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Abstract

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

reproductive investment depending on the attractiveness of its mate. A generalised version

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

of attractive males which are also predicted to be of higher quality, or negative, i.e. higher

for offspring of unattractive males thus compensating for inheriting poor paternal genes for

example. Moreover, investment can be allocated by the father as well as the mother. Few

studies have quantified both parental investment across reproductive stages and effects on

offspring survival and fecundity. Here, we tested the DAH by using red leg rings to increase

the attractiveness of male zebra finches *Taeniopygia guttata* and green leg rings to decrease

their attractiveness. All males within an aviary were given the same coloured ring to control

for assortative mating between treatments. Eggs were cross-fostered between and within

treatments to allow the differentiation of effects of egg investment and nestling-rearing

investment. Brood and clutch sizes were standardized. Both positive and negative changes

in investment were observed: Eggs from the red ringed group had higher yolk to albumen

ratios than eggs from green-ringed fathers. Cross-fostering revealed that nestlings from eggs

laid and incubated by red-ringed parents had higher hatching weights than those in the

green-ringed group. Both parents in the green-ringed group fed nestlings more frequently

than red-ringed parents. Ring colour was merely an experimental manipulation of male

attractiveness; so as red and green ringed males should be of the same quality on average,

we might expect additional investment to result in elevated offspring quality. Offspring

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

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

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

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

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

27

28   **Introduction**

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

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

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

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

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

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

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

36   from investing relative to the value of a particular event (Trivers and Willard, 1973) but see

37   (Jones et al., 2009). However, positive differential allocation may also occur, for example, if

38   attractive males invest less in offspring feeding than unattractive males, and the females

39   mated to attractive males then compensate by increasing their investment (e.g. (Witte,

40   1995). While the result of this is a pattern of positive differential allocation by the female,

41   this is because of compensatory investment rather than maximising the value of high quality

42   offspring. Data on investment by both parents at both egg and nestling stages is therefore

43   needed to identify the underlying causation, at least in species with parental care (Montoya

44   and Torres, 2015). More recently it has been recommended that the DAH is generalised

45   such that the investment could be allocated by the father as well as the mother, and

46   differential allocation could also be negative, i.e. parents may invest more in offspring of

47   unattractive than attractive, mates (Ratikainen and Kokko, 2010). Thus, parents may invest

48   more to compensate for a poor situation such as low genetic quality of their offspring due to

49   a poor quality mate, i.e. "making the best of a bad job".

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

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

52   males contribute less paternal care than less attractive males (e.g.(Mazuc et al., 2003; Sanz,

53   2001; Witte, 1995) then offspring with attractive fathers might benefit from good genes but

54   suffer from reduced paternal care, if mothers are unable to fully compensate. Under

55   negative differential allocation, if mothers invest heavily in offspring of unattractive fathers

56   then offspring may receive an overall benefit from having an unattractive father (Byers and

57   Waits, 2006; Griffith and Buchanan, 2010). In a socially monogamous species with biparental

58   care, an experimental system in which male attractiveness is manipulated independently of

59   genetic quality and also offspring are cross-fostered (Montoya and Torres, 2015) is

60   necessary to help us to tease apart some of these issues.

61   Theoretical models have predicted that a positive relationship between mate

62   attractiveness and reproductive investment should be the more common pattern of

63   differential allocation (Harris and Uller, 2009) but see (Ratikainen and Kokko, 2010). This

64   appears to be supported by empirical studies of investment in the pre-hatching {Rutstein,

65   2004 #3364; Cunningham, 2000 #956; Gilbert, 2006 #4629; (Saino et al., 2002; Uller et al.,

66   2005); but see (Horvathova et al., 2012) and post-hatching stages (e.g. (Burley, 1988;

67   Gorman et al., 2005; Hasegawa et al., 2012; Limbourg et al., 2004; Maguire and Safran,

68   2010). For offspring, such positive levels of investment can affect growth and development

69   {Gilbert, 2006 #4629} and have positive effects on fecundity and other fitness related traits

70   {Gilbert, 2012 #4624}.

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

72   models, has been reported less frequently (Harris and Uller, 2009). A number of studies

73   have shown decreased maternal expenditure in egg composition (Bolund et al., 2009; Michl

74   et al., 2005; Navara et al., 2006; Saino et al., 2002). However, few studies have looked at the

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

76   response to male attractiveness (but see (Montoya and Torres, 2015; Sheppard et al., 2013).

77   This is important in order to be able to differentiate whether females are allocating

78   investment based on male attractiveness or compensating for reduced parental care by

79   fathers (Witte, 1995). Crucially, even fewer studies have been able to assess the

80   consequences on offspring quality of such allocation decisions.

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

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

83   zebra finches (*Taeniopygia guttata*), the species used in the original test of the hypothesis

84   by Burley (1988). Importantly, we also relate the differential allocation to the phenotype,

85   survival and fecundity of the offspring. Using experimental manipulation of male

86   attractiveness and cross-fostering of the offspring which allows teasing apart the effects of

87   egg investment and nestling-rearing investment, we addressed the following questions: 1)

88   Do females adjust their investment into eggs based on the ring colour of their mate? 2) Do

89   either males or females provision nestlings differently based on male ring-colour? 3) Do the

90   offspring of red- or green-ringed biological or foster fathers differ in their begging behaviour

91   and growth rates? 4) Does the attractiveness of either the biological or foster father

92   influence the adult size, survival and fecundity of offspring?

93

94

95 **Methods**

96 *Husbandry*

97 All birds used in this experiment were approximately 9-18 months old, had bred at least

98 once with a mate wearing a neutral orange-coloured leg ring and had been housed indoors

99 since birth. Immediately prior to the experiment, all individuals were being housed indoors

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

101 were transported to our outside aviary facility and four breeding colonies each consisting of

102 20 males and 21 females were established in large outdoor aviaries (2.8 x 5.5 x 2.5m) in

103 2002. No bird was released in the same aviary as its previous breeding partner(s) or with

104 siblings. Birds were fed on a diet of *ad libitum* seed mix (foreign finch mix supplied by

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

106 mixed with vitamin supplement (Minavit) three times a week and fresh greens and millet

107 sprays once per week. Fresh drinking water, oystershell grit and cuttlebone were available

108 *ad libitum*. A calcium supplement (Calciform) was added to the water five times per week.

