzed in GeneMapper 4.0
202 (both Applied Biosystems, Rotkreuz, Switzerland).
203 We used the program Cervus 3.0 [41] to calculate allele frequencies and
204 exclusion probabilities based on the genetic data of 201 adult zebra finches (98 wild-
205 caught and 103 domesticated birds). Wild and domesticated birds were analyzed
206 separately. Exclusionary power over all loci was > 0.999 for the first parent and >
207 0.9999 for the second parent in both populations. The mean number of alleles was
208 30.1 (range 21 – 38) for wild-caught birds and 18.3 (range 11 – 23) for domesticated
209 birds.
210 Parentage assignment was carried out in Cervus 3.0. First, we assigned the
211 mothers to the nestlings. Paternity was then assigned using trio LOD score and
212 confirmed by exclusion. Nestlings with negative trio LOD scores were considered
213 extra-pair offspring (EPO). EPO mismatched their social father's genotype at two loci
214 or more. Nestlings with a positive trio LOD score and that mismatched their social
215 father's genotype at maximally one locus were classified as within-pair offspring
216 (WPO). Mismatches at only one locus are most likely due to null alleles, mutations,
217 spurious alleles or genotyping errors [42,43]. We determined the paternity status

(WPO or EPO) of 464 offspring originating from 157 broods.

Statistical Analyses

We calculated the relative attractiveness of a male by dividing his arcsine-transformed attractiveness by the mean arcsine-transformed attractiveness across all birds. We then used a stepwise backward linear regression approach to select the best model to describe the association between male phenotype and relative male attractiveness. Body size PC1, body mass, cheek patch size, breast band size, bill colour PC1, bill colour PC2, bill colour PC3, cheek patch colour PC1, cheek patch colour PC2, cheek patch colour PC3, song duration, mate choice test group (i.e. group of 10 females with which a male was tested), type (wild-caught / domestic) and all two-way interactions between traits and type were included in the initial model. Variables were sequentially removed from the model if $p > 0.1$, starting with the least significant term (n =132 males). All phenotypic traits were standardized to have a mean of zero and a standard deviation of one to obtain standardized selection gradients following [44]. Following this model selection procedure, only cheek patch size and type were retained in the final model (see Results).

We used father–son regressions to estimate the resemblance between father and sons in cheek patch size [45] (i.e. the only trait that was significantly associated with male attractiveness in the mate choice trials, see Results). Only sons that were not raised by their biological father (i.e. sons that were cross-fostered shortly after hatching or EPO) were included in this analysis to control for postnatal environmental factors that might contribute to father-son resemblance [46]. We used mean values of sons if more than one offspring of a particular father was measured to ensure that each father was included in the analysis only once. To account for variation in family

size, offspring means were weighted following [45]. Cheek patch sizes were standardized for fathers and sons to have a mean of zero and a standard deviation of one for wild-caught and domesticated birds separately. A (likely biased, see Discussion) estimate of the heritability (h^2) of cheek patch size was calculated as 2 * slope (b) of the regression between fathers and sons. The standard error of the heritability estimate was calculated as 2 * standard error of b. 39 father – (mid-) son pairs in the wild-caught population and 33 father – (mid-) son pairs in the domesticated population were included in the analysis.

Phenotypic differences between EP and WP male offspring (n =196 sons of 84 mothers) were analyzed in a mixed model ANCOVA including type (wild-caught / domestic), paternity status (WPO / EPO) and their two-way interaction as fixed effects, identity of the mother and breeding round as random effects, and offspring body size PC1 as a covariate. In addition, mean egg volume per clutch (i.e. mean of eggs laid by the genetic mother) was included as a covariate to estimate egg size-mediated maternal effects on offspring phenotype.

Phenotypic differences between the extra-pair and social partner of a female (n =29 social partner – extra-pair partner pairs) were analyzed in a repeated measures ANOVA including the measures of the social and extra-pair partner of a female as repeated measures (within-subject) and type as a fixed effect (between-subjects).

All tests were two-tailed with a significance level set at $p \leq 0.05$. Analyses followed a backward-stepwise procedure whereby all two-way interactions were initially included and non-significant interactions were sequentially removed to determine the final model. Normality of the residuals was ascertained using Shapiro-Wilk tests. We used the program JMP 8.0 (SAS Institute Inc., Cary, NC, 2009) for all

statistical analyses.

