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Paternal attractiveness and the effects of

differential allocation of parental investment

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24 **ABSTRACT**

25 The Differential Allocation Hypothesis (DAH) predicts that an individual should vary its

26 reproductive investment depending on the attractiveness of its mate. A recently revised

27 version of the DAH makes explicit that investment can be positive, i.e. higher for the

28 offspring of attractive males which should be of higher quality, or negative, i.e. higher for

29 offspring of unattractive males, e.g. compensating for inheriting poor paternal genes.

30 Moreover, investment can be made by the father and the mother. Here, we tested whether

31 experimental manipulation of male attractiveness affected parental investment at different

32 reproductive stages and thus influenced fitness-related traits in offspring. In two aviaries, all

33 male zebra finches *Taeniopygia guttata* were given red leg rings to increase attractiveness

34 and in two aviaries all males received green leg rings to decrease attractiveness. This

35 controlled for assortative mating between treatments. Ring colour was merely an

36 experimental manipulation of male attractiveness, not paternal quality, so we might expect

37 additional investment to elevate offspring quality. Eggs were cross-fostered between and

38 within treatments to allow differentiation of effects of investment in eggs and nestlings.

39 Clutch and brood sizes were standardized. Both positive and negative investment were

40 observed: Eggs from red ringed fathers had higher yolk to albumen ratios than eggs from

41 green-ringed fathers. Nestlings from eggs laid and incubated by parents in the red-ringed

42 group had higher hatching masses than those in the green-ringed group. Both parents in the

43 green-ringed group fed nestlings more frequently than red-ringed parents. Offspring

44 performance was influenced by the treatment of both foster and biological parents, but

45 combined effects of these different investment patterns on fitness-related traits were

46 ambiguous. Male attractiveness appeared to affect patterns of reproductive investment but

47   not consistently across all forms of reproductive investment suggesting that the costs and

48   benefits of differential allocation vary among individuals and across contexts.

49   **Key words:** androgens, begging, provisioning rates, transgenerational maternal effects,

50   Zebra finch.

51

52   The classical Differential Allocation Hypothesis (DAH) predicted that females should invest

53   more in offspring of attractive than unattractive males to maintain current and future pair

54   bond with an attractive mate (Burley, 1986a, 1988). This has subsequently been expanded

55   to a rationale that if male attractiveness is indicative of genetic quality or resource

56   availability, then a reproductive event with an attractive mate represents a higher value

57   reproductive event than one with an unattractive male (Sheldon, 2000). Therefore, since

58   females have a limited amount of resources to invest in reproduction, they would benefit

59   from investing relative to the value of a particular event (Trivers & Willard, 1973); but see

60   (Jones, Nakagawa, & Sheldon, 2009). However, positive differential allocation may also

61   occur if, for example, the females mated to attractive males increase their investment in

62   order compensate for attractive males investing less in offspring feeding than unattractive

63   males (e.g. Witte, 1995). While the result of this is a pattern of positive differential

64   allocation by the female, this is because of compensatory investment rather than

65   maximising the value of high quality offspring. Data on investment by both parents at both

66   egg and nestling stages is therefore needed to identify the underlying causation, at least in

67   species with biparental care (Montoya & Torres, 2015). More recently it has been

68   recommended that the DAH is generalised such that the investment could be allocated by

69   the father as well as the mother. Moreover, differential allocation could also be negative,

70   i.e. parents may invest more in offspring of unattractive than attractive, mates (Ratikainen &

71 Kokko, 2010). Thus, parents may invest more to compensate for a poor situation such as low

72 genetic quality of their offspring due to a poor quality mate, i.e. "making the best of a bad

73 job".

74   The impacts on offspring fitness of differential allocation are difficult to predict,

75 particularly in socially monogamous species with biparental care. If, for example, attractive

76 males contribute less paternal care than less attractive males (e.g.(Mazuc, Chastel, & Sorci,

77 2003; Sanz, 2001; Witte, 1995) then offspring with attractive fathers might benefit from

78 good genes but suffer from reduced paternal care, if mothers are unable to fully

79 compensate. Under negative differential allocation, if mothers invest heavily in offspring of

80 unattractive fathers then offspring may receive an overall benefit from having an

81 unattractive father (Byers & Waits, 2006; Griffith & Buchanan, 2010). Theoretical models

82 have predicted that a positive relationship between mate attractiveness and reproductive

83 investment should be the more common pattern of differential allocation (Harris & Uller,

84 2009; but see Ratikainen & Kokko, 2010). This appears to be supported by empirical studies

85 of investment in the pre-hatching (Cunningham & Russell, 2000; Gilbert, Williamson, Hazon,

86 & Graves, 2006; Rutstein, Gilbert, Slater, & Graves, 2004; Saino et al., 2002; Uller, Eklof, &

87 Andersson, 2005; but see Horvathova, Nakagawa, & Uller, 2012) and post-hatching stages

88 (e.g. Burley, 1988; Gorman, Arnold, & Nager, 2005; Hasegawa, Arai, Watanabe, &

89 Nakamura, 2012; Limbourg, Mateman, Andersson, & Lessells, 2004; Maguire & Safran,

90 2010). For offspring, such positive levels of investment can affect growth and development

91 (Gilbert et al., 2006) and have positive effects on fecundity and other fitness related

92 traits(Bowers et al., 2013; Cunningham & Russell, 2000; Gilbert, Williamson, & Graves, 2012;

93 Gilbert et al., 2006).

94   Negative differential allocation has received less attention and, as predicted by

95   models, has been reported less frequently (Harris & Uller, 2009). A number of studies have

96   shown decreased maternal expenditure in egg composition (Bolund, Schielzeth, &

97   Forstmeier, 2009; Michl, Torok, Peczely, Garamszegi, & Schwabl, 2005; Navara, Badyaev,

98   Mendonca, & Hill, 2006; Saino et al., 2002). However, few studies have looked at the

99   investment by both fathers and mothers at both pre- and post-hatching stages in the

100   response to male attractiveness (but see (Montoya & Torres, 2015; Sheppard, Clark,

101   Devries, & Brasher, 2013). This is important in order to be able to differentiate whether

102   females are allocating investment based on male attractiveness or compensating for

103   reduced parental care by fathers (Witte, 1995). Crucially, even fewer studies have been able

104   to assess the consequences on offspring quality of such allocation decisions. An

105   experimental system with biparental care, in which male attractiveness can be manipulated

106   independently of genetic quality and offspring can be cross-fostered (Montoya & Torres,

107   2015) is necessary to help us to tease apart some of these issues.

108   In this paper, we test for positive and negative differential allocation (Ratikainen &

109   Kokko, 2010) in egg formation and nestling-rearing in response to mate attractiveness in

110   zebra finches (*Taeniopygia guttata*). This is the species used in the original test of the

111   hypothesis by Burley (1988) and male attractiveness can be manipulated using leg rings with

112   red rings increasing attractiveness and green rings decreasing attractiveness. Importantly,

113   we also relate differential allocation to the phenotype, survival and fecundity of the

114   offspring. Using experimental manipulation of male attractiveness and cross-fostering of the

115   offspring, which allows teasing apart the effects of egg investment and nestling-rearing

116   investment, we addressed the following questions: (1) Do females adjust their investment

117   into eggs based on the ring colour of their mate? (2) Do either males or females provision

118 nestlings differently based on male ring-colour? (3) Do the offspring of red- or green-ringed

119 biological or foster fathers differ in their begging behaviour and growth rates? (4) Does the

120 attractiveness of either the biological or foster father influence the adult size, survival and

121 fecundity of offspring?