109 From hatching onwards we also provided daily *ad libitum* soaked seed mix.

110

111 *Manipulation of male attractiveness*

112 A great advantage of the zebra finch for the purposes of experimental design is that there is

113 a well-established technique to manipulate attractiveness by using coloured leg rings. In

114 mate choice trials of both captive and wild-caught zebra finches, females have consistently

115 demonstrated strong preferences for males with red leg rings over males with green leg

116 rings under 'natural' lighting conditions (either outside or inside under UV-rich lighting

117 tubes) (Burley, 1986b; Hunt et al., 1997). It has been suggested that red leg rings enhance

118 the red beak, which in zebra finches is a condition-dependent secondary sexual trait (Blount,

119 Metcalfe, Birkhead, et al., 2003). We thus ringed half the males with an individually

120 numbered red or a green leg ring at the start of the experiment. Moreover, there is

121 evidence that male zebra finches with red rings sing more and gain more weight suggesting

122 that ring colour alters other male traits as well as female behaviour (Pariser et al., 2010).

123 Red- and green-ringed males were kept in separate aviaries in order to control for

124 potentially assortative mating due to differential access of red-ringed males to high quality

125 females (Burley, 1986b) which would make it impossible to distinguish between increased

126 female effort due to differential allocation and that due to female quality. However, females

127 were still free to choose their mates within each attractiveness treatment group (Griffith et

128 al., 2011). Our experiment was done in outdoor aviaries, i.e. with a natural UV spectrum

129 (Hunt et al., 1997). All females were ringed with individually numbered orange leg-rings, a

130 neutral colour with respect to male mate preference (Burley, 1986b), for identification

131 purposes.

132   On the day males and females were released together into the aviaries, all birds

133 were weighed to the nearest 0.1 g and tarsus length measured to the nearest 0.1 mm. There

134 were no differences in either body mass or tarsus length of males and females between the

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

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

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

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

139 aviaries at the University of XXX.

140

Breeding design

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

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

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

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

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

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

allocated according to the expected hatching date. From the expected hatching date

onwards nests were checked twice a day to record from which egg nestlings hatched. For all

nestlings used in the experimental broods, it was known from which egg they hatched ('egg

of origin'), and thus, the treatment of their biological parents. Occurrence of hatching failure

meant that brood size at hatching had to be reduced to two nestlings, one each from the

two treatment groups, in order to be able to maintain constant brood size for all pairs. In

order to make up two-nestling broods, occasionally a hatchling, that had experienced the

same laying and incubation conditions as the un-hatched egg it had to replace, had to be

moved between nests. Thus, experimental broods consisted of two nestlings that hatched

on the same day, one of each colour ring group. No nestlings were related to either their

nestmate or their foster parents. A total of 23 experimental broods were set up (6 in each of

the two aviaries with red-ringed males and 5 and 6 in the two aviaries with green-ringed

males).

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

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

identification. Nestlings were reweighed and tarsus measured on days 3, 6 and 9 all by the

164   same observer and an instantaneous growth rate (slope of the regression of log(nestling

165   weight) on nestling age) calculated. The sex of the offspring was determined either

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

167   2003) if the bird died before adulthood; sex of 3 nestlings that died very early and could not

168   be recovered were not determined. There were no differences between nestlings hatching

169   from eggs laid in the red- or green-ringed groups in offspring sex (χ_1^2 = 0.19, P = 0.66) or egg

170   order (Wilcoxon matched pairs test: n = 23, Z = 0.63, P = 0.53). Offspring were left to fledge

171   naturally within the outdoor aviaries. All birds were brought back into indoor bird rooms in

172   August 2002 when offspring were nutritionally independent (approximately 6 weeks of age).

173   Parents and offspring were then housed in separate single-sex groups of six individuals in

174   cages 40 cm wide, 120 cm long and 40 cm high.

175

176   *Maternal investment into eggs*

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

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

179   and a subset of eggs (n = 98 from 31 clutches - 15 clutches from the red ringed treatment

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

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

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

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

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

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

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

187   in diameter {Gilbert, 2007 #4628} were further analysed for yolk androgen levels (see

188 below), and yolk and albumen weight. In more developed eggs with larger embryos, the yolk

189 and albumen could not be cleanly separated because after two days of incubation the

190 perivitelline membrane was easily broken, and these eggs were not used for analyses on

191 yolk androgen, yolk and albumen weight. The embryo or blood vessels, if present, were

192 removed for molecular sexing. The sexes of early embryo samples from eggs were assigned

193 using primers P2 and P17 (full methods outlined in (Arnold et al., 2003). The colour of the

194 yolk was scored using a Roche Yolk Fan, which correlates with carotenoid levels (Karadas et

195 al., 2006). The colour scores were square root transformed prior to analysis.

196   At YYY University, we analysed testosterone (T) and its derivative, 5α-

197 dihydrotestosterone (DHT) content for all eggs that contained blastocysts with no signs of

198 development or minute embryos < 0.01 g since they do not differ in yolk androgen levels (L.

199 Gilbert et al., 2007). The extraction and assay protocols used here are described elsewhere

200 {Gilbert, 2007 #4628} and follow the methods used in the commercially available T

201 radioimmunoassay (RIA) kit from Amersham Pharmacia Biotech (after (Gil et al., 1999).