3. RESULTS

Male attractiveness

Cheek patch size was the only significant predictor of male attractiveness in the mate choice trials (standardized selection gradient in final model β ± 1SE = 0.146 ± 0.055, $F_{1, 129}$ = 7.194, p = 0.008; standardized selection gradient in full model including all other, non-significant traits: β ± 1SE = 0.176 ± 0.066, $F_{1, 116}$ = 7.132, p = 0.009). Associations between attractiveness and all other morphological and behavioural traits were all substantially weaker and statistically non-significant (all p > 0.103; standardized selection gradient β ± 1SE in full model: body size PC1: 0.109 ± 0.082, body mass: -0.097 ± 0.092, breast band size: -0.102 ± 0.061, song rate: -0.049 ± 0.051, bill colour PC1: -0.025 ± 0.053, bill colour PC2: -0.046 ± 0.057, bill colour PC3: 0.044 ± 0.054, cheek colour PC1: -0.030 ± 0.057, cheek colour PC2: 0.001 ± 0.057, cheek colour PC3: 0.004 ± 0.059; test group: p = 0.130, type: p = 0.088; two-way interactions between type and traits: all p > 0.310). To provide further evidence that the association between cheek patch size and attractiveness was not due to females preferring larger males and larger males having larger cheek patches, we re-entered body size into the final model. Cheek patch size remained statistically significant in this model (standardized selection gradient β ± 1SE = 0.133 ± 0.059, $F_{1, 126}$ = 5.156, p = 0.025), whereas body size was not significantly associated with attractiveness (standardized selection gradient β ± 1SE = 0.036 ± 0.060, $F_{1, 126}$ = 0.364, p = 0.547; type: $F_{1, 126}$ = 3.063, p = 0.083).

Father – son resemblance in ornament size

We observed a strong resemblance in absolute cheek patch size between fathers and their sons, both in wild-caught ($F_{1, 37} = 13.946$, $p < 0.001$, $b \pm 1$ SE: 0.416 ± 0.111) and domesticated ($F_{1, 31} = 26.327$, $p < 0.001$, $b = 0.614 \pm 0.120$) birds (Fig. 1). The father–son resemblance in cheek patch size remained significant when analyzing cheek patch size corrected for overall body size, which is known to be heritable in zebra finches [47] (wild-caught: $F_{1, 30} = 5.734$, $p = 0.023$, $b = 0.313 \pm 0.131$, domesticated: $F_{1, 26} = 8.651$, $p = 0.007$, $b = 0.410 \pm 0.111$). Note that because body size was not available for all birds, the latter estimates are based on slightly less data.

If we assume an autosomal or Z-linked additive genetic basis of cheek patch size, as well an absence of any non-genetic sources of resemblance between fathers and sons, these slopes would suggest an exceptionally high heritability ($h^2 \pm 1$SE) of absolute cheek patch size of 0.83 ± 0.22 and 1.23 ± 0.24 in wild-caught and domesticated birds, respectively.

Parentage and ornament size

12.0% of the offspring (16 sons and 13 daughters) were sired by extra-pair partners in the wild-caught population and 15.3% of the offspring (19 sons and 15 daughters) were sired by extra-pair partners in the domesticated population (difference in extra-pair paternity rate between types: $\chi^2_1 = 1.095$, $p = 0.295$). Sons sired by an extra-pair partner (least squares mean ± 1SE: 110.4 ± 3.6 mm^2) had significantly larger cheek patches than their half-brothers sired by the social partner (least squares mean ± 1SE: 105.7 ± 3.0 mm^2; paternity status: $F_{1, 168} = 4.410$, $p = 0.039$; type: $F_{1, 64.5} = 16.812$, $p < 0.001$; type x paternity status: $F_{1, 167.3} = 0.988$, $p = 0.322$; body size PC1: $F_{1, 166.7} = 13.630$, $p < 0.001$; Fig. 2). However, they were not overall larger (body size