122

123 **METHODS**

124 *Husbandry*

125 All birds used in this experiment were between 9-18 months old, had been housed indoors

126 since birth and had bred at least once with a mate wearing a neutral orange-coloured leg

127 ring. Immediately prior to the experiment, all individuals were being housed indoors within

128 single-sex groups of typically 4-6 birds. At the start of the experiment, these birds were

129 transported to our outside aviary facility and four breeding colonies each consisting of 20

130 males and 21 females were established in large outdoor aviaries (2.8 x 5.5 x 2.5m) in 2002.

131 No bird was released in the same aviary as its previous breeding partner(s) or with siblings.

132 Birds were fed on a diet of *ad libitum* seed mix (foreign finch mix supplied by Haith's,

133 Cleethorpes, Lincolnshire, UK), supplemented with an egg food (Haith's egg biscuit) mixed

134 with vitamin supplement (Minavit) three times a week and fresh greens and millet sprays

135 once per week. Fresh drinking water, oystershell grit and cuttlebone were available *ad*

136 *libitum*. A calcium supplement (Calciform, Aviform, Wymondham, UK)) was added to the

137 water five times per week. From hatching onwards we also provided daily *ad libitum* soaked

138 seed mix.

139

Manipulation of Male Attractiveness

A great advantage of the zebra finch for the purposes of experimental design is that there is a well-established technique to manipulate attractiveness by using coloured leg rings. In mate choice trials of both captive and wild-caught zebra finches, females have consistently demonstrated strong preferences for males with red leg rings over males with green leg rings under 'natural' lighting conditions (either outside or inside under UV-rich lighting tubes) (Burley, 1986b; Hunt, Cuthill, Swaddle, & Bennett, 1997). It has been suggested that red leg rings enhance the red beak, which in zebra finches is a condition-dependent secondary sexual trait (Blount, Metcalfe, Birkhead, & Surai, 2003). We thus ringed half the males with an individually numbered red or a green leg ring at the start of the experiment. Moreover, there is evidence that male zebra finches with red rings sing more and gain more mass suggesting that ring colour alters other male traits as well as female behaviour (Pariser, Mariette, & Griffith, 2010). Red- and green-ringed males were kept in separate aviaries in order to control for potentially assortative mating due to differential access of red-ringed males to high quality females (Burley, 1986b) which would make it impossible to distinguish between increased female effort due to differential allocation and that due to female quality. However, females were still free to choose their mates within each attractiveness treatment group (Griffith, Pryke, & Buttemer, 2011). Our experiment was performed in four outdoor aviaries, i.e. with a natural UV spectrum (Hunt et al., 1997). All females were ringed with individually numbered orange leg-rings, a neutral colour with respect to male mate preference (Burley, 1986b), for identification purposes.

On the day that males and females were released together into the aviaries, all birds were weighed to the nearest 0.1 g and tarsus length measured to the nearest 0.1 mm. There were no differences in either body mass or tarsus length of males and females between the

7

164   two treatment groups (P > 0.21). Birds were released on the 20 May 2002 and allowed to

165   settle in their new environment for two weeks. Any birds that died during this acclimation

166   period were replaced with suitable birds of the same sex to maintain the group size. At the

167   end of the experiment, all birds were caught, re-measured and returned to the indoor

168   aviaries at the University of Glasgow.

169

170   *Breeding Design*

171   On the 6 June 2002, 24 nest boxes were installed in each aviary and nesting material (hemp

172   core and coconut fibres) provided. Nest boxes were then checked daily and each new egg

173   was individually marked and weighed. Once clutches were complete (no additional eggs had

174   been laid for two days) experimental clutches were formed by cross-fostering eggs between

175   nests. Each experimental clutch initially contained four eggs in total, two laid by pairs in the

176   green ring and two from the red ring groups. All eggs were transferred between nests and

177   assigned to foster parents according to the expected hatching date. From the expected

178   hatching date onwards nests were checked twice a day to record from which egg nestlings

179   hatched. For all nestlings used in the experimental broods, it was known from which egg

180   they hatched ('egg of origin'), and thus, the treatment of their biological parents.

181   Occurrence of hatching failure meant that brood size at hatching had to be reduced to two

182   nestlings, one from each treatment group. This maintained a constant brood size for all

183   pairs. In order to make up two-nestling broods, occasionally a hatchling that had

184   experienced the same laying and incubation conditions as the un-hatched egg it had to

185   replace had to be moved between nests. Thus, experimental broods consisted of two

186   nestlings that hatched on the same day, one of each colour ring group. No nestlings were

187   related to either their nestmate or their foster parents. A total of 23 experimental broods

188 were set up (6 in each of the two aviaries with red-ringed males and 5 and 6 in the two

189 aviaries with green-ringed males).

190   All nestlings were weighed to the nearest 0.1 g on the day they hatched (day 0) and

191 marked with a non-toxic colour marker pen on their down feathers to permit individual

192 identification. Nestlings were reweighed and tarsus length measured on days 3, 6 and 9 all

193 by the same observer. An instantaneous growth rate (slope of the regression of log(nestling

194 mass) on nestling age) was then calculated. The sex of the offspring was determined either

195 retrospectively from the adult plumage, or by a molecular sexing technique (Arnold et al.

196 2003) if the bird died before adulthood. The sex of 3 nestlings that died very early and could

197 not be recovered were not determined. There were no differences in sex between offspring

198 hatched from eggs laid in the red- or green-ringed groups $(\chi_1^2 = 0.19, P = 0.66)$ or with laying

199 order (Wilcoxon matched pairs test: $N = 23$, $Z = 0.63$, $P = 0.53$) (Rutstein et al., 2005).

200 Offspring were left to fledge naturally within the outdoor aviaries. All birds were brought

201 back into indoor bird rooms in August 2002 when offspring were nutritionally independent

202 (approximately 6 weeks of age). Parents and offspring were then housed in separate single-

203 sex groups of six individuals in cages 40 cm wide, 120 cm long and 40 cm high.

204

205 *Maternal Investment into Eggs*

206 To quantify maternal differential investment in primary reproductive effort, a range of egg

207 characteristics were measured. All eggs were individually marked on the day they were laid,

208 and a subset of eggs ($N = 98$ from 31 clutches - 15 clutches from the red ringed treatment

209 and 16 from the green ringed treatment) was collected approximately two days after onset

210 of incubation in order to allow the embryo to develop sufficiently to be sexed. We replaced

211 eggs with model eggs made from Fimo polymer clay (Eberhard Faber, Neumarkt, Germany)

212 which were similar in size, shape and colour to zebra finch eggs to ensure the birds did not

213 change their clutch size (Zann, 1996). Upon collection, each egg was weighed, then opened

214 and the yolk, embryo, albumen and shell were weighed separately. There was considerable

215 variation in embryo size and only yolks from eggs with blastocysts or minute embryos <2mm

216 in diameter (Gilbert, Bulmer, Arnold, & Graves, 2007) were further analysed for yolk colour

217 and androgen levels (see below), and yolk and albumen mass. In more developed eggs with

218 larger embryos, the yolk and albumen could not be cleanly separated because after two

219 days of incubation the perivitelline membrane was easily broken, and these eggs were not

220 used for analyses on yolk androgen, yolk and albumen masses. The embryo or blood vessels,

221 if present, were removed for molecular sexing. The sexes of early embryo samples from

222 eggs were assigned using primers P2 and P17 (full methods outlined in (Arnold et al., 2003).