202 Extraction recovery of total androgens (T+DHT) was 75.6 ± 9.0 % (mean ± SE) and of DHT

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

204 assayed by means of competitive binding RIA. We ran samples in duplicate and hormone

205 concentrations were compared to total (T+DHT) and DHT standard curves that ranged from

206 12.5-800 pg per assay tube. The degree to which the antiserum cross-reacted with DHT in

207 the RIA was 46%, so the T concentration was estimated as total-(0.46DHT). Minimal cross-

208 reactivity of this antiserum was found with ten other steroids (Nash et al., 2000). The intra-

209 assay coefficient of variation (± SE) was 2.9 ± 0.31% for total (T+DHT) and 2.1 ± 0.32 % for

210 DHT.

211

Parental care

We quantified differences in parental effort in relation to colour ring treatment by recording parental feeding behaviour on day 9 after hatching (day of hatching = day 0) in 18 experimental broods that still had both nestlings at that age. Day 9 is roughly mid-way through development and the point at which nestlings were large enough to distinguish on the camera and more reliably observed covered by the parents for less time than younger nestlings, but not too old that they were stimulated to fledge early when the nest box was opened. We recorded the behaviour using small infrared video cameras in the nest box. To allow birds to get used to the equipment, each camera was installed in the top of the nest box at least four hours before recording commenced. Breeding birds were observed to return to their nest boxes within minutes of setting up the camera.

Behaviours were recorded, always between 13:30 and 16:30 BST, coinciding with a minor peak of feeding. Average observation duration per nest was 2.88 ±0.08 hours (n = 18) because intense fighting on the nest between the breeding bird and an intruder in two cases meant that some observation time was lost in one nest each of the red and green-ringed group. Videos were watched by an observer unaware of the ring colour group. We recorded nest attentiveness (percent of total 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 chick'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

236 provided from 0 to 4 feeding bouts to their nestlings (mean = 0.8 ± 0.08 feeds/visit, n = 36)

237 and there was no relationship between nest visit rate and feeding bout rate (Spearman's

238 rank correlation: females: r_s = 0.18, P = 0.456; males: r_s = 0.37, P = 0.117, n = 18 each). Gilby

239 et al., 2011 also concluded that parental provisioning is more reliably quantified by rate of

240 feeding rates rather than number of nest visits. We therefore used the more informative

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

242

243 *Offspring behaviour and performance in the nest*

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

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

246 with white correction fluid to allow us to distinguish between the two nestlings on the video

247 recording. There was no difference in proportion of nestlings marked with non-toxic

248 correction fluid with respect to egg of origin (9 out of 19 hatched from an egg from the red-

249 ring treatment, binomial test: one-tailed P = 0.500), sex (χ_1^2 = 0.50, P = 0.480), hatching

250 order (Wilcoxon matched pairs test: Z = 0.63, P = 0.527), or weight on day 9 (paired t-test:

251 t_{18} = 0.59, P = 0.565). No preference was found for the provisioning of marked or unmarked

252 nestlings by foster fathers (paired t-test: t_{15} = 0.22, P = 0.83) or foster mothers (paired t-test:

253 t_{15} = 1.75, P = 0.10). Over the duration of the recording, the number of times each nestling

254 begged was recorded, regardless of the intensity of the begging (Kolliker et al., 1998).

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

256 when nestlings were 3, 6 and 9 days of age. Fledglings were weighed at the end of the

257 experiments, just prior to moving the birds from the outdoor aviaries back to the indoor

258 aviary complex, as an estimate of mass at independence.

Offspring performance as adults

Finally, we assessed the growth, survival and reproductive effort at first breeding of F1s in relation to colour ring group of both their biological and foster parents. This was carried out in the indoor bird facilities at the University of XXX. At the age of 4-5 months, each offspring was paired with an experienced breeder of the opposite sex from our stock population which was in breeding cages 40 cm wide, 60 cm long and 40 cm high and with a nest box provided. A total of 38 experimental offspring were paired up. Birds were weighed and their tarsus length measured on pairing. All pairs were provided with a standard breeding diet for birds breeding indoors including *ad libitum* seed mix (foreign finch mix supplied by Haith's, Cleethorpes, Lincolnshire, UK), cuttlebone and grit, supplemented once per week with half a teaspoon per bird of a protein supplement (Haith's egg biscuit) mixed with a vitamin supplement (Minavit) and with a calcium supplement (Calcivet) in the drinking water. We recorded the number of paired-up birds that produced eggs within 20 days of pairing, their clutch size and size and composition of their eggs. Each egg was removed from the nest on the day of laying and replaced with an artificial egg. Eggs were weighed to the nearest 0.01 g on the day of laying and the weight of all eggs per clutch summed to give clutch mass. Egg composition was assayed as above.

Ethical Information

This project was approved by the University of XXX's ethical review committee and carried out under licence from the UK Home Office (Animal [Scientific Procedures] Act 1986). The protocols adhered to ASAB/ABS Guidelines for the Use of Animals in Research. All the birds were sourced from the University of XX's stock colony which included some birds that had

283 been hatched *in situ* and some that had been acquired from local bird breeders. The birds

284 were transported 5km from the main Department to Home Office Licenced outdoor aviaries

285 on a campus of the University of XXX and then back again in groups of 20 - 25 in cages 40 cm

286 wide, 60 cm long and 40 cm high. The cages contained perches and bowls of seed but no

287 water as the journey was ca. 20 minutes and we did not want water to soak the floors of the

288 cages. The fronts of the cages were covered to minimise the light entering the cages during

289 transport.

290

291 *Statistical analyses*

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

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

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

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

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

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

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

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

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

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

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

303 and Torres, 2015). As offspring from the same biological parents or raised by the same foster

304 parents cannot be considered independent we used general linear mixed models with the

305 identity of biological and foster parents as a random effect in order to account for this.

306 Preliminary analyses showed that there were no differences between aviaries and we here

307   present only models with biological and foster parents as the random factor which gave us

308   greater degrees of freedom and therefore greater statistical power. In these statistical models

309   we also included offspring sex, latency to lay and egg order. Similar general linear mixed

310   models were used when analysing the composition of eggs.