318 PC1: paternity status: $F_{1, 166} = 1.028$, $p = 0.309$; type: $F_{1, 73.8} = 20.620$, $p < 0.001$; type
319 x paternity status: $F_{1, 164.1} = 0.117$, $p = 0.733$) than their maternal half-brothers, nor
320 did they differ in any other measured trait (ESM 3).
321
322 *Ornament size of social vs. extra-pair partners*
323 No significant difference in cheek patch size between the social (mean ± 1SE: 112.2
324 ± 3.0 mm^2) and the extra-pair partner (113.0 ± 2.2 mm^2) of a female was observed
325 (difference extra-pair – social partner: $F_{1, 27} = 0.063$, $p = 0.805$; difference x type: $F_{1,}$
326 $_{27} = 0.331$, $p = 0.570$; Fig. 3). These results did not change when excluding the one
327 domestic male with exceptionally large cheek patches (see Fig. 3) from the analyses
328 (difference extra-pair – social partner: $F_{1, 26} = 0.834$, $p = 0.370$; difference x type: $F_{1,}$
329 $_{26} = 0.001$, $p = 0.994$). Furthermore, the results did not change when analysing
330 residual cheek patch size corrected for overall body size PC1 (difference extra-pair –
331 social partner: $F_{1, 25} = 0.004$, $p = 0.949$; difference x type: $F_{1, 25} = 0.622$, $p = 0.438$).
332 Extra-pair and social partners did not differ significantly in any other measured trait
333 either (ESM 4).
334
335 Assuming that the difference in cheek patch size between WPO and EPO is genetic
336 (as is expected under the genetic benefit hypothesis), we would expect to find a
337 difference between extra-pair and social partners that is two times larger (if $h^2 = 1$;
338 more if $h^2 < 1$) than the difference in cheek patch size between EPO and WPO
339 (because offspring get only half of their genes from the father). Twice the difference
340 in cheek patch size observed in the offspring is 9.4mm^2, which is well outside the
341 95% confidence interval of the cheek patch size difference between extra-pair and
342 social fathers (95% CI: -4.8 – 6.5 mm^2). The difference between EPO and WPO is

thus larger than what would be expected from the difference between extra-pair and social males. This shows that the lack of a significant difference between social and extra-pair partners is unlikely to be explained by a lack of statistical power, and that non-genetic effects are likely to contribute to the difference in cheek patch size expression between EPO and WPO.

Maternal effects on ornament size of sons

92% of the variation in egg volume was explained by differences between mothers. Cheek patch size of sons was positively associated with the mean egg volume of the clutch they came from ($F_{1, 66.8} = 4.311$, p = 0.042; type: $F_{1, 64.5} = 16.812$, $p < 0.001$; egg volume x type: $F_{1, 64.6} = 0.000$, $p = 0.984$). Such an association was not observed between egg volume and overall body size PC1 ($F_{1, 86.9} = 1.028$, $p = 0.313$; type: $F_{1, 73.8} = 20.620$, p < 0.001; egg volume x type: $F_{1, 85} = 1.379$, $p = 0.244$).

4. DISCUSSION

The size of the rufous cheek patch of zebra finch males was the best predictor of male attractiveness in our study population. This finding is consistent with an early study by Price and Burley (1994) [31], which found strong positive selection gradients for cheek patch size for the number of independent offspring produced and reproductive rate in zebra finches. Because sons resemble their father, and cheek patch size thus appears to be ‘heritable’, females could – in theory – obtain ‘sexy’ genes for their sons by engaging in extra-pair copulations with highly ornamented males. Indeed, we found that sons sired by an extra-pair partner had significantly larger cheek patches, but were not otherwise larger than their maternal half-brothers,

368 seemingly supporting the hypothesis that good (or rather ‘sexy’) gene effects on
369 offspring sexual attractiveness favoured the evolution of extra-pair mating behaviour
370 [3,5]. However, unlike most studies that examine morphological, physiological or life
371 history traits between EPO and WPO in wild populations (e.g. [11-16]), we had the
372 opportunity to directly compare the phenotype of the extra-pair and social partners of
373 all females. Surprisingly, and counter to the predictions of the good gene hypothesis,
374 no difference in their cheek patch size was observed. Moreover, the difference in
375 cheek patch size between extra-pair and social fathers was significantly smaller than
376 required to generate the observed difference in cheek patch size between WPO and
377 EPO.

378 It is generally assumed that EP and WP maternal half-sibs differ only in
379 relation to the genetic contribution of their fathers [12,15]. Yet, since we found no
380 evidence for a difference in ornament size between fathers, genetic effects are
381 unlikely to explain the differences in cheek patch size between EPO and WPO. If
382 these differences do not have a genetic basis, they are most parsimoniously
383 explained by environmental factors, most likely mediated by prenatal maternal
384 effects.

385 Differential maternal investment of resources in the offspring, either pre- or
386 postnatal, is known to occur in response to a large number of environmental or social
387 cues, including partner attractiveness [48-53]. Such maternal investment biases can
388 have pronounced and long-lasting effects on offspring performance [54], including
389 offspring attractiveness [55,56] and fecundity [53]. Although we cannot pinpoint the
390 exact mechanism by which female zebra finches promote ornament expression of
391 extra-pair sons, the quantity and / or quality of resources transferred from the mother
392 to the eggs are likely to play an important role. In support of this hypothesis, we

found that cheek patch size of sons was positively associated with the mean egg volume of the clutch they came from. Unfortunately, however, as we do not have information on egg volume for each individual nestling, we cannot directly show that EPO hatched from larger eggs than WPO. Alternatively, or additionally, females might not invest differently in egg size, but allocate more specific resources (e.g. maternal yolk androgens [49]) into the eggs sired by an extra-pair and social partner (either actively, passively or because they are forced to do so), and that these resources specifically favour the expression of sexually selected ornaments [57,58]. Because we used a cross-fostering approach, which randomized EPO and WPO across broods and thereby broke up potential biases within broods, differential investment of resources after hatching or hatching order effects (as observed in [21,22]) are unlikely to explain the differences in cheek patch size between EPO and WPO in our study.

