

This is a repository copy of *Paternal attractiveness and the effects of differential allocation of parental investment*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/95226/>

Version: Accepted Version

Article:

Arnold, Kathryn E. orcid.org/0000-0002-6485-6065, Gilbert, Lucy, Gorman, Helen E. et al. (3 more authors) (2016) Paternal attractiveness and the effects of differential allocation of parental investment. ANIMAL BEHAVIOUR. pp. 69-78. ISSN: 0003-3472

<https://doi.org/10.1016/j.anbehav.2015.12.013>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1

2 **Paternal attractiveness and the effects of**

3 **differential allocation of parental investment**

4 Kathryn E. Arnold¹, Lucy Gilbert², Helen E. Gorman³, Kate Griffiths³, Aileen

5 Adam³ and Ruedi G. Nager³

6

7 ¹ Environment Department, University of York, YORK YO10 5DD, UK

8 ² Ecological Sciences Group, The James Hutton Institute, Craigiebuckler, Aberdeen AB15

9 8QH, UK

10 ³ Institute of Biodiversity, Animal Health and Comparative Medicine, School of Life Sciences,

11 College of Medical, Veterinary and Life Sciences Graham Kerr Building University of Glasgow

12 Glasgow G12 8QQ, UK

13

14 Author for correspondence: Dr Kathryn Arnold, Environment Department, University of

15 York, Heslington, YORK YO10 5DD, UK

16 Tel. +44 (0) 1904 322997 Email: Kathryn.Arnold@york.ac.uk

17

18 Short title: Mate attractiveness and offspring fitness

19 Word count: 7798 (excluding references and legends)

20 Final version published online: 25-JAN-2016

21 Full bibliographic details: Animal Behaviour (2016), pp. 69-78

22 DOI information: 10.1016/j.anbehav.2015.12.013

23

ABSTRACT

The Differential Allocation Hypothesis (DAH) predicts that an individual should vary its reproductive investment depending on the attractiveness of its mate. A recently revised version of the DAH makes explicit that investment can be positive, i.e. higher for the offspring of attractive males which should be of higher quality, or negative, i.e. higher for offspring of unattractive males, e.g. compensating for inheriting poor paternal genes. Moreover, investment can be made by the father and the mother. Here, we tested whether experimental manipulation of male attractiveness affected parental investment at different reproductive stages and thus influenced fitness-related traits in offspring. In two aviaries, all male zebra finches *Taeniopygia guttata* were given red leg rings to increase attractiveness and in two aviaries all males received green leg rings to decrease attractiveness. This controlled for assortative mating between treatments. Ring colour was merely an experimental manipulation of male attractiveness, not paternal quality, so we might expect additional investment to elevate offspring quality. Eggs were cross-fostered between and within treatments to allow differentiation of effects of investment in eggs and nestlings. Clutch and brood sizes were standardized. Both positive and negative investment were observed: Eggs from red ringed fathers had higher yolk to albumen ratios than eggs from green-ringed fathers. Nestlings from eggs laid and incubated by parents in the red-ringed group had higher hatching masses than those in the green-ringed group. Both parents in the green-ringed group fed nestlings more frequently than red-ringed parents. 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

not consistently across all forms of reproductive investment suggesting that the costs and benefits of differential allocation vary among individuals and across contexts.

Key words: androgens, begging, provisioning rates, transgenerational maternal effects, Zebra finch.

The classical Differential Allocation Hypothesis (DAH) predicted that females should invest more in offspring of attractive than unattractive males to maintain current and future pair bond with an attractive mate (Burley, 1986a, 1988). This has subsequently been expanded to a rationale that if male attractiveness is indicative of genetic quality or resource availability, then a reproductive event with an attractive mate represents a higher value reproductive event than one with an unattractive male (Sheldon, 2000). Therefore, since females have a limited amount of resources to invest in reproduction, they would benefit from investing relative to the value of a particular event (Trivers & Willard, 1973); but see (Jones, Nakagawa, & Sheldon, 2009). However, positive differential allocation may also occur if, for example, the females mated to attractive males increase their investment in order compensate for attractive males investing less in offspring feeding than unattractive males (e.g. Witte, 1995). While the result of this is a pattern of positive differential allocation by the female, this is because of compensatory investment rather than maximising the value of high quality offspring. Data on investment by both parents at both egg and nestling stages is therefore needed to identify the underlying causation, at least in species with biparental care (Montoya & Torres, 2015). More recently it has been recommended that the DAH is generalised such that the investment could be allocated by the father as well as the mother. Moreover, differential allocation could also be negative, i.e. parents may invest more in offspring of unattractive than attractive, mates (Ratikainen &

Kokko, 2010). Thus, parents may invest more to compensate for a poor situation such as low genetic quality of their offspring due to a poor quality mate, i.e. “making the best of a bad job”.