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

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

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

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

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

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

317

318   **Results**

319

320   *Maternal investment into eggs*

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

322   = 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

323   ± 0.2 eggs, N = 38; green rings: 4.3 ± 0.2 eggs, N = 38; $F_{1,74} = 1.75$, $P = 0.19$). Egg volume

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

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

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

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

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

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

330    $F_{1,24.9} = 5.87$, $P = 0.023$; latency: $F_{1,25.6} = 5.71$, $P = 0.025$; egg order and interactions $P > 0.7$).

331   Eggs from the red-ring treatment did not have significantly larger yolks or albumens (P >

332   0.2), but had significantly larger yolks relative to albumen mass (mean ratio = 3.28 ± 0.20, N

333   = 60) than those from the green-ring group (mean ratio = 2.09 ± 0.40; N = 71; ring colour of

334   biological father: $F_{1,24.9}$ = 5.87, P = 0.023). Paternal ring colour did not influence yolk colour

335   (a proxy for carotenoid content), but yolk colour declined with egg order ($F_{1,75.1}$ = 30.77, P <

336   0.0001) and latency to lay ($F_{1,26.2}$ = 4.92, P = 0.04; nest (random factor): Z = 2.47, N = 96, P =

337   0.007). Finally we found that DHT concentrations in freshly laid eggs increased with latency

338   to lay (latency to lay: $F_{1,9.26}$ = 7.15, P = 0.025; nest (random factor) Z = 0.32, N = 32, P > 0.3).

339   Egg order, paternal ring colour and embryo sex did not contribute to the model.

340   Testosterone concentrations in eggs did not vary with any variable.

341

342   *Parental care*

343   On day 9 post-hatching, when parental care behaviour was recorded, nest attentiveness

344   (the percentage of time a parent spent on the nest brooding their nestlings) decreased with

345   increasing total brood mass (estimate = -0.01 ± 0.005 % of time spent brooding per g of

346   brood mass) and females had higher attentiveness (54.6 ± 4.2 %, n = 18) than males (29.4 ±

347   3.8 %, n = 18), irrespective of ring colour treatment (Table 1). Parents in the green-ringed

348   group fed their nestlings more frequently (mean = 3.36 ± 0.22 feeds per hour) than parents

349   in the red-ringed group (mean = 2.36 ± 0.16 feeds per hour) and feeding rate decreased with

350   increasing total brood mass on day 9 (estimate = -0.05 ± 0.02 feeds $h^{-1} g^{-1}$, Table 1). A

351   feeding bout lasted on average 15.0 ± 0.99 s (n = 157 feeding bouts) and its average length

352   did not differ between the colour ring treatments (Table 1). Mothers did not differ from

353   fathers in their rate of feeding or the duration of their feeding bouts and there was no

354   significant interaction between treatment and sex of the feeding parent (Table 1).

Parental condition and survival

All parent birds lost mass between being first released into the aviaries and the end of the experiment and this differed between ring colour groups (ANOVA F $_{1, 143}$ = 2.98, P = 0.034). Post-hoc tests showed that females paired to green-ringed males (5.2 ± 1.9 %) lost less mass than all other birds (red-ringed males (11.6 ± 1.3 %), green-ringed males (11.8 ± 2.4 %) and females paired to red-ringed males (9.5 ± 1.4 %; Tukey b P = 0.05)). Females paired to red-ringed males were more likely to die during the study period than males (10 females versus 2 males; χ^2_1 = 5.33, P = 0.021). Mortality of males and females in the green treatment over the course of the experiment was even (6 females versus 6 males).

Offspring behaviour and performance in the nest

Nestlings hatched from eggs laid by parents in the green-ringed group and incubated by green-ringed foster parents (0.9 ± 0.05 g, n = 10) were marginally (P = 0.06) smaller than hatchlings from all other groups (1.1 ± 0.06 g, N = 31; Table 2). During the first nine days post-hatching, nestling growth rate was not influenced by ring colour treatment. Female nestlings grew faster (1.09 ± 0.05 g day^{-1}, N = 24) than male nestlings (0.96 ± 0.08 g day^{-1}, N = 15; Table 2).

Nestlings begged with an average rate of 1.1 ± 0.16 begs h^{-1} (N = 18 broods) and this was independent of the treatment of the biological and foster parents, its sex, the order of the egg it hatched from and other variables investigated (GLMM, ring colour treatment of biological parent: $F_{1,51}$ = 0.04, P = 0.852; ring colour treatment of foster parent: $F_{1,15}$ = 1.56, P = 0.231; latency: $F_{1,16}$ = 0.94, P = 0.347; egg order: $F_{1,59.7}$ = 0.01, P = 0.925; offspring sex: $F_{1,61.6}$ < 0.01 P = 0.993; nestling's size relative to its nest mate: $F_{1,13}$ = 0.17, P = 0.684; brood

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

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

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

382   foster parent did not affect the distribution of food between nestlings with red- or green-

383   ringed biological fathers ($F_{1,102}$ < 0.01, P = 0.979).

384

385   *Offspring performance at adulthood*

386   Offspring's body mass and tarsus length at first breeding differed between ring colour

387   treatments (Table 2). Offspring body mass as adults declined with increasing order of the

388   egg they hatched from when raised by foster parents from the green ring treatment but not

389   when raised by foster parents from the red ring treatment, irrespective of the colour ring

390   treatment of the biological parents (Fig 1a). In contrast, individuals raised by red-ringed

391   foster parents had longer tarsi than birds raised by green-ringed foster parents but only

392   when the biological parents were from the green-ring treatment (Table 2; Fig. 1b).

393   Offspring survival from hatching to their first breeding attempt was high (84.8 %, N =

394   46). Five nestlings died during the first 10 days (for the two where sex was identified, one

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

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

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

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

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

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

401   0.05; colour ring of foster parent: $F_{1,39}$ < 0.01, P = 0.99; colour ring of biological parent: $F_{1,8.64}$

402   = 0.24, P = 0.64; latency to lay: $F_{1,29.8}$ = 0.45, P = 0.51).