Although the exact nature of maternal resources that cause the observed difference in ornament expression remains speculative at this point, our finding that EPO develop larger cheek patches than WPO – despite no evidence for a difference in cheek patch size among their fathers – is difficult to explain without invoking some sort of prenatal maternal favouritism (either active, passive or forced) for EPO. Similarly, the unrealistically high heritability estimates for cheek patch size observed in our study are likely inflated by differential maternal allocation of resources to clutches produced with an attractive male. This again highlights the important role of maternal effects in mediating the expression of sexually selected ornaments, but also the problems associated with estimating heritabilities based on parent-offspring regression, even when using cross-fostering approaches.

Why would females invest more resources into the eggs sired by an extra-pair partner?

First, whereas our study shows that differential maternal egg investment is likely to contribute to the enhanced ornamentation of EPO, our results do not preclude the possibility that females gain additional genetic benefits for their offspring by engaging in extra-pair matings. Genetic benefits might manifest themselves in other than the measured traits and / or become apparent at later life stages only [13,16]. In particular, sons sired by an extra-pair mate might inherit the ability to gain extra-pair copulations themselves, thereby increasing their lifetime reproductive success [59], but see [60]. Increasing the investment in such highly valuable offspring will pay for mothers, and will amplify differences between EPO and WPO [51,61]. Under such a scenario, maternal effects will thus exaggerate rather than substitute good gene effects on EPO quality. However, a mechanism that would allow females to differentially allocate resources to eggs bearing EPO, either voluntarily or involuntarily, might be difficult to envisage [17].

Alternatively, females might not gain genetic benefits by engaging in extra-pair matings, but still invest more resources in EPO. This could occur if females invest more resources into eggs laid early in the laying sequence because offspring from these eggs will – regardless of who sires them – hatch earlier and be therefore more competitive, heavier, and more likely to recruit to the local breeding population (i.e. more valuable for the mother) [23,62]. If, for some reason, early laid eggs are more likely to be fertilized by extra-pair partners, as has recently been found [21,22,63], then mothers will – indirectly, and not necessarily adaptively – invest more resources in EPO. Indeed, maternal egg investment has been consistently found to vary across the laying sequence, with, for example, levels of yolk androgens and carotenoids

decreasing with laying order in zebra finch clutches [64,65]. This scenario could explain why there were no phenotypic differences between social and extra-pair mates in our study, and it illustrates that selection might act on a male's ability to obtain paternity over certain eggs in a female's laying sequence (via sperm competition, for example), rather than on females to choose particular males as extra-pair partners [7,9,10], but see [66]. A particular strength of this second scenario is that it does not invoke any complicated and unlikely maternal allocation mechanisms [17].

In conclusion, our study indicates that maternal effects mediated by differential resource investment into the eggs promote the expression of a sexually selected ornament in extra-pair sons. It highlights that maternal effects can influence sexual attractiveness, mate choice decisions, and the process of sexual selection in general [61,67], and suggests that (prenatal) maternal effects might play a more important role in creating differences between EPO and WPO than has previously been appreciated. By not accounting for differential maternal investment, we might therefore considerably overestimate the role of good gene benefits in the evolution of extra-pair mating behaviour.