The impacts on offspring fitness of differential allocation are difficult to predict, particularly in socially monogamous species with biparental care. If, for example, attractive males contribute less paternal care than less attractive males (e.g. (Mazuc, Chastel, & Sorci, 2003; Sanz, 2001; Witte, 1995) then offspring with attractive fathers might benefit from good genes but suffer from reduced paternal care, if mothers are unable to fully compensate. Under negative differential allocation, if mothers invest heavily in offspring of unattractive fathers then offspring may receive an overall benefit from having an unattractive father (Byers & Waits, 2006; Griffith & Buchanan, 2010). Theoretical models have predicted that a positive relationship between mate attractiveness and reproductive investment should be the more common pattern of differential allocation (Harris & Uller, 2009; but see Ratikainen & Kokko, 2010). This appears to be supported by empirical studies of investment in the pre-hatching (Cunningham & Russell, 2000; Gilbert, Williamson, Hazon, & Graves, 2006; Rutstein, Gilbert, Slater, & Graves, 2004; Saino et al., 2002; Uller, Eklof, & Andersson, 2005; but see Horvathova, Nakagawa, & Uller, 2012) and post-hatching stages (e.g. Burley, 1988; Gorman, Arnold, & Nager, 2005; Hasegawa, Arai, Watanabe, & Nakamura, 2012; Limbourg, Mateman, Andersson, & Lessells, 2004; Maguire & Safran, 2010). For offspring, such positive levels of investment can affect growth and development (Gilbert et al., 2006) and have positive effects on fecundity and other fitness related traits (Bowers et al., 2013; Cunningham & Russell, 2000; Gilbert, Williamson, & Graves, 2012; Gilbert et al., 2006).

Negative differential allocation has received less attention and, as predicted by models, has been reported less frequently (Harris & Uller, 2009). A number of studies have shown decreased maternal expenditure in egg composition (Bolund, Schielzeth, & Forstmeier, 2009; Michl, Torok, Peczely, Garamszegi, & Schwabl, 2005; Navara, Badyaev, Mendonca, & Hill, 2006; Saino et al., 2002). However, few studies have looked at the investment by both fathers and mothers at both pre- and post-hatching stages in the response to male attractiveness (but see (Montoya & Torres, 2015; Sheppard, Clark, Devries, & Brasher, 2013). This is important in order to be able to differentiate whether females are allocating investment based on male attractiveness or compensating for reduced parental care by fathers (Witte, 1995). Crucially, even fewer studies have been able to assess the consequences on offspring quality of such allocation decisions. An experimental system with biparental care, in which male attractiveness can be manipulated independently of genetic quality and offspring can be cross-fostered (Montoya & Torres, 2015) is necessary to help us to tease apart some of these issues.

In this paper, we test for positive and negative differential allocation (Ratikainen & Kokko, 2010) in egg formation and nestling-rearing in response to mate attractiveness in zebra finches (*Taeniopygia guttata*). This is the species used in the original test of the hypothesis by Burley (1988) and male attractiveness can be manipulated using leg rings with red rings increasing attractiveness and green rings decreasing attractiveness. Importantly, we also relate differential allocation to the phenotype, survival and fecundity of the offspring. Using experimental manipulation of male attractiveness and cross-fostering of the offspring, which allows teasing apart the effects of egg investment and nestling-rearing investment, we addressed the following questions: (1) Do females adjust their investment into eggs based on the ring colour of their mate? (2) Do either males or females provision

nestlings differently based on male ring-colour? (3) Do the offspring of red- or green-ringed biological or foster fathers differ in their begging behaviour and growth rates? (4) Does the attractiveness of either the biological or foster father influence the adult size, survival and fecundity of offspring?

METHODS

Husbandry

All birds used in this experiment were between 9-18 months old, had been housed indoors since birth and had bred at least once with a mate wearing a neutral orange-coloured leg ring. Immediately prior to the experiment, all individuals were being housed indoors within single-sex groups of typically 4-6 birds. At the start of the experiment, these birds were transported to our outside aviary facility and four breeding colonies each consisting of 20 males and 21 females were established in large outdoor aviaries (2.8 x 5.5 x 2.5m) in 2002. No bird was released in the same aviary as its previous breeding partner(s) or with siblings. Birds were fed on a diet of *ad libitum* seed mix (foreign finch mix supplied by Haith's, Cleethorpes, Lincolnshire, UK), supplemented with an egg food (Haith's egg biscuit) mixed with vitamin supplement (Minavit) three times a week and fresh greens and millet sprays once per week. Fresh drinking water, oystershell grit and cuttlebone were available *ad libitum*. A calcium supplement (Calciform, Aviform, Wymondham, UK) was added to the water five times per week. From hatching onwards we also provided daily *ad libitum* soaked seed mix.

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

two treatment groups ($P > 0.21$). Birds were released on the 20 May 2002 and allowed to settle in their new environment for two weeks. Any birds that died during this acclimation period were replaced with suitable birds of the same sex to maintain the group size. At the end of the experiment, all birds were caught, re-measured and returned to the indoor aviaries at the University of Glasgow.

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 assigned to foster parents 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 from each treatment group. This maintained a 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 length measured on days 3, 6 and 9 all by the same observer. An instantaneous growth rate (slope of the regression of log(nestling mass) on nestling age) was then calculated. The sex of the offspring was determined either retrospectively from the adult plumage, or by a molecular sexing technique (Arnold et al. 2003) if the bird died before adulthood. The sex of 3 nestlings that died very early and could not be recovered were not determined. There were no differences in sex between offspring hatched from eggs laid in the red- or green-ringed groups ($\chi^2 = 0.19$, $P = 0.66$) or with laying order (Wilcoxon matched pairs test: $N = 23$, $Z = 0.63$, $P = 0.53$) (Rutstein et al., 2005). Offspring were left to fledge naturally within the outdoor aviaries. All birds were brought back into indoor bird rooms in August 2002 when offspring were nutritionally independent (approximately 6 weeks of age). Parents and offspring were then housed in separate single-sex groups of six individuals in cages 40 cm wide, 120 cm long and 40 cm high.