223 The colour of the yolk was scored using a Roche Yolk Fan, which correlates with carotenoid

224 levels (Karadas, Grammenidis, Surai, Acamovic, & Sparks, 2006). The colour scores were

225 square root transformed prior to analysis.

226   At the University of St Andrews, we analysed testosterone (T) and its derivative 5α-

227 dihydrotestosterone (DHT) contents for all eggs that contained blastocysts with either no

228 signs of development or minute embryos < 0.01 g since they do not differ in yolk androgen

229 levels (Gilbert et al., 2007). The extraction and assay protocols used here are described

230 elsewhere (Gilbert et al., 2007) and follow the methods used in the commercially available T

231 radioimmunoassay (RIA) kit from Amersham Pharmacia Biotech (after Gil, Graves, Hazon, &

232 Wells, 1999). Extraction recovery of total androgens (T+DHT) was 75.6 ± 9.0 % (mean ± SE)

233 and of DHT alone was 59.8 ± 0.9 %. The two resulting extracts (total (T + DHT) and DHT only)

234 were assayed by means of competitive binding RIA. We ran samples in duplicate and

235 hormone concentrations were compared to total (T+DHT) and DHT standard curves that

236 ranged from 12.5-800 pg per assay tube. The degree to which the antiserum cross-reacted

237 with DHT in the RIA was 46%, so the T concentration was estimated as total-(0.46DHT).

238 Minimal cross-reactivity of this antiserum was found with ten other steroids (Nash et al.,

239 2000). The intra-assay coefficient of variation ($\pm$ SE) was 2.9 $\pm$ 0.31% for total (T+DHT) and

240 2.1 $\pm$ 0.32 % for DHT.

241

242 *Parental Care*

243 We quantified differences in parental effort in relation to colour ring treatment by

244 recording parental feeding behaviour on day 9 after hatching (day of hatching = day 0) in 18

245 experimental broods that still had both nestlings at that age. Day 9 is roughly mid-way

246 through development and the point at which nestlings were large enough to distinguish on

247 the camera. At this age the nestlings were also not too old that they were stimulated to

248 fledge early when the nest box was opened. We recorded the behaviour using small infrared

249 video cameras in the nest box. To allow birds to get used to the equipment, each camera

250 was installed in the top of the nest box at least four hours before recording commenced.

251 Breeding birds were observed to return to their nest boxes within minutes of setting up the

252 camera.

253 Behaviours were recorded, always between 13:30 and 16:30 BST, coinciding with a

254 minor peak of feeding (K.E.A. & R.G.N Unpublished data). Average observation duration per

255 nest was 2.88 $\pm$0.08 hours (N = 18) because intense fighting on the nest between the

256 breeding bird and an intruder in two cases meant that some observation time was lost in

257 one nest each of the red and green-ringed group. Videos were watched by an observer

258 unaware of the treatment groups. We recorded nest attentiveness (percent of total

259 observation time that the parent was present on the nest), and the number and duration of

feeding bouts per nestling by each parent. Feeding bouts were easily recognisable on the

videos, and they were counted and timed. In a feeding bout regurgitated seed mixed with

water is transferred to the young. The parent's gaping bill is interlocked with the nestling's

bill and, using its tongue, the parent pushes portions of food into the mouth of the nestling,

which swallows the food into its crop. The duration of a continuous period of conspicuous

feeding behaviour was defined as a feeding bout and one or both nestlings may receive food

within a single feeding bout. Per nest visit, parents provided from 0 to 4 feeding bouts to

their nestlings (mean = 0.8 ± 0.08 feeds/visit, N = 36) and there was no relationship between

nest visit rate and feeding bout rate (Spearman's rank correlation: females: r_s = 0.18, P =

0.456; males: r_s = 0.37, P = 0.117, N = 18 each). Gilby et al., 2011 also concluded that

parental provisioning is more reliably quantified by feeding rates rather than number of nest

visits (Gilby, Mainwaring, Rollins, & Griffith, 2011). We therefore used the more informative

feeding bout rate as a measure of reproductive expenditure into nestling rearing.

Offspring Behaviour and Performance in the Nest

Nestling behaviour and begging were assessed from the same video recordings. Prior to

video recording, one nestling in each brood was randomly selected and its upper bill marked

with white correction fluid to allow us to distinguish between the two nestlings. There was

no difference in proportion of nestlings marked with non-toxic correction fluid with respect

to egg of origin (9 out of 19 hatched from an egg from the red-ring treatment, binomial test:

one-tailed P = 0.500), sex (χ_1^2 = 0.50, P = 0.480), hatching order (Wilcoxon matched pairs

test: Z = 0.63, P = 0.527), or body mass on day 9 (paired t-test: T_{18} = 0.59, P = 0.565). No

preference was found for the provisioning of marked or unmarked nestlings by foster

fathers (paired t-test: T_{15} = 0.22, P = 0.83) or foster mothers (paired t-test: T_{15} = 1.75, P =

284 0.10). Over the duration of the recording, the number of times each nestling begged was

285 recorded, regardless of the intensity of the begging (Kolliker, Richner, Werner, & Heeb,

286 1998).

287   Nestling mass and tarsus length were recorded between 09:00 and 12:00 on days

288 when the eldest nestling/s were 3, 6 and 9 days of age. Fledglings were weighed at the end

289 of the experiment, just prior to moving the birds from the outdoor aviaries back to the

290 indoor aviary complex, as an estimate of mass at independence. Sample sizes vary between

291 analyses due to missing samples or failed video recordings.

292

293 *Offspring Performance as Adults*

294 Finally, we assessed the growth, survival and reproductive effort at first breeding of

295 offspring in relation to colour ring group of both their biological and foster parents. This was

296 carried out in the indoor bird facilities at the University of Glasgow. At the age of 4-5

297 months, each of the 38 surviving offspring was paired with an experienced breeder of the

298 opposite sex from our stock population. Breeding cages were 40 cm wide, 60 cm long and

299 40 cm high and provided with a nest box. Birds were weighed and their tarsus length

300 measured on pairing. All pairs were provided with a standard breeding diet for birds

301 breeding indoors including *ad libitum* seed mix (foreign finch mix supplied by Haith's,

302 Cleethorpes, Lincolnshire, UK), cuttlebone and grit, supplemented once per week with half a

303 teaspoon per bird of a protein supplement (Haith's egg biscuit) mixed with a vitamin

304 supplement (Minavit) and with a calcium supplement (Calcivet) in the drinking water. We

305 recorded the number of paired-up birds that produced eggs within 20 days of pairing, their

306 clutch size and size and composition of their eggs. Each egg was removed from the nest on

307 the day of laying and replaced with an artificial egg. Eggs were weighed to the nearest 0.01 g

308 on the day of laying and the mass of all eggs per clutch summed to give clutch mass. Egg

309 composition was assayed as above.

310

311 *Ethical Note*

312 This project was approved by the University of Glasgow's ethical review committee and

313 carried out under licence from the UK Home Office (Animal [Scientific Procedures] Act

314 1986). The protocols adhered to ASAB/ABS Guidelines for the Use of Animals in Research.