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476

476 **REFERENCES**

477

478 1 Petrie, M. & Kempenaers, B. 1998 Extra-pair paternity in birds: explaining
479 variation between species and populations. *Trends Ecol. Evol.* **13**, 52-58.
480 2 Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002 Extra pair paternity in
481 birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* **11**, 2195-
482 2212.
483 3 Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of
484 the genetic benefits. *Biol. Rev.* **75**, 21-64.
485 4 Møller, A. P. & Alatalo, R. V. 1999 Good-genes effects in sexual selection.
486 *Proc. R. Soc. Lond. B* **266**, 85-91.
487 5 Neff, B. D. & Pitcher, T. E. 2005 Genetic quality and sexual selection: an
488 integrated framework for good genes and compatible genes. *Mol. Ecol.* **14**, 19-38.
489 (DOI 10.1111/j.1365-294X.2004.02395.x.)
490 6 Griffith, S. C. & Immler, S. 2009 Female infidelity and genetic compatibility in
491 birds: the role of the genetically loaded raffle in understanding the function of
492 extrapair paternity. *J. Avian Biol.* **40**, 97-101. (DOI 10.1111/j.1600-
493 048X.2009.04562.x.)
494 7 Westneat, D. F. & Stewart, I. R. K. 2003 Extra-pair paternity in birds: Causes,
495 correlates, and conflict. *Annu. Rev. Ecol. Evol. Syst.* **34**, 365-396. (DOI
496 10.1146/annurev.ecolsys.34.011802.132439.)
497 8 Akçay, E. & Roughgarden, J. 2007 Extra-pair paternity in birds: review of the
498 genetic benefits. *Evol. Ecol. Res.* **9**, 855-868.
499 9 Arnqvist, G. & Kirkpatrick, M. 2005 The evolution of infidelity in socially
500 monogamous passerines: The strength of direct and indirect selection on extrapair

copulation behavior in females. *Am. Nat.* **165**, S26-S37.

10 Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H. & Kempenaers, B. 2011 Female extrapair mating behavior can evolve via indirect selection on males. *Proc. Natl. Acad. Sci. U S A* **108**, 10608-10613. (DOI 10.1073/pnas.1103195108.)

11 Forsman, A. M., Vogel, L. A., Sakaluk, S. K., Johnson, B. G., Masters, B. S., Johnson, L. S. & Thompson, C. F. 2008 Female house wrens (*Troglodytes aedon*) increase the size, but not immunocompetence, of their offspring through extra-pair mating. *Mol. Ecol.* **17**, 3697-3706. (DOI 10.1111/j.1365-294X.2008.03860.x.)

12 Sheldon, B. C., Merilä, J., Qvarnström, A., Gustafsson, L. & Ellegren, H. 1997 Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proc. R. Soc. Lond. B* **264**, 297-302.

13 Johnsen, A., Andersen, V., Sunding, C. & Lifjeld, J. T. 2000 Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**, 296-299.

14 Garvin, J. C., Abroe, B., Pedersen, M. C., Dunn, P. O. & Whittingham, L. A. 2006 Immune response of nestling warblers varies with extra-pair paternity and temperature. *Mol. Ecol.* **15**, 3833-3840. (DOI 10.1111/j.1365-294X.2006.03042.x.)

15 Kempenaers, B., Verheyren, G. R. & Dhondt, A. A. 1997 Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behav. Ecol.* **8**, 481-492.

16 Schmoll, T., Dietrich, V., Winkel, W., Epplen, J. T., Schurr, F. & Lubjuhn, T. 2005 Paternal genetic effects on offspring fitness are context dependent within the extrapair mating system of a socially monogamous passerine. *Evolution* **59**, 645-657.

17 Birkhead, T., Schwabl, H. & Burke, T. 2000 Testosterone and maternal effects - integrating mechanisms and function. *Trends Ecol. Evol.* **15**, 86-87.

526 18 Whittingham, L. A., Dunn, P. O. & Clotfelter, E. D. 2003 Parental allocation of
527 food to nestling tree swallows: the influence of nestling behaviour, sex and paternity.
528 *Anim. Behav.* **65**, 1203-1210. (DOI 10.1006/anbe.2003.2178.)
529 19 Kempenaers, B. & Sheldon, B. C. 1996 Why do male birds not discriminate
530 between their own and extra-pair offspring? *Anim. Behav.* **51**, 1165-1173.
531 20 Westneat, D. F., Clark, A. B. & Rambo, K. C. 1995 Within brood patterns of
532 paternity and paternal behavior in red-winged blackbirds. *Behav. Ecol. Sociobiol.* **37**,
533 349-356.
534 21 Magrath, M. J. L., Vedder, O., van der Velde, M. & Komdeur, J. 2009 Maternal
535 effects contribute to the superior performance of extra-pair offspring. *Curr. Biol.* **19**,
536 792-797. (DOI 10.1016/j.cub.2009.03.068.)
537 22 Ferree, E. D., Dickinson, J., Rendell, W., Stern, C. & Porter, S. 2010 Hatching
538 order explains an extrapair chick advantage in western bluebirds. *Behav. Ecol.* **21**,
539 802-807. (DOI 10.1093/beheco/arq056.)
540 23 Magrath, R. D. 1990 Hatching ssynchrony in altricial birds. *Biol. Rev. Camb.*
541 *Philos. Soc.* **65**, 587-622.
542 24 Slagsvold, T., Amundsen, T. & Dale, S. 1995 Costs and benefits of hatching
543 asynchrony in blue tits *Parus caeruleus*. *J. Anim. Ecol.* **64**, 563-578.
544 25 Gebhardt-Henrich, S. G. & Richner, H. 1998 Causes of growth variation and
545 its consequences for fitness. In *Avian Growth and Development: Evolution within the*
546 *Altricial-Precocial Spectrum* (eds. J. M. Stark & R. E. Ricklefs), pp. 324-339. New
547 York: Oxford University Press.
548 26 Tschirren, B., Rutstein, A. N., Postma, E., Mariette, M. & Griffith, S. C. 2009
549 Short- and long-term consequences of early developmental conditions: a case study
550 on wild and domesticated zebra finches. *J. Evol. Biol.* **22**, 387-395. (DOI