Maternal Investment into Eggs

To quantify maternal differential investment in primary reproductive effort, a range of egg characteristics were measured. All eggs were individually marked on the day they were laid, and a subset of eggs ($N = 98$ from 31 clutches - 15 clutches from the red ringed treatment and 16 from the green ringed treatment) was collected approximately two days after onset of incubation in order to allow the embryo to develop sufficiently to be sexed. We replaced eggs with model eggs made from Fimo polymer clay (Eberhard Faber, Neumarkt, Germany)

which were similar in size, shape and colour to zebra finch eggs to ensure the birds did not change their clutch size (Zann, 1996). Upon collection, each egg was weighed, then opened and the yolk, embryo, albumen and shell were weighed separately. There was considerable variation in embryo size and only yolks from eggs with blastocysts or minute embryos <2mm in diameter (Gilbert, Bulmer, Arnold, & Graves, 2007) were further analysed for yolk colour and androgen levels (see below), and yolk and albumen mass. In more developed eggs with larger embryos, the yolk and albumen could not be cleanly separated because after two days of incubation the perivitelline membrane was easily broken, and these eggs were not used for analyses on yolk androgen, yolk and albumen masses. The embryo or blood vessels, if present, were removed for molecular sexing. The sexes of early embryo samples from eggs were assigned using primers P2 and P17 (full methods outlined in (Arnold et al., 2003). The colour of the yolk was scored using a Roche Yolk Fan, which correlates with carotenoid levels (Karadas, Grammenidis, Surai, Acamovic, & Sparks, 2006). The colour scores were square root transformed prior to analysis.

At the University of St Andrews, we analysed testosterone (T) and its derivative 5 α -dihydrotestosterone (DHT) contents for all eggs that contained blastocysts with either no signs of development or minute embryos < 0.01 g since they do not differ in yolk androgen levels (Gilbert et al., 2007). The extraction and assay protocols used here are described elsewhere (Gilbert et al., 2007) and follow the methods used in the commercially available T radioimmunoassay (RIA) kit from Amersham Pharmacia Biotech (after Gil, Graves, Hazon, & Wells, 1999). Extraction recovery of total androgens (T+DHT) was 75.6 ± 9.0 % (mean \pm SE) and of DHT alone was 59.8 ± 0.9 %. The two resulting extracts (total (T + DHT) and DHT only) were assayed by means of competitive binding RIA. We ran samples in duplicate and hormone concentrations were compared to total (T+DHT) and DHT standard curves that

ranged from 12.5-800 pg per assay tube. The degree to which the antiserum cross-reacted with DHT in the RIA was 46%, so the T concentration was estimated as total-(0.46DHT). Minimal cross-reactivity of this antiserum was found with ten other steroids (Nash et al., 2000). The intra-assay coefficient of variation (\pm SE) was $2.9 \pm 0.31\%$ for total (T+DHT) and $2.1 \pm 0.32 \%$ for DHT.

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. At this age the nestlings were also 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 (K.E.A. & R.G.N Unpublished data). 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 treatment groups. 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 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 ($\chi^2 = 0.50$, $P = 0.480$), 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: $T_{15} = 1.75$, $P =$

0.10). Over the duration of the recording, the number of times each nestling begged was recorded, regardless of the intensity of the begging (Kolliker, Richner, Werner, & Heeb, 1998).

Nestling mass and tarsus length were recorded between 09:00 and 12:00 on days when the eldest nestling/s were 3, 6 and 9 days of age. Fledglings were weighed at the end of the experiment, just prior to moving the birds from the outdoor aviaries back to the indoor aviary complex, as an estimate of mass at independence. Sample sizes vary between analyses due to missing samples or failed video recordings.

Offspring Performance as Adults

Finally, we assessed the growth, survival and reproductive effort at first breeding of offspring 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 Glasgow. At the age of 4-5 months, each of the 38 surviving offspring was paired with an experienced breeder of the opposite sex from our stock population. Breeding cages were 40 cm wide, 60 cm long and 40 cm high and provided with a nest box. 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 mass of all eggs per clutch summed to give clutch mass. Egg composition was assayed as above.

Ethical Note

This project was approved by the University of Glasgow'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 Glasgow's stock colony which included some birds that had been hatched *in situ* and some that had been acquired from local bird breeders. The birds were transported 5km from the main Department to Home Office Licenced outdoor aviaries on a campus of the University of Glasgow and then back again in groups of 20 - 25 in cages 40 cm wide, 60 cm long and 40 cm high. The cages contained perches and bowls of seed but no water as the journey was ca. 20 minutes and we did not want water to soak the floors of the cages. The fronts of the cages were covered to minimise the light entering the cages during transport.

Statistical Analyses

We analysed parental care behaviour for males and females together by including pair identity as a random effect into a general linear mixed model and included the sex of the parent contributing to the parental care as a factor in the model. As the size of the offspring and ambient temperature might affect parental care behaviour, we included in the statistical model total brood mass and ambient temperature as covariates. In a previous study on different birds using the same experimental design we found that the female's

response to ring colour may depend on the timing of breeding (Gorman et al., 2005) and we therefore also included in the statistical model latency to lay as another covariate.

Our cross-fostering design allowed us to separate out the effect of differences in egg quality (due to ring colour of the biological father), incubation and nestling rearing environment (due to ring colour of the foster father) on offspring performance (Montoya & Torres, 2015). As offspring produced by the same biological parents or raised by the same foster parents cannot be considered independent, we used general linear mixed models with the identity of biological and foster parents as a random effect. Preliminary analyses showed that there were no differences between aviaries, so we here present only models with biological and foster parents as the random factor which gave us greater degrees of freedom and therefore greater statistical power. In these statistical models we also included offspring sex, latency to lay and laying order. Similar general linear mixed models were used when analysing the composition of eggs.