315 All the birds were sourced from the University of Glasgow's stock colony which included

316 some birds that had been hatched *in situ* and some that had been acquired from local bird

317 breeders. The birds were transported 5km from the main Department to Home Office

318 Licenced outdoor aviaries on a campus of the University of Glasgow and then back again in

319 groups of 20 - 25 in cages 40 cm wide, 60 cm long and 40 cm high. The cages contained

320 perches and bowls of seed but no water as the journey was ca. 20 minutes and we did not

321 want water to soak the floors of the cages. The fronts of the cages were covered to

322 minimise the light entering the cages during transport.

323

324 *Statistical Analyses*

325 We analysed parental care behaviour for males and females together by including pair

326 identity as a random effect into a general linear mixed model and included the sex of the

327 parent contributing to the parental care as a factor in the model. As the size of the offspring

328 and ambient temperature might affect parental care behaviour, we included in the

329 statistical model total brood mass and ambient temperature as covariates. In a previous

330 study on different birds using the same experimental design we found that the female's

331 response to ring colour may depend on the timing of breeding (Gorman et al., 2005) and we

332 therefore also included in the statistical model latency to lay as another covariate.

333   Our cross-fostering design allowed us to separate out the effect of differences in egg

334 quality (due to ring colour of the biological father), incubation and nestling rearing

335 environment (due to ring colour of the foster father) on offspring performance (Montoya &

336 Torres, 2015). As offspring produced by the same biological parents or raised by the same

337 foster parents cannot be considered independent, we used general linear mixed models with

338 the identity of biological and foster parents as a random effect. Preliminary analyses showed

339 that there were no differences between aviaries, so we here present only models with

340 biological and foster parents as the random factor which gave us greater degrees of freedom

341 and therefore greater statistical power. In these statistical models we also included offspring

342 sex, latency to lay and laying order. Similar general linear mixed models were used when

343 analysing the composition of eggs.

344   All mixed models were run on SAS, version 9 using either PROC MIXED or the macro

345 GLIMMIX (for the logistic regressions in the analysis of survival and breeding propensity). We

346 tested for all two-way interactions between main effects and covariates, and removed non-

347 significant factors from the full model stepwise beginning with the interaction terms. Only

348 statistically significant interactions and main effects are reported. We used $P < 0.05$ for

349 statistical significance and report mean values ± 1 S.E. throughout the text.

350

351 **RESULTS**

352

353 *Maternal Investment into Eggs*

354 The ring colour treatment did not affect the timing of breeding (red rings: 22.6 ± 1.5 June, N

355 = 38; green rings: 21.7 ± 1.1 June, N = 38; $F_{1,74}$ = 0.21, P = 0.65), or clutch size (red rings: 4.7

356 ± 0.2 eggs, N = 38; green rings: 4.3 ± 0.2 eggs, N = 38; $F_{1,74}$ = 1.75, P = 0.19). Egg volume

357 increased with increasing laying order (laying order: $F_{1,108}$ = 35.11, P < 0.0001; nest (random

358 factor): Z = 4.54, P < 0.0001), but colour ring of biological father, sex of egg and latency to

359 lay did not contribute to the models. No aspect of egg composition differed between male

360 and female eggs (GLMM, all P > 0.2).

361   Among the subset of collected eggs, the ratio of yolk to albumen varied significantly

362 with paternal ring colour and also decreased with increasing latency to lay (ring colour:

363 $F_{1,24.9}$ = 5.87, P = 0.023; latency: $F_{1,25.6}$ = 5.71, P = 0.025; laying order and interactions P >

364 0.7). Eggs from the red-ring treatment had significantly larger yolks relative to albumen

365 mass (mean ratio = 3.28 ± 0.20, N = 60) than those from the green-ring group (mean ratio =

366 2.09 ± 0.40; N = 71; ring colour of biological father: $F_{1,24.9}$ = 5.87, P = 0.023). Yolks and

367 albumens from the red treatment weighed on average 0.59g ± 0.03 and 0.32g ± 0.02

368 respectively compared with 0.57g ± 0.02 and 0.40g ± 0.03 in the green group. So, although

369 eggs from the red-ring treatment did not have significantly larger yolks or albumens (P > 0.2)

370 than those from the green-ring group, the differences in the ratio of yolk to albumen seems

371 to be driven by both over-investment in yolk and under-investment in albumen by the red-

372 ringed group.

373   Paternal ring colour did not influence yolk colour (a proxy for carotenoid content),

374 but yolk colour declined with laying order ($F_{1,75.1}$ = 30.77, P < 0.0001) and latency to lay

16

375   ($F_{1,26.2}$ = 4.92, P = 0.04; nest (random factor): Z = 2.47, N = 96, P = 0.007). Finally we found

376   that DHT concentrations in freshly laid eggs increased with latency to lay (latency to lay:

377    $F_{1,9.26}$ = 7.15, P = 0.025; nest (random factor) Z = 0.32, N = 32, P > 0.3). Laying order, paternal

378   ring colour and embryo sex did not contribute to the model. Testosterone concentrations in

379   eggs did not vary with any variable.

380

381   *Parental Care*

382   On day 9 post-hatching, video recordings revealed that nest attentiveness (the percentage

383   of time a parent spent brooding their nestlings) decreased with increasing total brood mass

384   (estimate = -0.01 ± 0.005 % of time spent brooding per g of brood mass) and females had

385   higher attentiveness (54.6 ± 4.2 %, N = 18) than males (29.4 ± 3.8 %, N = 18), irrespective of

386   ring colour treatment (Table 1). Parents in the green-ringed group fed their nestlings more

387   frequently (mean = 3.36 ± 0.22 feeds per hour) than parents in the red-ringed group (mean

388   = 2.36 ± 0.16 feeds per hour) and feeding rate decreased with increasing total brood mass

389   on day 9 (estimate = -0.05 ± 0.02 feeds $h^{-1} g^{-1}$, Table 1). A feeding bout lasted on average

390   15.0 ± 0.99 s (N = 157 feeding bouts) and its average length did not differ between the

391   colour ring treatments (Table 1). Mothers did not differ from fathers in their rate of feeding

392   or the duration of their feeding bouts. Also there was no significant interaction between

393   treatment and sex of the feeding parent, so mothers and fathers were not investing in

394   provisioning differently based on paternal attractiveness (Table 1).

395

396   *Parental Condition and Survival*

397   All parent birds lost mass between being first released into the aviaries and the end of the

398   experiment and this differed between treatment groups (ANOVA $F_{1, 143}$ = 2.98, P = 0.034).

399   Post-hoc tests showed that females paired to green-ringed males lost less mass (5.2 ± 1.9 %)

400   than all other birds (red-ringed males (11.6 ± 1.3 %), green-ringed males (11.8 ± 2.4 %) and

401   females paired to red-ringed males (9.5 ± 1.4 %; Tukey's B test $P = 0.05$)). Females paired to

402   red-ringed males were more likely to die during the study period than males (10 females

403   versus 2 males; $\chi^2_1 = 5.33$, $P = 0.021$). Mortality of males and females in the green

404   treatment over the course of the experiment was even (6 females versus 6 males).

405

406   *Offspring Behaviour and Performance in the Nest*

407   There was a non-significant trend (P = 0.06) towards nestlings hatched from eggs laid by

408   parents in the green-ringed group and incubated by green-ringed foster parents to be

409   smaller (0.9 ± 0.05 g, $N = 10$) than hatchlings from all other groups (1.1 ± 0.06 g, $N = 31$;

410   Table 2). During the first nine days post-hatching, nestling growth rate was not influenced

411   by ring colour treatment. Female nestlings grew faster (1.09 ± 0.05 g day^{-1}, $N = 24$) than

412   male nestlings (0.96 ± 0.08 g day^{-1}, $N = 15$; Table 2).