551 10.1111/j.1420-9101.2008.01656.x.)
552 27 Rutstein, A. N., Brazill-Boast, J. & Griffith, S. C. 2007 Evaluating mate choice
553 in the zebra finch. *Anim. Behav.* **74**, 1277-1284.
554 28 Birkhead, T. R., Pellatt, E. J., Matthews, I. M., Roddis, N. J., Hunter, F. M.,
555 McPhie, F. & Castillo-Juarez, H. 2006 Genic capture and the genetic basis of
556 sexually selected traits in the zebra finch. *Evolution* **60**, 2389-2398.
557 29 Blount, J. D., Metcalfe, N. B., Birkhead, T. R. & Surai, P. F. 2003 Carotenoid
558 modulation of immune function and sexual attractiveness in zebra finches. *Science*
559 **300**, 125-127.
560 30 Burley, N. & Coopersmith, C. B. 1987 Bill color preferences of zebra finches.
561 *Ethology* **76**, 133-151.
562 31 Price, D. K. & Burley, N. T. 1994 Constraints on the evolution of attractive
563 traits - selection in male and female zebra finches. *Am. Nat.* **144**, 908-934.
564 32 Riebel, K. 2009 Song and female mate choice in zebra finches: a review In
565 *Adv. Study Behav.* (eds. M. Naguib, K. Zuberbühler, N. S. Clayton & V. M. Janik), pp.
566 197-238. Elsevier.
567 33 Hadfield, J. D. SPEC: Processing Spectral data. Ascot, UK: Imperial College
568 at Silwood Park; 2005. Available from
569 http://www.bio.ic.ac.uk/research/iowens/spec/welcome.htm.
570 34 Hadfield, J. D. & Owens, I. P. F. 2006 Strong environmental determination of a
571 carotenoid-based plumage trait is not mediated by carotenoid availability. *J. Evol.*
572 *Biol.* **19**, 1104-1114.
573 35 Endler, J. A. & Mielke, P. W. 2005 Comparing entire colour patterns as birds
574 see them. *Biol. J. Linn. Soc.* **86**, 405-431.
575 36 Abramoff, M. D., Magelhaes, P. J. & Ram, S. J. 2004 Image Processing with

576 ImageJ. *Biophotonics International* **11**, 36-42.
577 37 Hoyt, D. F. 1979 Practical methods of estimating volume and fresh weight of
578 bird eggs. *Auk* **96**, 73-77.
579 38 Forstmeier, W., Schielzeth, H., Schneider, M. & Kempenaers, B. 2007
580 Development of polymorphic microsatellite markers for the zebra finch (*Taeniopygia*
581 *guttata*). *Mol. Ecol. Notes* **7**, 1026-1028. (DOI 10.1111/j.1471-
582 8286.2007.01762.x|ISSN 1471-8278.)
583 39 Sefc, K. M., Payne, R. B. & Sorenson, M. D. 2001 Characterization of
584 microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species
585 amplification in estrildid and ploceid finches. *Mol. Ecol. Notes* **1**, 252-254.
586 40 Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. &
587 Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite
588 loci polymorphic in Sylviidae species and their cross-species amplification in other
589 passerine birds. *Mol. Ecol.* **9**, 2226-2231.
590 41 Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007 Revising how the
591 computer program CERVUS accommodates genotyping error increases success in
592 paternity assignment. *Mol. Ecol.* **16**, 1099-1106. (DOI 10.1111/j.1365-
593 294X.2007.03089.x.)
594 42 Vandeputte, M., Mauger, S. & Dupont-Nivet, M. 2006 An evaluation of
595 allowing for mismatches as a way to manage genotyping errors in parentage
596 assignment by exclusion. *Mol. Ecol. Notes* **6**, 265-267. (DOI 10.1111/j.1471-
597 8286.2005.01167.x.)
598 43 Slate, J., Marshall, T. & Pemberton, J. 2000 A retrospective assessment of the
599 accuracy of the paternity inference program CERVUS. *Mol. Ecol.* **9**, 801-808.
600 44 Lande, R. & Arnold, S. J. 1983 The measurement of selection on correlated