All mixed models were run on SAS, version 9 using either PROC MIXED or the macro GLIMMIX (for the logistic regressions in the analysis of survival and breeding propensity). We tested for all two-way interactions between main effects and covariates, and removed non-significant factors from the full model stepwise beginning with the interaction terms. Only statistically significant interactions and main effects are reported. We used $P < 0.05$ for statistical significance and report mean values ± 1 S.E. throughout the text.

RESULTS

Maternal Investment into Eggs

The ring colour treatment did not affect the timing of breeding (red rings: 22.6 ± 1.5 June, $N = 38$; green rings: 21.7 ± 1.1 June, $N = 38$; $F_{1,74} = 0.21$, $P = 0.65$), or clutch size (red rings: 4.7 ± 0.2 eggs, $N = 38$; green rings: 4.3 ± 0.2 eggs, $N = 38$; $F_{1,74} = 1.75$, $P = 0.19$). Egg volume increased with increasing laying order (laying order: $F_{1,108} = 35.11$, $P < 0.0001$; nest (random factor): $Z = 4.54$, $P < 0.0001$), but colour ring of biological father, sex of egg and latency to lay did not contribute to the models. No aspect of egg composition differed between male and female eggs (GLMM, all $P > 0.2$).

Among the subset of collected eggs, the ratio of yolk to albumen varied significantly with paternal ring colour and also decreased with increasing latency to lay (ring colour: $F_{1,24.9} = 5.87$, $P = 0.023$; latency: $F_{1,25.6} = 5.71$, $P = 0.025$; laying order and interactions $P > 0.7$). Eggs from the red-ring treatment had significantly larger yolks relative to albumen mass (mean ratio = 3.28 ± 0.20 , $N = 60$) than those from the green-ring group (mean ratio = 2.09 ± 0.40 ; $N = 71$; ring colour of biological father: $F_{1,24.9} = 5.87$, $P = 0.023$). Yolks and albumens from the red treatment weighed on average $0.59g \pm 0.03$ and $0.32g \pm 0.02$ respectively compared with $0.57g \pm 0.02$ and $0.40g \pm 0.03$ in the green group. So, although eggs from the red-ring treatment did not have significantly larger yolks or albumens ($P > 0.2$) than those from the green-ring group, the differences in the ratio of yolk to albumen seems to be driven by both over-investment in yolk and under-investment in albumen by the red-ringed group.

Paternal ring colour did not influence yolk colour (a proxy for carotenoid content), but yolk colour declined with laying order ($F_{1,75.1} = 30.77$, $P < 0.0001$) and latency to lay

($F_{1,26.2} = 4.92$, $P = 0.04$; nest (random factor): $Z = 2.47$, $N = 96$, $P = 0.007$). Finally we found that DHT concentrations in freshly laid eggs increased with latency to lay (latency to lay: $F_{1,9.26} = 7.15$, $P = 0.025$; nest (random factor) $Z = 0.32$, $N = 32$, $P > 0.3$). Laying order, paternal ring colour and embryo sex did not contribute to the model. Testosterone concentrations in eggs did not vary with any variable.

Parental Care

On day 9 post-hatching, video recordings revealed that nest attentiveness (the percentage of time a parent spent brooding their nestlings) decreased with increasing total brood mass (estimate = -0.01 ± 0.005 % of time spent brooding per g of brood mass) and females had higher attentiveness (54.6 ± 4.2 %, $N = 18$) than males (29.4 ± 3.8 %, $N = 18$), irrespective of ring colour treatment (Table 1). Parents in the green-ringed group fed their nestlings more frequently (mean = 3.36 ± 0.22 feeds per hour) than parents in the red-ringed group (mean = 2.36 ± 0.16 feeds per hour) and feeding rate decreased with increasing total brood mass on day 9 (estimate = -0.05 ± 0.02 feeds $\text{h}^{-1} \text{g}^{-1}$, Table 1). A feeding bout lasted on average 15.0 ± 0.99 s ($N = 157$ feeding bouts) and its average length did not differ between the colour ring treatments (Table 1). Mothers did not differ from fathers in their rate of feeding or the duration of their feeding bouts. Also there was no significant interaction between treatment and sex of the feeding parent, so mothers and fathers were not investing in provisioning differently based on paternal attractiveness (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 treatment groups (ANOVA $F_{1,143} = 2.98$, $P = 0.034$).

Post-hoc tests showed that females paired to green-ringed males lost less mass ($5.2 \pm 1.9 \%$) than all other birds (red-ringed males ($11.6 \pm 1.3 \%$), green-ringed males ($11.8 \pm 2.4 \%$) and females paired to red-ringed males ($9.5 \pm 1.4 \%$; Tukey's B test $P = 0.05$)). Females paired to red-ringed males were more likely to die during the study period than males (10 females versus 2 males; $\chi^2_1 = 5.33$, $P = 0.021$). 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

There was a non-significant trend ($P = 0.06$) towards nestlings hatched from eggs laid by parents in the green-ringed group and incubated by green-ringed foster parents to be smaller (0.9 ± 0.05 g, $N = 10$) 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: $F_{1,16} = 0.94$, $P = 0.347$; laying 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 sex composition: $F_{1,14} = 0.36$, $P = 0.557$; marking of the nestling: $F_{1,52} = 0.45$, $P = 0.503$). The treatment of neither the biological ($F_{1,2.1} = 0.03$, $P = 0.871$) nor foster parents ($F_{1,3.02} = 0.97$, $P = 0.397$) affected the distribution of feeds to nestlings. Similarly, the sex of the feeding

foster parent did not interact with the treatment of the biological father to affect the distribution of food to nestlings ($F_{1,102} < 0.01$, $P = 0.979$).