403   When paired with an experienced breeder from our stock population, 73.7% (N = 38)

404   of the offspring produced eggs. There was no difference in breeding propensity between the

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

406   parent: $F_{1,35}$ = 1.06, P = 0.31; colour ring of foster parent: $F_{1,34}$ = 0.72, P = 0.40; egg order:

407     $F_{1,33}$ = 0.37, P = 0.55; sex: $F_{1,32}$ < 0.01, P = 0.95). This GLIMMIX model would not run with

408   identity of biological parent as a random factor because there were a large number of

409   families for which there was only one offspring included in the model, so we only included

410   data from one daughter per brood to avoid pseudoreplication. The analyses of the

411   daughters' reproductive efforts during their first breeding attempt are presented in Table 3.

412   When breeding for the first time, daughters with green ringed biological fathers laid

413   clutches with a larger mass than daughters from red ringed biological fathers (Fig. 2) due to

414   them laying both more (red: 3.7 ± 0.47 eggs, N = 7; green: 4.9 ± 0.51 eggs, N = 7; $F_{1,8.1}$ = 4.85,

415   P = 0.06) and larger eggs (red: 1.21 ± 0.05 g, n = 7; green: 1.29 ± 0.02 g, N = 7; $F_{1,9.07}$ = 4.17, P

416   = 0.07). There was no difference in the ratio between wet yolk mass to wet albumen mass

417   suggesting all eggs were of similar gross composition irrespective of egg size. Between

418   pairing and clutch completion, daughters raised by foster parents in the red ring group lost

419   significantly more weight (15.8 ± 2.86 %, N = 7) than daughters raised by foster parents in

420   the green ring group (12.4 ± 1.76 %, N = 7; Table 3). Daughters that hatched from eggs laid

421   late in the sequence produced heavier clutches than daughters that hatched from eggs laid

422   early in the laying sequence (Table 3).

423   **Discussion**

424

425   Overall, both mothers and breeding pairs differentially allocated resources into offspring

426   based on male attractiveness (ring colour)(summarised in Table 4). The evidence for

427   differential allocation by fathers was less clear cut. Moreover, there appeared to be

428   evidence for both positive and negative levels of investment that may be related to

429   differential allocation, which depended on the resource being invested and the stage of

430   reproduction. There was also evidence of differential consequences for the offspring from

431   the different treatment groups; which must have been due to changes in parental

432   investment as only the perceived attractiveness of males was experimentally altered but no

433   other qualities of the males should have differed between treatment groups. While we

434   found no evidence for a difference between treatment groups in egg size or yolk micro-

435   nutrients (androgens and carotenoids), there was some evidence that mothers invested

436   more in the eggs of red-ringed than green-ringed males: females paired to red-ringed males

437   did lay eggs with a higher yolk to albumen ratio. Our cross-fostering design revealed that

438   this was associated with an effect on offspring phenotype (summarised in table 5): nestlings

439   that hatched from eggs laid by parents in the green-ringed group that were also incubated

440   by green-ringed parents were lighter at hatching than all other groups. Both egg quality, for

441   example the nutrients available for embryo development, and incubation environment

442   interact to impact upon nestling quality. Interestingly the patterns of maternal expenditure

443   during the pre-hatching stage appeared to have been reversed during nestling rearing. Pairs

444   in the red-ringed group provisioned their nestlings less frequently than pairs in the green-

445   ringed group. So, how did positive differential allocation into yolk mass, but negative

446   differential allocation into nestling provisioning affect offspring phenotype? Even though

447   nestlings which had received a relatively poor pre-hatching environment (green biological

448   and incubation parents) were smaller at hatching than all other groups, they appeared to be

449   able to compensate for this in the nest; offspring body size at independence did not differ

450   between treatment groups. However, despite hatching from eggs with a lower yolk to

451 albumen ratio, daughters with green-ringed biological fathers laid more and heavier eggs

452 compared with those with red-ringed fathers. Thus, non-exclusive alternative explanations

453 are that a poor pre-hatching environment has a positive effect on female fecundity, or does

454 not negatively affect female fecundity and can be more than compensated for during the

455 nestling phase (Arnold et al., 2007; Metcalfe and Monaghan, 2001). In terms of the

456 consequences for fitness-related traits due to differential allocation at the nestling-rearing

457 stage, female offspring raised by green-ringed foster parents lost less mass during their first

458 breeding attempt than those with red-ringed foster fathers, although their eggs did not

459 differ in mass or composition. Previous studies have also shown that zebra finches,

460 daughters in particular, experience long term consequences of nestling nutrition in terms of

461 their final body size and also various reproductive traits (Arnold et al., 2007; Blount et al.,

462 2006; Martins, 2003).

463 Differential maternal allocation is expected whenever males differ in attractiveness

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

465 our study, male attractiveness was manipulated independently of male quality. As all males

466 within the same aviary were subject to the same treatment, in contrast to Burley's classic

467 studies in which both green- and red-ringed males were simultaneously present in an aviary

468 (Burley, 1988); see also (Sheppard et al., 2013). Thus, in our study high quality females could

469 not pair assortatively with red-ringed males and low quality females with green-ringed

470 males, which could otherwise have been an alternative explanation to the higher breeding

471 expenditure in the red-ringed group. So, in our design any differences in maternal

472 investment due to ring colour were not confounded by female quality, but were the result

473 of adjustments in investment in response to perceived male attractiveness.

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

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

476   of the DAH in birds some of which have found negative differential allocation in egg

477   composition (Bolund et al., 2009; Michl et al., 2005; Navara et al., 2006; Saino et al., 2002).