601 characters. *Evolution* **37**, 1210-1226.
602 45 Lynch, M. & Walsh, B. 1998 *Genetics and analysis of quantitative traits*.
603 Sunderland: Sinauer Associates, Inc.
604 46 Griffith, S. C., Owens, I. P. F. & Burke, T. 1999 Environmental determination
605 of a sexually selected trait. *Nature* **400**, 358-360.
606 47 Tschirren, B. & Postma, E. 2010 Quantitative genetics research in Zebra
607 Finches: where we are and where to go. *Emu* **110**, 268–278
608 48 Cunningham, E. J. A. & Russell, A. F. 2000 Egg investment is influenced by
609 male attractiveness in the mallard. *Nature* **404**, 74-77.
610 49 Gil, D., Graves, J., Hazon, N. & Wells, A. 1999 Male attractiveness and
611 differential testosterone investment in zebra finch eggs. *Science* **286**, 126-128.
612 50 Pryke, S. R. & Griffith, S. C. 2009 Genetic incompatibility drives sex allocation
613 and maternal investment in a polymorphic finch. *Science* **323**, 1605-1607. (DOI
614 10.1126/science.1168928.)
615 51 Sheldon, B. C. 2000 Differential allocation: tests, mechanisms and
616 implications. *Trends Ecol. Evol.* **15**, 397-402.
617 52 Burley, N. 1986 Sexual selection for aesthetic traits in species with biparental
618 care. *Am. Nat.* **127**, 415-445.
619 53 Gilbert, L., Williamson, K. A. & Graves, J. A. in press Male attractiveness
620 regulates daughter fecundity non-genetically via maternal investment. *Proc. R. Soc.*
621 *B*
622 54 Mousseau, T. A. & Fox, C. W. 1998 *Maternal Effects As Adaptations*. New
623 York: Oxford University Press.
624 55 Meikle, D. B., Kruper, J. H. & Browning, C. R. 1995 Adult male house mice
625 born to undernurished mothers are unattractive to estrous females. *Anim. Behav.* **50**,

626 753-758.
627 56 Hunt, J. & Simmons, L. W. 2000 Maternal and paternal effects on offspring
628 phenotype in the dung beetle *Onthophagus taurus*. *Evolution* **54**, 936-941.
629 57 Strasser, R. & Schwabl, H. 2004 Yolk testosterone organizes behavior and
630 male plumage coloration in house sparrows (*Passer domesticus*). *Behav. Ecol.*
631 *Sociobiol.* **56**, 491-497.
632 58 Eising, C. M., Müller, W. & Groothuis, T. G. G. 2006 Avian mothers create
633 different phenotypes by hormone deposition in their eggs. *Biol. Lett.* **2**, 20-22. (DOI
634 10.1098/rsbl.2005.0391.)
635 59 Gerlach, N. M., McGlothlin, J. W., Parker, P. G. & Ketterson, E. D. in press
636 Promiscuous mating produces offspring with higher lifetime fitness. *Proc. R. Soc. B*
637 60 Reid, J. M., Arcese, P., Sardell, R. J. & Keller, L. F. 2011 Additive genetic
638 variance, heritability, and inbreeding depression in male extra-pair reproductive
639 success. *Am. Nat.* **177**, 177-187. (DOI 10.1086/657977.)
640 61 Kotiaho, J. S., Simmons, L. W., Hunt, J. & Tomkins, J. L. 2003 Males influence
641 maternal effects that promote sexual selection: A quantitative genetic experiment
642 with dung beetles *Onthophagus taurus*. *Am. Nat.* **161**, 852-859.
643 62 Both, C., Visser, M. E. & Verboven, N. 1999 Density-dependent recruitment
644 rates in great tits: the importance of being heavier. *Proc. R. Soc. Lond. B* **266**, 465-
645 469.
646 63 Krist, M., Nadvornik, P., Uvirova, L. & Bures, S. 2005 Paternity covaries with
647 laying and hatching order in the collared flycatcher *Ficedula albicollis*. *Behav. Ecol.*
648 *Sociobiol.* **59**, 6-11. (DOI 10.1007/s00265-005-0002-2.)
649 64 Griffith, S. C., Pariser, E. C., Tschirren, B. & Astheimer, L. B. in press
650 Resource allocation across the laying sequence in the wild zebra finch. *J. Avian Biol.*