Offspring Performance at Adulthood

Offspring survival from hatching to their first breeding attempt was high (84.8 %, $N = 46$). Five nestlings died during the first 10 days (for the two where sex was identified, one was male and one was female); after fledging two more nestlings died, one of each sex. We therefore did not include offspring sex in the statistical analysis of offspring mortality. Offspring mortality was independent of the ring colour treatment of the foster and biological father and the latency to lay, but offspring from eggs laid later in the laying sequence were more likely to die than eggs laid early in the laying sequence (GLIMMIX with identity of biological parent as random effect: $Z = 1.32$, $P = 0.19$; laying order: $F_{1,38.9} = 4.17$, $P < 0.05$; colour ring of foster parent: $F_{1,39} < 0.01$, $P = 0.99$; colour ring of biological parent: $F_{1,8.64} = 0.24$, $P = 0.64$; latency to lay: $F_{1,29.8} = 0.45$, $P = 0.51$).

Offspring body mass and tarsus length at adulthood prior to first breeding differed between ring colour treatments (Table 2). Specifically, offspring body mass as adults declined with increasing order of the egg they hatched from when raised by foster parents from the green ring treatment but not when raised by foster parents from the red ring treatment, irrespective of the colour ring treatment of the biological parents (Table 2; Fig 1a). In contrast, individuals raised by red-ringed foster parents had longer tarsi than birds raised by green-ringed foster parents but only when the biological parents were from the green-ring treatment (Table 2; Fig. 1b).

When paired with an experienced breeder from our stock population, 73.7% of the surviving 38 offspring produced eggs (sample size for offspring with treatment of

biological+treatment of foster fathers: Red+Red = 9, Red+Green = 11, Green+Red = 8,
 Green+Green = 9). There was no difference in breeding propensity between the treatment
 groups (GLIMMIX; latency to lay: $F_{1,36} = 1.83$, $P = 0.19$; colour ring of biological parent: $F_{1,35} =$
 1.06, $P = 0.31$; colour ring of foster parent: $F_{1,34} = 0.72$, $P = 0.40$; laying order: $F_{1,33} = 0.37$, $P =$
 0.55; sex: $F_{1,32} < 0.01$, $P = 0.95$). This GLIMMIX model would not run with identity of
 biological parent as a random factor because there were a large number of families for
 which there was only one offspring included in the model, so we only included data from
 one daughter per from a family of biological siblings to avoid pseudo-replication. The
 analyses of the daughters' reproductive efforts during their first breeding attempt are
 presented in Table 3. When breeding for the first time, daughters with green ringed
 biological fathers laid clutches with a larger mass than daughters from red ringed biological
 fathers (Fig. 2) due to them laying both more (red: 3.7 ± 0.47 eggs, $N = 7$; green: 4.9 ± 0.51
 eggs, $N = 7$; $F_{1,8.1} = 4.85$, $P = 0.06$) and larger eggs (red: 1.21 ± 0.05 g, $N = 7$; green: $1.29 \pm$
 0.02 g, $N = 7$; $F_{1,9.07} = 4.17$, $P = 0.07$). There was no difference in the ratio between wet yolk
 mass to wet albumen mass suggesting all eggs were of similar gross composition
 irrespective of egg size. Between pairing and clutch completion, daughters raised by foster
 parents in the red ring group lost significantly more body mass (15.8 ± 2.86 %, $N = 7$) than
 daughters raised by foster parents in the green ring group (12.4 ± 1.76 %, $N = 7$; Table 3).
 Daughters that hatched from eggs laid late in the sequence produced heavier clutches than
 daughters that hatched from eggs laid early in the laying sequence (Table 3).

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

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

Differential maternal allocation is expected whenever males differ in attractiveness which is predicted to be an honest signal of genetic or phenotypic quality (Sheldon 2000). In our study, male attractiveness was manipulated independently of male quality. Also, in contrast to Burley's classic studies, all males within the same aviary were subject to the same treatment, (Burley, 1988); see also (Sheppard et al., 2013). Thus, in our study high quality females could not pair assortatively with red-ringed males and low quality females with green-ringed males. So, in our design any differences in maternal investment due to ring colour were not confounded by female quality, but were the results of adjustments in investment due to perceived male attractiveness.

Our finding that females mated to red-ringed males laid eggs with relatively larger yolks than those with green-ringed mates is difficult to compare directly with previous tests of the DAH in birds, some of which found negative differential allocation but into different egg components (Bolund et al., 2009; Michl et al., 2005; Navara et al., 2006; Saino et al., 2002). In contrast to Bolund et al. (2009), we also found no modulation of egg carotenoids or hormones in response to male attractiveness (see also Grenna, Avidano, Malacarne, Leboucher, & Cucco, 2014). Compared with albumen, yolk comprises higher levels and diversity of lipids, minerals, vitamins and other substances vital for embryo development (Klasing, 2000). While albumen contributes to nestling structural size, yolk supports survivorship after hatching, suggesting that relative investment into these two egg components will have different impacts on the resulting nestling (Klasing, 2000). One potential explanation for this, based on the 'silver spoon' hypothesis (Bateson et al., 2004), 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. Maignet & 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

males. In our study, females in the green-ringed group, which 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 nestlings), although the sample sizes were small for the mortality rates. Although the relative costs of egg production to nestling rearing (Monaghan & Nager, 1997; Nager, 2006) 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 of females with red-ringed mates may have been because their body reserves were relatively more exhausted by egg production than in females with green-ringed males. This was despite the fact that we standardised the brood size to two nestlings which is lower than the typical brood size (~ 4 nestlings) of successful zebra finch parents in our aviaries (see also Zann, 1996). Perhaps females paired to unattractive males were altering the composition and/or size of their eggs to cope best with assumed poor genetic quality? However, in this experimental context, attractiveness was actually unrelated to genetic quality and thus daughters from matings with unattractive males happened to fare better than expected. Alternatively, or in addition, since parents in the green-ringed group provisioned nestlings more frequently, the best strategy was to invest less at the egg stage but more at the nestling rearing stage. Similarly, female brown boobies *Sula leucogaster* paired to more colourful males laid smaller eggs and did not increase parental care, but the colouration of foster fathers was positively related to paternal care and nestling growth (Montoya & Torres, 2015). Overall, we found some evidence that in zebra finches that females can differentially invest resources into offspring at different stages and that such investment

differs in costs to survival, breeding success and condition of parents. Next, we determined whether the “differential allocation” affected fitness related traits in the offspring.