413   Nestlings begged with an average rate of 1.1 ± 0.16 begs h^{-1} (N = 18 broods) and this

414   was independent of the treatment of the biological and foster parents, its sex, the order of

415   the egg it hatched from and other variables investigated (GLMM, ring colour treatment of

416   biological parent: $F_{1,51} = 0.04$, $P = 0.852$; ring colour treatment of foster parent: $F_{1,15} = 1.56$,

417     $P = 0.231$; latency: $F_{1,16} = 0.94$, $P = 0.347$; laying order: $F_{1,59.7} = 0.01$, $P = 0.925$; offspring sex:

418     $F_{1,61.6} < 0.01$ $P = 0.993$; nestling's size relative to its nest mate: $F_{1,13} = 0.17$, $P = 0.684$; brood

419   sex composition: $F_{1,14} = 0.36$, $P = 0.557$; marking of the nestling: $F_{1,52} = 0.45$, $P = 0.503$). The

420   treatment of neither the biological ($F_{1,2.1} = 0.03$, $P = 0.871$) nor foster parents ($F_{1,3.02} = 0.97$,

421     $P = 0.397$) affected the distribution of feeds to nestlings. Similarly, the sex of the feeding

422 foster parent did not interact with the treatment of the biological father to affect the

423 distribution of food to nestlings ($F_{1,102} < 0.01$, $P = 0.979$).

424

425 *Offspring Performance at Adulthood*

426 Offspring survival from hatching to their first breeding attempt was high (84.8 %, $N = 46$).

427 Five nestlings died during the first 10 days (for the two where sex was identified, one was

428 male and one was female); after fledging two more nestlings died, one of each sex. We

429 therefore did not include offspring sex in the statistical analysis of offspring mortality.

430 Offspring mortality was independent of the ring colour treatment of the foster and

431 biological father and the latency to lay, but offspring from eggs laid later in the laying

432 sequence were more likely to die than eggs laid early in the laying sequence (GLIMMIX with

433 identity of biological parent as random effect: $Z = 1.32$, $P = 0.19$; laying order: $F_{1,38.9} = 4.17$, P

434 < 0.05; colour ring of foster parent: $F_{1,39} < 0.01$, $P = 0.99$; colour ring of biological parent:

435 $F_{1,8.64} = 0.24$, $P = 0.64$; latency to lay: $F_{1,29.8} = 0.45$, $P = 0.51$).

436   Offspring body mass and tarsus length at adulthood prior to first breeding differed

437 between ring colour treatments (Table 2). Specifically, offspring body mass as adults

438 declined with increasing order of the egg they hatched from when raised by foster parents

439 from the green ring treatment but not when raised by foster parents from the red ring

440 treatment, irrespective of the colour ring treatment of the biological parents (Table 2; Fig

441 1a). In contrast, individuals raised by red-ringed foster parents had longer tarsi than birds

442 raised by green-ringed foster parents but only when the biological parents were from the

443 green-ring treatment (Table 2; Fig. 1b).

444   When paired with an experienced breeder from our stock population, 73.7% of the

445 surviving 38 offspring produced eggs (sample size for offspring with treatment of

446 biological+treatment of foster fathers: Red+Red = 9, Red+Green = 11, Green+Red = 8,

447 Green+Green = 9). There was no difference in breeding propensity between the treatment

448 groups (GLIMMIX; latency to lay: $F_{1,36}$ = 1.83, P = 0.19; colour ring of biological parent: $F_{1,35}$ =

449 1.06, P = 0.31; colour ring of foster parent: $F_{1,34}$ = 0.72, P = 0.40; laying order: $F_{1,33}$ = 0.37, P =

450 0.55; sex: $F_{1,32}$ < 0.01, P = 0.95). This GLIMMIX model would not run with identity of

451 biological parent as a random factor because there were a large number of families for

452 which there was only one offspring included in the model, so we only included data from

453 one daughter per from a family of biological siblings to avoid pseudo-replication. The

454 analyses of the daughters' reproductive efforts during their first breeding attempt are

455 presented in Table 3. When breeding for the first time, daughters with green ringed

456 biological fathers laid clutches with a larger mass than daughters from red ringed biological

457 fathers (Fig. 2) due to them laying both more (red: 3.7 ± 0.47 eggs, N = 7; green: 4.9 ± 0.51

458 eggs, N = 7; $F_{1,8.1}$ = 4.85, P = 0.06) and larger eggs (red: 1.21 ± 0.05 g, N = 7; green: 1.29 ±

459 0.02 g, N = 7; $F_{1,9.07}$ = 4.17, P = 0.07). There was no difference in the ratio between wet yolk

460 mass to wet albumen mass suggesting all eggs were of similar gross composition

461 irrespective of egg size. Between pairing and clutch completion, daughters raised by foster

462 parents in the red ring group lost significantly more body mass (15.8 ± 2.86 %, N = 7) than

463 daughters raised by foster parents in the green ring group (12.4 ± 1.76 %, N = 7; Table 3).

464 Daughters that hatched from eggs laid late in the sequence produced heavier clutches than

465 daughters that hatched from eggs laid early in the laying sequence (Table 3).

466

467

DISCUSSION

Overall, both mothers and breeding pairs differentially invested resources into offspring based on our experimental manipulation of male attractiveness (summarised in Table 4). The evidence for differential allocation by fathers was less clear cut. Moreover, there appeared to be evidence for both positive and negative levels of investment, that may be related to differential allocation, which depended on the resource being invested and the stage of reproduction. While we found no evidence for a difference between treatment groups in egg size or yolk micro-nutrients (androgens and carotenoids), there was some evidence that mothers invested more in the eggs of red-ringed than green-ringed males: females paired to red-ringed males did lay eggs with a higher yolk to albumen ratio. Our cross-fostering design revealed that this was associated with an effect on offspring phenotype (summarised in Table 4): nestlings that hatched from eggs laid by parents in the green-ringed group that were also incubated by green-ringed parents were lighter at hatching than all other groups. In contrast, pairs in the red-ringed group provisioned their nestlings less frequently than pairs in the green-ringed group. Even though nestlings which had received a relatively poor pre-hatching environment (green biological and incubation parents) were smaller at hatching than all other groups, they appeared to be able to compensate for this in the nest as offspring body size at independence did not differ between treatment groups. However, despite hatching from eggs with a lower yolk to albumen ratio, daughters with green-ringed biological fathers laid more and heavier eggs compared with those with red-ringed fathers. In terms of the consequences for fitness-related traits due to differential investment at the nestling-rearing stage, female offspring raised by green-ringed foster parents lost less mass during their first breeding attempt than

492   those with red-ringed foster fathers, although their eggs did not differ in mass or

493   composition.

494   Differential maternal allocation is expected whenever males differ in attractiveness

495   which is predicted to be an honest signal of genetic or phenotypic quality (Sheldon 2000). In

496   our study, male attractiveness was manipulated independently of male quality. Also, in

497   contrast to Burley's classic studies, all males within the same aviary were subject to the

498   same treatment, (Burley, 1988); see also (Sheppard et al., 2013). Thus, in our study high

499   quality females could not pair assortatively with red-ringed males and low quality females

500   with green-ringed males. So, in our design any differences in maternal investment due to

501   ring colour were not confounded by female quality, but were the results of adjustments in

502   investment due to perceived male attractiveness.