651 65 Griffith, S. C. & Buchanan, K. L. 2010 Maternal effects in the Zebra Finch: a
652 model mother reviewed. *Emu* **110**, 251–267.
653 66 Griffith, S. C. 2007 The evolution of infidelity in socially monogamous
654 passerines: Neglected components of direct and indirect selection. *Am. Nat.* **169**,
655 274-281.
656 67 Qvarnström, A. & Price, T. D. 2001 Maternal effects, paternal effects and
657 sexual selection. *Trends Ecol. Evol.* **16**, 95-100.
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660 **FIGURE LEGENDS**

661

662 **Fig. 1** Relationship between the cheek patch size (mm^2) of biological fathers and
663 their sons in wild (grey dots and line) and domesticated (black dots and line) zebra
664 finches. Only sons reared by another male than their biological father were included
665 in the analysis. Dashed lines represent OLS regression lines. Note that absolute
666 values are presented for illustrative purposes, but that standardised values were
667 used in the statistical analyses.

668

669 **Fig. 2** Residual cheek patch size of sons sired by a female's social (WPO; grey dots)
670 or extra-pair partner (EPO; black dots) in wild and domesticated zebra finches.
671 Residual cheek patch size is corrected for breeding round, brood and body size.
672 Means + 1 SE are shown.

673

674 **Fig. 3** Cheek patch size (mm^2) of the extra-pair and social partner of wild-caught
675 (grey dots and lines) and domesticated (black dots and lines) females.

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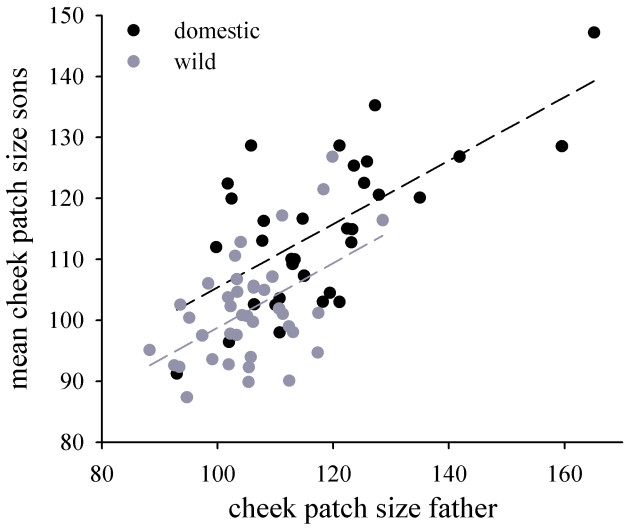
domestic
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mean cheek patch size sons
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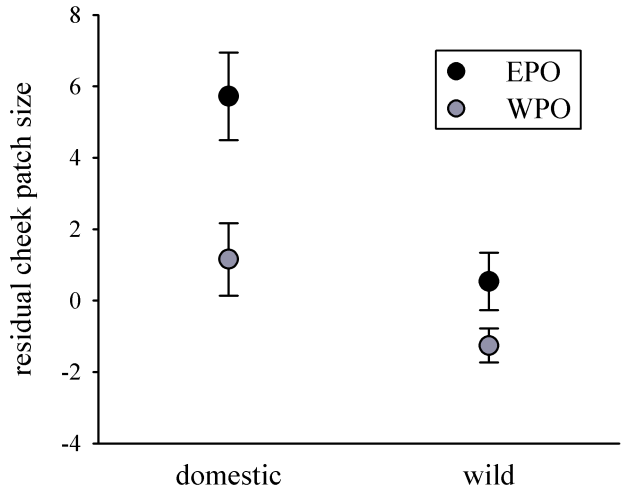

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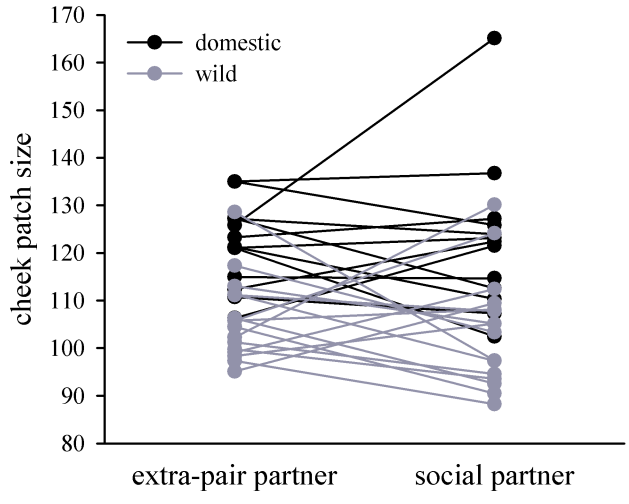
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cheek patch size
domestic
wild
extra-pair partner
social partner

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