Offspring from eggs laid by and incubated by parents in the green-ringed treatment were shown to have low yolk to albumen ratios and were also smaller at hatching but, compared with hatchlings from the other treatment groups, did not differ in mortality. Daughters from green-ringed biological parents laid heavier clutches at sexual maturity. Furthermore, daughters reared under the relatively poor feeding regime of red-ringed foster parents lost more mass during their first breeding attempt than those with green-ringed foster parents, despite producing similar numbers and quality of eggs. So we do have some evidence that differential breeding expenditure, at least in eggs, in response to mate attractiveness results in trans-generational effects on fitness-related traits. Notably, the effects were dependent on breeding stage. Thus, more experimental data are required to tease out whether females are able to strategically invest in nestlings as well as eggs or whether investment in later reproductive stages is limited, for example energetically, by previous investment decisions (Bowers et al., 2013). One issue with our data is that our sample size of offspring which bred was relatively small ($N = 38$). Other studies on zebra finches have also shown that conditions experienced during either the embryo (Gorman & Nager, 2004; Tobler & Sandell, 2009; von Engelhardt, Carere, Dijkstra, & Groothuis, 2006) or nestling stage (Blount, Metcalfe, Arnold, et al., 2003; Blount et al., 2006; Boag, 1987; Spencer, Heidinger, D’Alba, Evans, & Monaghan, 2010) can affect fitness-related traits but studies like ours that can directly link parental investment with offspring phenotypic or life history traits at both pre- and post-hatching stages are largely lacking (but see (Bowers et al., 2013; Cunningham & Russell, 2000; Gilbert et al., 2012; Gilbert et al., 2006).

Our results suggest both negative and positive investment, but how this balances out to be positive, negative or indeed any overall differential allocation is unclear. There are several alternative explanations. Previous studies have suggested that such a reduction in male provisioning effort may be due to the attractive trait handicapping the male (Witte, 1995). For example, increasing the attractiveness of some males could increase the frequency of dominance interactions between red-ringed males (Cuthill, Hunt, Cleary, & Clark, 1997), permit males to become polygynous (Burley, 1986b) and/or lead to more intense male competition (Qvarnström., 1997). Arguments against such behavioural mechanisms are that a red ring should not handicap a male any more than a green ring and also using aviaries where all males had the same ring colour should minimise the issue of red-ringed *versus* green-ringed male competition or polygyny since treatment groups could not interact or see each other (but see (Cuthill et al., 1997). That females with red-ringed males did not increase their provisioning rate in response to the low input by their mates suggests that a compensatory mechanism is not at play here (but see Witte, 1995). Alternatively, given that females with red-ringed males had already invested heavily in eggs, they might have been in poorer condition, and thus unable to compensate. It is also possible that compensatory feeding was not necessary if they had already prepared their offspring for a poor quality rearing environment, through changing egg resources (e.g. Gilbert et al. 2012).

Our result that female offspring of green-ringed biological fathers laid heavier clutches is, interestingly, the opposite to that found by Gilbert et al. (2012) which used a similar manipulation and cross-fostering design. The only clear differences between the two studies are that we standardised our brood size to two nestlings and also our offspring were reared in outdoor aviaries, in contrast to Gilbert et al. (2012) who used a separate cage per

pair of birds kept indoors with constant temperature, humidity and daylight regime. Subtle environmental differences may result in differences in investment patterns (e.g. Mousseau and Fox 1998; Williamson et al. 2008), and this can mean that using experiments to generalise about avian investment decisions can be difficult. Comparing differences between two standard groups of females exposed to males that differ only in their perceived quality demonstrates the ability to alter investment and the direction in which it occurs at different stages. Ultimately, however, the important thing is what individuals do across their individual lifetime and the consequences of these different possible investment strategies for their own fitness. So while in our study we found evidence for positive differential investment at the egg stage, negative investment at the nestling rearing stage and corresponding fitness-related offspring traits, we cannot conclude that passerine birds, or even zebra finches specifically, will always behave like this. An individual is likely to benefit by changing investment patterns depending on a range of environment cues (Mousseau and Fox 1998; Williamson et al. 2008), often not yet quantified or understood by researchers. To conclude, our study illustrates how patterns of reproductive investment can be complex (see also Gorman et al., 2005; Michl et al., 2005; Rutstein et al., 2005) and not consistent across all forms of maternal investment (Balzer & Williams, 1998).

Acknowledgements

We thank Graham Law, Paul McGowan, Davina Graham, John Laurie, Kat Fellerman and Alister Kirk for caring for the birds; the Electronic Workshop for assistance with the video equipment. H.E.G. was funded by a studentship from the Natural Environment Research Council. K.E.A. was funded by a Royal Society University Research Fellowship.