503   Our finding that females mated to red-ringed males laid eggs with relatively larger

504   yolks than those with green-ringed mates is difficult to compare directly with previous tests

505   of the DAH in birds, some of which found negative differential allocation but into different

506   egg components (Bolund et al., 2009; Michl et al., 2005; Navara et al., 2006; Saino et al.,

507   2002). In contrast to Bolund et al. (2009), we also found no modulation of egg carotenoids

508   or hormones in response to male attractiveness (see also Grenna, Avidano, Malacarne,

509   Leboucher, & Cucco, 2014). Compared with albumen, yolk comprises higher levels and

510   diversity of lipids, minerals, vitamins and other substances vital for embryo development

511   (Klasing, 2000). While albumen contributes to nestling structural size, yolk supports

512   survivorship after hatching, suggesting that relative investment into these two egg

513   components will have different impacts on the resulting nestling (Klasing, 2000). One

514   potential explanation for this, based on the 'silver spoon' hypothesis (Bateson et al., 2004),

515   is that females are able to tailor eggs, so nestlings are better able to cope with predicted

conditions in the nest, e.g. low provisioning rates. We have previously demonstrated, using

the same experimental set-up, that earlier laying females with red-ringed partners

contributed significantly more to incubation than late breeding mothers, but no such

relationship was found in females mated to green ringed males. Incubation attentiveness of

the pair was correlated with hatching success (Gorman et al., 2005). Thus, our finding

suggests that egg composition interacted with incubation environment such that eggs with

relatively smaller yolks had prolonged embryonic development and reduced hatching

success. Finally, parents in the green-ringed treatment fed their nestlings more frequently

than those in the red-ringed aviaries (see also Limbourg, Mateman, & Lessells, 2013), even

though the nestlings in these nests did not differ in begging behaviour. Females in the

green-ringed treatment group were potentially compensating for relatively small yolk size as

opposed to under-investment by males because fathers did not feed at a significantly lower

rate than mothers.

Strictly speaking, to qualify as differential allocation, the investment into

provisioning eggs and nestlings should be costly to the individual and advantageous to the

offspring (Sheldon 2000). Egg production in zebra finches has been shown to be costly, for

example breeding females show a 22% increase in resting metabolic rate (Vezina &

Williams, 2005) and decreased flight performance (Veasey, Houston, & Metcalfe, 2001)

Moreover, better nourished mothers are able to produce heavier clutches at a lower cost to

themselves (Arnold et al., 2003). Provisioning nestlings is costly in terms of increased

susceptibility to oxidative stress (Alonso-Alvarez et al., 2004), and in some species of birds,

reduced future fecundity and survival (e.g. Maigret & Murphy, 1997; Owens & Bennett,

1994; Reid, Bignal, Bignal, McCracken, & Monaghan, 2003). Burley (Burley, 1986b, 1988) has

shown that increased parental effort decreased the survival of females mated to attractive

540   males. In our study, females in the green-ringed group, which provisioned their nestlings at

541   a higher rate than females in the red-ringed group, lost less mass during breeding than all

542   other birds. There is some evidence that mothers in the red-ringed treatment had higher

543   mortality than red-ringed males, which might be a consequence of heavy investment into

544   eggs (but less so into nestlings), although the sample sizes were small for the mortality

545   rates. Although the relative costs of egg production to nestling rearing (Monaghan & Nager,

546   1997; Nager, 2006) are unknown for zebra finches, our data suggest that differential

547   investment into eggs, but not nestlings, was costly to females at least in terms of mass loss

548   and potentially mortality. Moreover, the relatively low provisioning rates of females with

549   red-ringed mates may have been because their body reserves were relatively more

550   exhausted by egg production than in females with green-ringed males. This was despite the

551   fact that we standardised the brood size to two nestlings which is lower than the typical

552   brood size (~ 4 nestlings) of successful zebra finch parents in our aviaries (see also Zann,

553   1996). Perhaps females paired to unattractive males were altering the composition and/or

554   size of their eggs to cope best with assumed poor genetic quality? However, in this

555   experimental context, attractiveness was actually unrelated to genetic quality and thus

556   daughters from matings with unattractive males happened to fare better than expected.

557   Alternatively, or in addition, since parents in the green-ringed group provisioned nestlings

558   more frequently, the best strategy was to invest less at the egg stage but more at the

559   nestling rearing stage. Similarly, female brown boobies *Sula leucogaster* paired to more

560   colourful males laid smaller eggs and did not increase parental care, but the colouration of

561   foster fathers was positively related to paternal care and nestling growth (Montoya &

562   Torres, 2015). Overall, we found some evidence that in zebra finches that females can

563   differentially invest resources into offspring at different stages and that such investment

564 differs in costs to survival, breeding success and condition of parents. Next, we determined

565 whether the "differential allocation" affected fitness related traits in the offspring.

566   Offspring from eggs laid by and incubated by parents in the green-ringed treatment

567 were shown to have low yolk to albumen ratios and were also smaller at hatching but,

568 compared with hatchlings from the other treatment groups, did not differ in mortality.

569 Daughters from green-ringed biological parents laid heavier clutches at sexual maturity.

570 Furthermore, daughters reared under the relatively poor feeding regime of red-ringed

571 foster parents lost more mass during their first breeding attempt than those with green-

572 ringed foster parents, despite producing similar numbers and quality of eggs. So we do have

573 some evidence that differential breeding expenditure, at least in eggs, in response to to

574 mate attractiveness results in trans-generational effects on fitness-related traits. Notably,

575 the effects were dependent on breeding stage. Thus, more experimental data are required

576 to tease out whether females are able to strategically invest in nestlings as well as eggs or

577 whether investment in later reproductive stages is limited, for example energetically, by

578 previous investment decisions (Bowers et al., 2013). One issue with our data is that our

579 sample size of offspring which bred was relatively small (N = 38). Other studies on zebra

580 finches have also shown that conditions experienced during either the embryo (Gorman &

581 Nager, 2004; Tobler & Sandell, 2009; von Engelhardt, Carere, Dijkstra, & Groothuis, 2006) or

582 nestling stage (Blount, Metcalfe, Arnold, et al., 2003; Blount et al., 2006; Boag, 1987;

583 Spencer, Heidinger, D'Alba, Evans, & Monaghan, 2010) can affect fitness-related traits but

584 studies like ours that can directly link parental investment with offspring phenotypic or life

585 history traits at both pre- and post-hatching stages are largely lacking (but see (Bowers et

586 al., 2013; Cunningham & Russell, 2000; Gilbert et al., 2012; Gilbert et al., 2006).

587   Our results suggest both negative and positive investment, but how this balances out

588   to be positive, negative or indeed any overall differential allocation is unclear. There are

589   several alternative explanations.Previous studies have suggested that such a reduction in

590   male provisioning effort may be due to the attractive trait handicapping the male (Witte,

591   1995). For example, increasing the attractiveness of some males could increase the

592   frequency of dominance interactions between red-ringed males (Cuthill, Hunt, Cleary, &

593   Clark, 1997), permit males to become polygynous (Burley, 1986b) and/or lead to more

594   intense male competition (Qvarnström., 1997). Arguments against such behavioural

595   mechanisms are that a red ring should not handicap a male any more than a green ring and

596   also using aviaries where all males had the same ring colour should minimise the issue of

597   red-ringed *versus* green-ringed male competition or polygyny since treatment groups could

598   not interact or see each other (but see (Cuthill et al., 1997). That females with red-ringed

599   males did not increase their provisioning rate in response to the low input by their mates

600   suggests that a compensatory mechanism is not at play here (but see Witte, 1995).