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

479   hormones in response to male attractiveness (see also (Grenna et al., 2014). Compared with

480   albumen, yolk comprises higher levels and diversity of lipids, minerals, vitamins and other

481   substances vital for embryo development (Klasing, 2000). While albumen contributes to

482   nestling structural size, yolk supports survivorship after hatching, suggesting that relative

483   investment into these two egg components will have different impacts on the resulting

484   nestling (Klasing, 2000). One potential explanation for this, based on the 'silver spoon'

485   hypothesis (Bateson et al., 2004), is that females are able to tailor eggs, so nestlings are

486   better able to cope with predicted conditions in the nest, e.g. low provisioning rates. We

487   have previously demonstrated, using the same experimental set-up, that earlier laying

488   females with red-ringed partners contributed significantly more to incubation than late

489   breeding mothers, but no such relationship was found in females mated to green ringed

490   males. In terms of incubation overall, similar levels were seen across both parents between

491   treatment groups, suggesting some compensation within the pair, but incubation

492   attentiveness of the pair was correlated with hatching success (Gorman et al., 2005). Thus,

493   our finding suggests that egg quality also interacted with incubation environment and

494   relatively poor quality eggs incubated in apparently suboptimal conditions appear to have

495   negative impacts on embryonic development and hatchling quality. Finally, parents in the

496   green-ringed treatment fed their nestlings more frequently than those in the red-ringed

497   aviaries (see also Limbourg et al., 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 poor egg quality 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 there is a 22% increase in resting metabolic rate in female zebra finches (Vézina &

Williams 2005), decreased flight performance (Veasey et al., 2001) and 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 bird reduced future fecundity and

survival (e.g. (Maigret and Murphy, 1997; Owens and Bennett, 1994; Reid et al., 2003).

Burley (Burley, 1986b, 1988) has shown that increased parental effort decreased the

survival of females mated to attractive males. In our study, females in the green-ringed

group, that provisioned their nestlings at a higher rate than females in the red-ringed group,

lost less mass during breeding than all other birds. There is some evidence that mothers in

the red-ringed treatment had higher mortality than red-ringed males which might be a

consequence of heavy investment into eggs (but less so into chicks), although the sample

sizes are small for the mortality rates. Both egg production and chick rearing are known to

be costly, and can be comparable both in terms of energetic expemditure and consequences

on reproductive performance (Monaghan & Nager 1998; Nager 2006). Although the relative

costs of egg production to chick rearing are unknown for zebra finches, our data suggest

that differential investment into eggs, but not nestlings, was costly to females at least in

terms of mass loss and potentially mortality. Moreover, the relatively low provisioning rates

522 of females with red-ringed mates may have been because their body reserves were

523 relatively more exhausted by egg production than in females with green-ringed males. This

524 was despite the fact that we standardised the brood size to two nestlings which is lower

525 than the typical brood size (~ 4 nestlings) of successful zebra finch parents in our aviaries

526 (see also (Zann, 1996). Perhaps females paired to unattractive males were tailor-making

527 their eggs to cope best with assumed poor genetic quality. However, in this experimental

528 context, attractiveness was actually unrelated to genetic quality and thus daughters from

529 matings with unattractive males happened to fare better than expected. Alternatively, or in

530 addition, since parents in the green-ringed group provisioned nestlings more frequently, the

531 best strategy was to invest less at the egg stage but more at the nestling rearing stage (but

532 see (Montoya and Torres, 2015). While it is not possible to test these ideas with our data,

533 we found some evidence that in zebra finches that females can differentially allocate

534 resources into offspring at different stages and that such investment differs in costs to

535 survival, breeding success and condition. Do we also have evidence that the "differential

536 allocation" affected fitness related traits in the offspring?

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

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

539 compared with other hatchlings from the other treatment groups, did not differ in

540 mortality. Daughters from green-ringed biological parents laid heavier clutches at sexual

541 maturity. Daughters reared under the relatively poor feeding regime of red-ringed foster

542 parents (negative differential allocation) lost more mass during their first breeding attempt

543 than those with green-ringed foster parents despite producing similar numbers and quality

544 of eggs. So we do have some evidence that differential breeding expenditure, at least in

545 eggs, relative to mate attractiveness results in trans-generational effects on fitness-related

546   traits. Notably, the effects were dependent on breeding stage and more experimental data

547   are required to tease out whether females are able to strategically invest in nestlings as well

548   as eggs or whether investment in later reproductive stages are limited, for example

549   energetically, by previous investment decisions (Bowers et al., 2013). One issue with our

550   data is that our sample size of offspring which bred was relatively small (N = 38). In support,

551   other studies on zebra finches have also shown that conditions experienced during either

552   the embryo (Gorman and Nager, 2004; Tobler and Sandell, 2009; von Engelhardt et al.,

553   2006) or nestling stage (Blount, Metcalfe, Arnold, et al., 2003; Blount et al., 2006; Boag,

554   1987; Spencer et al., 2010) can affect fitness-related traits but studies like ours that can

555   directly link parental investment with offspring phenotypic or life history traits at both pre-

556   and post-hatching stages are largely lacking (but see {Bowers, 2013 #4425;Cunningham,

557   2000 #956; {Gilbert, 2012 #4624;Gilbert, 2006 #4629}.

558   Some of our results suggest both negative and positive investment, but how this

559   balances out to be positive, negative or indeed any overall differential allocation is unclear.

560   Both males and females in the red-ringed treatment had a lower provision rate than those

561   in the green-ringed treatment. Previous studies have suggested that such a reduction in

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

563   1995). For example, increasing the attractiveness of some males could increase the

564   frequency of dominance interactions between red-ringed males (Cuthill et al., 1997), permit

565   males to become polygynous (Burley, 1986b) or lead to more intense male competition

566   (Qvarnström, 1997). Arguments against such behavioural mechanisms are that a red ring

567   should not handicap a male any more than a green ring and also using aviaries where all

568   males had the same ring colour should minimise the issue of red-ringed *versus* green-ringed

569   male competition or polygyny since treatment groups could not interact or see each other

570   (but see Cuthill et al., 1997). That females with red-ringed males did not increase their

571   provisioning rate in response to the low input by their mates suggests that a compensatory

572   mechanism is not at play here, unlike the compensatory feeding observed by Witte (1995).