636 REFERENCES

- 637 Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., & Sorci, G. (2004). Increased susceptibility to
638 oxidative stress as a proximate cost of reproduction. *Ecology Letters*, 7, 363-368.
- 639 Arnold, K. E., Blount, J. D., Metcalfe, N. B., Orr, K. J., Adam, A., Houston, D., & Monaghan, P. (2007). Sex-
640 specific differences in compensation for poor neonatal nutrition in the zebra finch *Taeniopygia*
641 *guttata*. *Journal of Avian Biology*, 38(3), 356-366.
- 642 Arnold, K. E., Griffiths, R., Stevens, D. J., Orr, K. J., Adam, A., & Houston, D. C. (2003). Subtle manipulation of
643 egg sex ratio in birds. *Proceedings Of The Royal Society Of London. Series B. Biological Sciences*
644 *Biology Letters., Suppl 2*, 216 - 219.
- 645 Balzer, A. L., & Williams, T. D. (1998). Do female zebra finches vary primary reproductive effort in relation to
646 mate attractiveness? *Behaviour*, 135, 297-309.
- 647 Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A., . . . Sultan, S. E. (2004).
648 Developmental plasticity and human health. *Nature*, 430(6998), 419-421.
- 649 Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., Devevey, G. L., & Monaghan, P. (2003). Neonatal
650 nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proceedings of the*
651 *Royal Society of London: Series B*, 270, 1691-1696.
- 652 Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., & Monaghan, P. (2006). Effects of neonatal nutrition on
653 adult reproduction in a passerine bird. *Ibis*, 148(3), 509-514.
- 654 Blount, J. D., Metcalfe, N. B., Birkhead, T. R., & Surai, P. F. (2003). Carotenoid modulation of immune function
655 and sexual attractiveness in zebra finches. *Science*, 300, 125-127.
- 656 Boag, P. J. (1987). Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). *The Auk*,
657 104, 155 - 166.
- 658 Bolund, E., Schielzeth, H., & Forstmeier, W. (2009). Compensatory investment in zebra finches: females lay
659 larger eggs when paired to sexually unattractive males. *Proceedings of the Royal Society B-Biological*
660 *Sciences*, 276(1657), 707-715.
- 661 Bowers, E. K., Munclinger, P., Bureš, S., Kučerová, L., Nádvorník, P., & Krist, M. (2013). Cross-fostering eggs
662 reveals that female collared flycatchers adjust clutch sex ratios according to parental ability to invest
663 in offspring. *Molecular Ecology*, 22(1), 215-228. doi: 10.1111/mec.12106
- 664 Burley, N. (1986a). Sex-ratio manipulation in color-banded populations of zebra finches. *Evolution*, 40, 1191-
665 1206.
- 666 Burley, N. (1986b). Sexual selection for aesthetic traits in species with biparental care. *American Naturalist*,
667 127, 415-445.
- 668 Burley, N. (1988). The differential-allocation hypothesis: an experimental test. *American Naturalist*, 132, 611-
669 628.
- 670 Byers, J. A., & Waits, L. (2006). Good genes sexual selection in nature. *Proceedings of the National Academy of*
671 *Sciences of the United States of America*, 103(44), 16343-16345. doi: 10.1073/pnas.0608184103
- 672 Cunningham, E. J. A., & Russell, A. F. (2000). Egg investment is influenced by male attractiveness in the mallard.
673 *Nature*, 404, 74 - 77.
- 674 Cuthill, I. C., Hunt, S., Cleary, C., & Clark, C. (1997). Colour bands, dominance, and body mass regulation in male
675 zebra finches (*Taeniopygia guttata*). *Proceedings of the Royal Society of London Series B-Biological*
676 *Sciences*, 264(1384), 1093-1099.
- 677 Gil, D., Graves, J., Hazon, N., & Wells, A. (1999). Male attractiveness and differential testosterone investment
678 in zebra finch eggs. *Science*, 286, 126-128.
- 679 Gilbert, L., Bulmer, E., Arnold, K. E., & Graves, J. A. (2007). Yolk androgens and embryo sex: Maternal effects or
680 confounding factors? *Hormones and Behavior*, 51(2), 231-238.
- 681 Gilbert, L., Williamson, K. A., & Graves, J. A. (2012). Male attractiveness regulates daughter fecundity non-
682 genetically via maternal investment. *Proceedings of the Royal Society B-Biological Sciences*, 279(1728),
683 523-528. doi: 10.1098/rspb.2011.0962
- 684 Gilbert, L., Williamson, K. A., Hazon, N., & Graves, J. A. (2006). Maternal effects due to male attractiveness
685 affect offspring development in the zebra finch. *Proceedings of the Royal Society B-Biological Sciences*,
686 273(1595), 1765-1771. doi: 10.1098/rspb.2006.3520