601   Alternatively, given that females with red-ringed males had already invested heavily in eggs,

602   they might have been in poorer condition, and thus unable to compensate. It is also possible

603   that compensatory feeding was not necessary if they had already prepared their offspring

604   for a poor quality rearing environment, through changing egg resources (e.g. Gilbert et al.

605   2012).

606   Our result that female offspring of green-ringed biological fathers laid heavier

607   clutches is, interestingly, the opposite to that found by Gilbert et al. (2012) which used a

608   similar manipulation and cross-fostering design. The only clear differences between the two

609   studies are that we standardised our brood size to two nestlings and also our offspring were

610   reared in outdoor aviaries, in contrast to Gilbert et al. (2012) who used a separate cage per

611   pair of birds kept indoors with constant temperature, humidity and daylight regime. Subtle

612   environmental differences may result in differences in investment patterns (e.g. Mousseau

613   and Fox 1998; Williamson et al. 2008), and this can mean that using experiments to

614   generalise about avian investment decisions can be difficult. Comparing differences

615   between two standard groups of females exposed to males that differ only in their

616   perceived quality demonstrates the ability to alter investment and the direction in which it

617   occurs at different stages. Ultimately, however, the important thing is what individuals do

618   across their individual lifetime and the consequences of these different possible investment

619   strategies for their own fitness. So while in our study we found evidence for positive

620   differential investment at the egg stage, negative investment at the nestling rearing stage

621   and corresponding fitness-related offspring traits, we cannot conclude that passerine birds,

622   or even zebra finches specifically, will always behave like this. An individual is likely to

623   benefit by changing investment patterns depending on a range of environment cues

624   (Mousseau and Fox 1998; Williamson et al. 2008), often not yet quantified or understood by

625   researchers. To conclude, our study illustrates how patterns of reproductive investment can

626   be complex (see also Gorman et al., 2005; Michl et al., 2005; Rutstein et al., 2005) and not

627   consistent across all forms of maternal investment (Balzer & Williams, 1998).

628

634

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801 **Figure 1.** **(a)** Adult body mass at first breeding of birds raised by red-ringed foster parents

802 (closed symbols) and by green-ringed foster parents (open symbols) in relation to laying

803 order. **(b)** Mean (± 1 SE) adult tarsus length in relation to ring colour treatment of the

804 biological father. Open bars show the tarsus length of birds raised by foster parents in the

805 green-ring group and the shaded bars of birds raised by foster parents in the red-ring group.

806 See table 2 for results of the statistical analysis. Numbers above the bars represent the

807 numbers of offspring.

808

809 **Figure 2.** Mean (± 1 SE) clutch mass (number of eggs * mean egg mass) at first breeding of

810 daughters that hatched from eggs laid by red-ringed biological parents (shaded bars) and by

811 green-ringed biological parents (open bars) in relation to laying order. For presentation,

812 daughters hatched from early-laid eggs (first two eggs) and later-laid eggs (eggs 3 to 5) are

813 shown separately, but laying order was used as a continuous variable in the analysis (see

814 Table 3 for results of the statistical analysis). Numbers above the bars indicate the numbers

815 of daughters.

	Attentiveness (%)	Feeds per hour	Feeding bout length (s)
Foster father ring colour	$F_{1,16} = 1.24$ $P = 0.28$	$F_{1,15} = 9.60$ **P = 0.007**	$F_{1,13} = 0.95$ $P = 0.35$
Foster parent sex	$F_{1,18} = 12.09$ **P = 0.003**	$F_{1,17} = 3.00$ $P = 0.10$	$F_{1,17} = 2.85$ $P = 0.11$
Latency to lay	$F_{1,15} = 1.90$ $P = 0.19$	$F_{1,13} = 0.03$ $P = 0.87$	$F_{1,14} = 0.63$ $P = 0.44$
Total brood mass	$F_{1,17} = 8.08$ **P = 0.011**	$F_{1,15} = 5.80$ **P = 0.029**	$F_{1,15} = 0.18$ $P = 0.68$
Ambient temperature	$F_{1,14} = 0.56$ $P = 0.47$	$F_{1,14} = 1.22$ $P = 0.29$	$F_{1,16} = 4.05$ $P = 0.061$
Nest (random factor)	$Z = 3.29$, $P = 0.001$	$Z = 0.19$, $P = 0.85$	$Z = 0.92$, $P = 0.36$

820   All broods ($N = 18$) consisted of two nestlings. Measures of parental care behaviour include
821   nest attentiveness (percentage of observation time when nestlings are brooded by one
822   parent), feeding rate (number of feeds per hour per brood) and the average length of the
823   feeding bout per nestling (i.e. the time a parent spent regurgitating seeds into the mouth of
824   a nestling, see methods for details). *P* > 0.06 for all interactions.

825

826

827 **Table 2.** Results for mixed models on the effect of the colour ring treatment on hatchling

828 mass, nestling growth, and body mass and length of offspring tarsus at adulthood when

829 breeding the first time.

830

	Hatchling Mass (N = 41)	Nestling Growth (N = 39)	Adult body mass (N = 37)	Adult tarsus length (N = 37)
Treatment of biological parent	$F_{1,21.3}=0.40$ P=0.54	$F_{1,13.5}=0.25$ P=0.63	$F_{1,24.6}=0.70$ P=0.41	$F_{1,15}=0.01$ P=0.92
Treatment of foster parent	$F_{1,16}=1.49$ P=0.24	$F_{1,19.4}=0.35$ P=0.56	$F_{1,19.2}=5.61$ P=0.03	$F_{1,10.5}=0.06$ P=0.81
Offspring sex	$F_{1,33.4}<0.01$ P=0.95	$F_{1,23.9}=5.72$ P=0.03	$F_{1,15}=3.58$ P=0.08	$F_{1,17.4}=1.41$ P=0.25
Latency to lay	$F_{1,18.1}=0.84$ P=0.37	$F_{1,120.4}=3.35$ P=0.08	$F_{1,3.21}=4.38$ P=0.12	$F_{1,17.3}=0.12$ P=0.74
Laying order	$F_{1,30.6}=0.32$ P=0.57	$F_{1,25.5}=0.10$ P=0.75	$F_{1,10.8}=0.15$ P=0.71	$F_{1,26}=0.34$ P=0.56
Laying order * foster parent treatment			$F_{1,18.9}=6.50$ P=0.02	
Foster * biological parent treatments	$F_{1,20.6}=4.14$ P=0.06			$F_{1,11.1}=8.33$ P=0.02
Identity of biological nest	Z=2.25 P=0.02	Z=1.06 P=0.29	Z= 1.46 P=0.14	Z=1.03 P=0.30
Identity of foster nest	Z=0.51 P=0.61	Z= 2.40 P=0.02	Z=2.27 P=0.02	Z=2.35 P=0.02

831 These models contained the ring colour of biological and foster parents, sex of the offspring

832 and from what laying order it hatched (laying order) and the latency to lay with identity of

833 the biological 'nest' and the foster 'nest' as random factors. All other interactions $P > 0.23$.

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Table 3. Results for mixed models on the effect of the colour ring treatment of the father on reproductive effort of their daughters when breeding the first time.