573   Alternatively, given that females with red-ringed males produced eggs with higher yolk to

574   albumen ratios these females had already invested heavily in eggs and might have been in

575   poorer condition, and thus unable to compensate. It is also possible that compensatory

576   feeding was not necessary if they had already prepared their offspring for a poor quality

577   rearing environment, through changing egg resources (e.g. Gilbert et al. 2012).

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

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

580   similar manipulation and cross-fostering design. However, in contrast to our study, they

581   found that female offspring of red-ringed, not green-ringed, biological fathers (and foster

582   fathers) laid heavier clutches and that this was due to differences in offspring body size at

583   fledging (larger females of red-ringed fathers were able to lay larger eggs). The only clear

584   differences between the two studies are that we standardised our brood size to two chicks

585   and also our offspring were reared in outdoor aviaries, in contrast to Gilbert et al. (2012)

586   who used a separate cage per pair of birds kept indoors with constant temperature,

587   humidity and daylight regime. Subtle environmental differences may result in differences in

588   investment patterns (e.g. Mousseau and Fox 1998; Williamson et al. 2008), and this can

589   mean that using experiments to generalise about avian investment decisions can be

590   difficult. Moreover, the DAH is also about individual females altering their allocation in

591   response to the perceived value of their current mating opportunity to optimise their

592   lifetime reproductive success when they may mate more than once. In our experiment,

593   levels of investment were only measured across one breeding attempt per female, however,

594 it should be noted that due to the high mortality rates in the wild, very few female zebra

595 finches would survive to mate more than once if ever (Zann, 1996). So while in our study we

596 found evidence for positive differential investment at the egg stage and negative

597 investment at the nestling rearing stage in response to male attractiveness, and we found

598 corresponding fitness-related offspring traits, we cannot conclude that passerine birds, or

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

600 by changing investment patterns depending on a range of environment cues (Mousseau and

601 Fox 1998; Williamson et al. 2008), often not yet quantified or understood by researchers. To

602 conclude, our study illustrates how patterns of reproductive investment can be complex

603 (see also (Gorman et al., 2005; Michl et al., 2005; Rutstein et al., 2005) and not consistent

604 across all forms of maternal investment (Balzer and Williams, 1998).

605

606

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771 **Figure 1. (a)** Body weight at first breeding of birds raised by red-ringed foster parents

772 (closed symbols) and by green-ringed foster parents (open symbols) in relation to egg order.

773 **(b)** Mean (± 1 SE) fully-grown tarsus length in relation to ring colour treatment of the

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

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

776 Birds from biological parents in the green-ringed group had the longest tarsi when raised by

777 foster parents in the red-ring group. See table 2 for results of the statistical analysis.

778 Numbers above the bars present the numbers of offspring.

779

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

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

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

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

784 shown separately, but egg order was used as a continuous variable in the analysis (see Table

785 3 for results of the statistical analysis). Numbers above the bars present numbers of

786 daughters.

787 **Table 1.** Results of general linear mixed models on parental care behaviour at day 9 post-

788 hatching including the ring colour of foster fathers and sex of foster parent as factors,

789 latency to lay, total brood mass and ambient temperature at the day of the behavioural

790 recording as covariates and identity of the 'nest' as a random factor. All broods (n = 18)

791 consisted of two nestlings. Measures of parental care behaviour include nest attentiveness

792 (percentage of observation time when nestlings are brooded by one parent), feeding rate

793 (number of feeds per hour per brood) and the average length of the feeding bout per

794 nestling (i.e. the time a parent spent regurgitating seeds into the mouth of a nestling, see

795 methods for details). P > 0.06 for all interactions.

796

	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

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798

Table 2. Results for mixed models on the effect of the colour ring treatment on hatchling mass (N = 41), nestling growth (N = 39), and body mass (N = 37) and length of offspring tarsus at adulthood (N = 37) when breeding the first time. These models contained the ring colour of biological and foster parents, sex of the offspring and from what egg order it hatched (egg order) and the latency to lay with identity of the biological 'nest' and the foster 'nest' as random factors. All other interactions P > 0.23.

	Hatchling mass	Nestling Growth	Adult body mass	Adult tarsus length
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	$F_{1,18.1}=0.84$ $P=0.37$	$F_{1,120.4}=3.35$ $P=0.08$	$F_{1,3.21}=4.38$ $P=0.12$	$F_{1,17.3}=0.12$ $P=0.74$
Egg order	$F_{1,30.6}=0.32$ $P=0.57$	$F_{1,25.5}=0.10$ $P=0.75$	$F_{1,10.8}=0.15$ $P=0.71$	$F_{1,26}=0.34$ $P=0.56$
Egg 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	$Z=0.51$ $P=0.61$	$Z=2.40$ $P=0.02$	$Z=2.27$ $P=0.02$	$Z=2.35$ $P=0.02$

809 **Table 3.** Results for mixed models on the effect of the colour ring treatment of the father
810 on reproductive effort of their daughters when breeding the first time (N = 14). Independent
811 variables were latency to lay (number of days between pairing and laying the first egg),
812 clutch mass (number of eggs laid * mean egg mass), egg composition (ratio between wet
813 yolk mass and wet albumen mass) and mass loss between pairing and clutch completion.
814 These models contained the ring colour of biological and foster parents, the order of the egg
815 from which it hatched (egg order) and the latency of parents to lay. Only one daughter per
816 rearing nest was used in the analysis (see methods) and therefore the model contains only
817 identity of the biological 'nest' as a random factor. All interactions P > 0.22.