	Latency to lay	Clutch mass	Egg composition	Mass loss
Ring colour of biological parent	$F_{1,11}=0.79$ $P=0.39$	$F_{1,8.94}=6.82$ $P=0.03$	$F_{1,9.74}=0.89$ $P=0.37$	$F_{1,8}=0.19$ $P=0.67$
Ring colour of foster parent	$F_{1,6.03}=1.16$ $P=0.32$	$F_{1,4.58}=0.14$ $P=0.72$	$F_{1,9.47}=0.91$ $P=0.36$	$F_{1,11}=24.48$ $P<0.001$
Latency to lay	$F_{1,7}=0.25$ $P=0.63$	$F_{1,9.35}=2.41$ $P=0.15$	$F_{1,8.97}=0.02$ $P=0.88$	$F_{1,7}=0.01$ $P=0.92$
Laying order	$F_{1,1}=0.18$ $P=0.74$	$F_{1,10.6}=5.84$ $P=0.03$	$F_{1,10}=1.09$ $P=0.32$	$F_{1,11}=0.84$ $P=0.38$
Identity of biological parent	$Z=2.34$ $P=0.02$	$Z=0.07$ $P=0.95$	$Z=0.23$ $P=0.82$	$Z=2.35$ $P=0.02$

Independent variables were latency to lay (number of days between pairing and laying the first egg), clutch mass (number of eggs laid * mean egg mass), egg composition (ratio between wet yolk mass and wet albumen mass) and mass loss between pairing and clutch completion. These models contained the ring colour of biological and foster parents, the order of the egg from which it hatched (laying order) and the latency of parents to lay. Only one daughter per rearing nest was used in the analysis (see methods) and therefore the model contains only identity of the biological 'nest' as a random factor. All interactions *P* > 0.22.
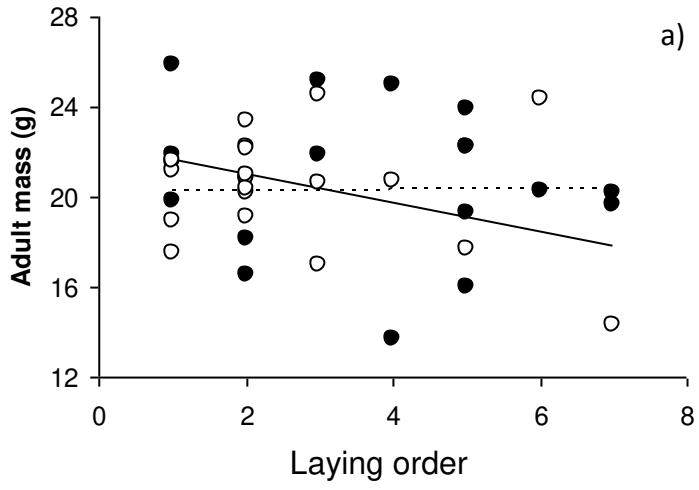
Table 4: Summary of the effects of paternal ring colour on parental investment in different

stages of reproduction, parental condition and fitness-related traits in offspring.

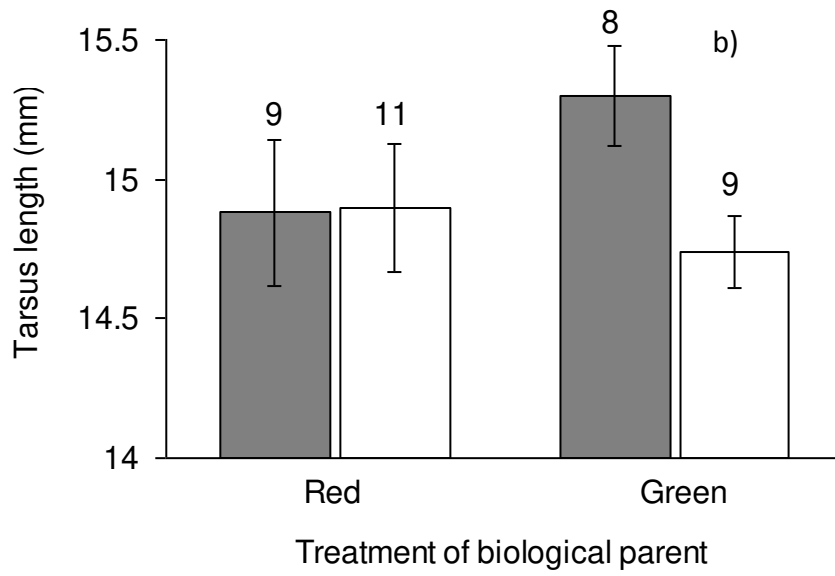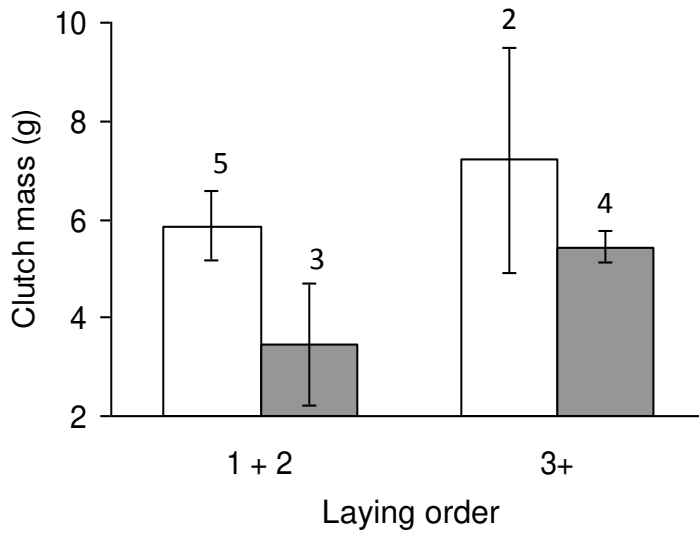| Parameter	Red or Green ring effect
Maternal investment into eggs	
Timing of breeding	NS
Clutch size	NS
Egg volume	NS
Yolk:albumen mass	Higher for eggs of red-ringed fathers
Yolk colour	NS
Yolk DHT concentration	NS
Yolk T concentration	NS
Parental care	
Nest attentiveness	NS
Nestling feeding rate	Higher for green-ringed parents
Parental condition and survival	
Adult mass loss over breeding period	Lower in females paired to green-ringed males than in other birds
Adult mortality over breeding period	Red-ringed pairs: females had higher mortality than males. Green-ringed pairs: females and males had equal mortality.
Offspring behaviour and performance in the nest	
Mass at hatching	Smaller if green-ringed biological and foster parents
Growth rate	NS
Begging rate	NS
Distribution of feeds to nestling	NS
Offspring performance at adulthood	
Survival from hatching to first breeding	NS
Offspring adult body mass	Body mass declined with laying order for offspring raised by red-ringed (but not green-ringed) foster parents.
Offspring adult tarsus length	Longer tarsi if offspring raised by red-ringed (compared to green-ringed) foster parents but only if biological father was from green-ringed treatment.
Latency to lay	NS
Clutch mass	Larger for daughters of red-ringed biological father
Clutch size	Larger for daughters of red-ringed biological father
Egg mass	Larger for daughters of red-ringed biological father
Yolk:albumen mass	NS
Daughters' mass loss during laying	Greater for females raised by red-ringed (compared to green-ringed) foster father

853   Fig. 1.

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857   **(b)**

b)

858

859   Fig. 2.

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