818

	Latency to lay	Clutch mass	Egg composition	Weight 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	$F_{1,8.97}=0.02$ $P=0.88$	$F_{1,7}=0.01$ $P=0.92$
Egg 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

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820

821   Table 4: Summary of the effects of paternal ring colour on parental investment in different

822   stages of reproduction. + = significant positive effects, - = significant negative effects, 0 = no

823   significant effect, N/A = not tested for or not applicable.

	Biological Parents' treatment		**Foster Parents' Treatment**	
	Red	Green	Red	Green
Egg volume	0	0	N/A	N/A
Yolk:albumen ratio	+	-	N/A	N/A
Yolk carotenoid index	0	0	N/A	N/A
Testosterone	0	0	N/A	N/A
DHT	0	0	N/A	N/A
Nest attentiveness	N/A	N/A	0	0
Feeding rate	N/A	N/A	-	+
Feeding bout duration	N/A	N/A	0	0
Maternal mass loss	N/A	N/A	-	+
Maternal mortality	N/A	N/A	-	+

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825

826   Table 6: Summary of the significant effects of the treatment groups of the biological and

827   foster parents (red-ringed fathers or green-ringed fathers) on offspring traits, see text for

828   further details. + = significant positive effects, - = significant negative effects, 0 = no

829   significant effect, N/A = not tested for or not applicable.

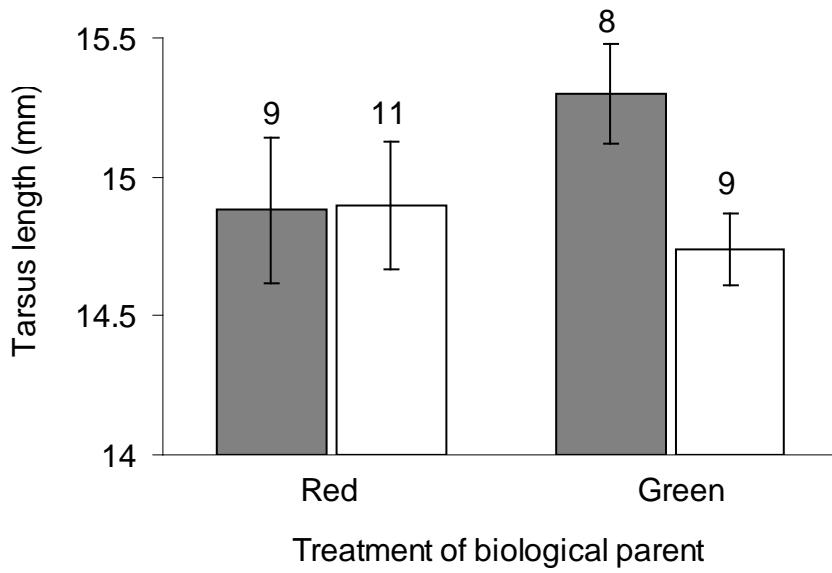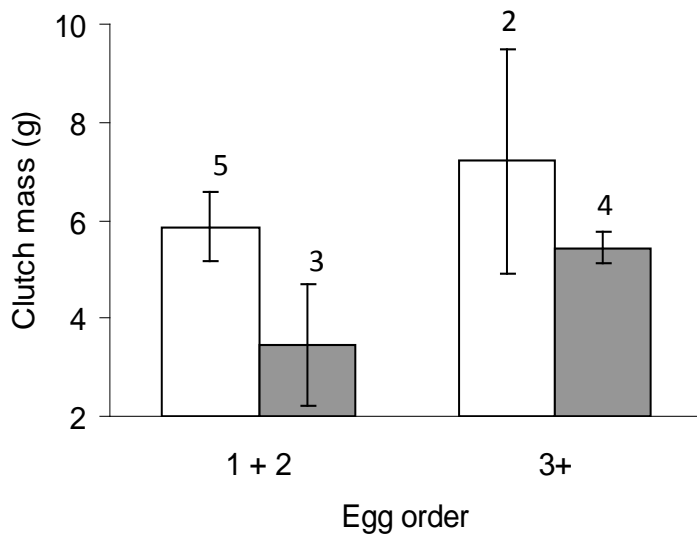|	Biological parent: Red		Biological parent: Green	
	Foster: Red	Foster: Green	Foster: Red	Foster: Green
Hatchling mass	+	+	+	-
Begging rate	0	0	0	0
Growth rate in nest	0	0	0	0
F1 adult mass	0	0	0	0
F1 tarsus length	-	-	+	-
Propensity of F1s to breed	0	0	0	0
Daughters' clutch mass	-	-	+	+
Daughters' clutch size	-	-	+	+
Daughters' yolk:albumen	0	0	0	0
Daughters' breeding mass loss	-	+	-	+

830

831   Fig. 1.

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833   **(a)**
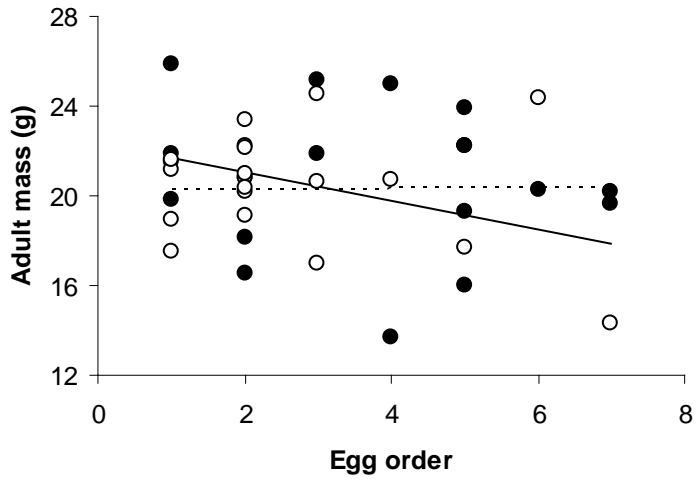
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836   **(b)**

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838   Fig. 2.

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