- Gilby, A. J., Mainwaring, M. C., Rollins, L. A., & Griffith, S. C. (2011). Parental care in wild and captive zebra finches: measuring food delivery to quantify parental effort. *Animal Behaviour*, 81(1), 289-295. doi: 10.1016/j.anbehav.2010.10.020
- Gorman, H. E., Arnold, K. E., & Nager, R. G. (2005). Incubation effort in relation to male attractiveness in zebra finches *Taeniopygia guttata*. *Journal Of Avian Biology*, 36, 413-420.
- Gorman, H. E., & Nager, R. G. (2004). Prenatal developmental conditions have long-term effects on offspring fecundity. *Proceedings of the Royal Society B-Biological Sciences*, 271, 1923-1928.
- Grenna, M., Avidano, L., Malacarne, G., Leboucher, G., & Cucco, M. (2014). Influence of Male Dominance on Egg Testosterone and Antibacterial Substances in the Egg of Grey Partridges. *Ethology*, 120(2), 149-158. doi: 10.1111/eth.12189
- Griffith, S. C., & Buchanan, K. L. (2010). Maternal effects in the Zebra Finch: a model mother reviewed. *Emu*, 110(3), 251-267.
- Griffith, S. C., Pryke, S. R., & Buttemer, W. A. (2011). Constrained mate choice in social monogamy and the stress of having an unattractive partner. *Proceedings of the Royal Society B: Biological Sciences*. doi: 10.1098/rspb.2010.2672
- Harris, W. E., & Uller, T. (2009). Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 364(1520), 1039-1048. doi: 10.1098/rstb.2008.0299
- Hasegawa, M., Arai, E., Watanabe, M., & Nakamura, M. (2012). High incubation investment of females paired to attractive males in barn swallows. *Ornithological Science*, 11(1), 1-8.
- Horvathova, T., Nakagawa, S., & Uller, T. (2012). Strategic female reproductive investment in response to male attractiveness in birds. *Proceedings of the Royal Society B-Biological Sciences*, 279(1726), 163-170. doi: 10.1098/rspb.2011.0663
- Hunt, S., Cuthill, I. C., Swaddle, J. P., & Bennett, A. T. D. (1997). Ultraviolet vision and band-colour preferences in female zebra finches, *Taeniopygia guttata*. *Animal Behaviour*, 54, 1383-1392.
- Jones, K. S., Nakagawa, S., & Sheldon, B. C. (2009). Environmental Sensitivity in Relation to Size and Sex in Birds: Meta-Regression Analysis. *American Naturalist*, 174(1), 122-133. doi: 10.1086/599299
- Karadas, F., Grammenidis, E., Surai, P. F., Acamovic, T., & Sparks, C. (2006). Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *British Poultry Science*, 47(5), 561-566.
- Klasing, K. C. (2000). *Comparative Avian Nutrition*. Wallingford, UK: CAB International.
- Kolliker, M., Richner, H., Werner, I., & Heeb, P. (1998). Begging signals and biparental care: nestling choice between parental feeding locations. *Animal Behaviour*, 55, 215-222.
- Limbou, T., Mateman, A. C., Andersson, S., & Lessells, C. M. (2004). Female blue tits adjust parental effort to manipulated male UV attractiveness. *Proceedings of the Royal Society B-Biological Sciences*, 271(1551), 1903-1908. doi: 10.1098/rspb.2004.2825
- Limbou, T., Mateman, A. C., & Lessells, C. M. (2013). Opposite differential allocation by males and females of the same species. *Biology Letters*, 9(1), 20120835. doi: 10.1098/rsbl.2012.0835
- Maguire, S. E., & Safran, R. J. (2010). Morphological and genetic predictors of parental care in the North American barn swallow *Hirundo rustica erythrogaster*. *Journal of Avian Biology*, 41(1), 74-82. doi: 10.1111/j.1600-048X.2009.04806.x
- Maigret, J. L., & Murphy, M. T. (1997). Costs and benefits of parental care in eastern kingbirds. *Behavioral Ecology*, 8(3), 250-259.
- Martins, T. L. F. (2003). Sex specific growth rates in Zebra Finch nestlings: a possible mechanism for sex-ratio adjustment in a non-size dimorphic species? *Behavioral Ecology*, 15, 174-180.
- Mazuc, J., Chastel, O., & Sorci, G. (2003). No evidence for differential maternal allocation to offspring in the house sparrow (*Passer domesticus*). *Behavioral Ecology*, 14(3), 340-346. doi: 10.1093/beheco/14.3.340
- Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution*, 16, 254-260.
- Michl, G., Torok, J., Pecze, P., Garamszegi, L. Z., & Schwabl, H. (2005). Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. *Behavioral Ecology*, 16(2), 383-388.
- Monaghan, P., & Nager, R. G. (1997). Why don't birds lay more eggs? *Trends in Ecology & Evolution*, 12(7), 270-274.
- Montoya, B., & Torres, R. (2015). Male skin color signals direct and indirect benefits in a species with biparental care. *Behavioral Ecology*, 26(2), 425-434. doi: 10.1093/beheco/aru204
- Nager, R. G. (2006). The challenges of making eggs. *Ardea*, 94(3), 323-346.

- Nash, J. P., Cuisset, B. D., Bhattacharyya, S., Suter, H. C., Le Menn, F., & Kime, D. E. (2000). An enzyme linked immunosorbant assay (ELISA) for testosterone, estradiol, and 17,20 beta-dihydroxy-4-pregnen-3-one using acetylcholinesterase as tracer: application to measurement of diel patterns in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, 22(4), 355-363.
- Navara, K. J., Badyaev, A. V., Mendonca, M. T., & Hill, G. E. (2006). Yolk antioxidants vary with male attractiveness and female condition in the house finch (*Carpodacus mexicanus*). *Physiological and Biochemical Zoology*, 79(6), 1098-1105.
- Owens, I. P. F., & Bennett, P. M. (1994). Mortality costs of parental care and sexual dimorphism in birds. *Proceedings of the Royal Society B-Biological Sciences*, 257, 1-8.
- Pariser, E. C., Mariette, M. M., & S C Griffith, S. C. (2010). Artificial ornaments manipulate intrinsic male quality in wild-caught zebra finches (*Taeniopygia guttata*). *Behavioral Ecology*, 21(2), 264-269.
- Qvarnström, A. (1997). Experimentally enlarged badge size increases male competition and reduces male parental care in the collared flycatcher. *Proceedings of the Royal Society series B* . , 264, 1225-1231.
- Ratikainen, I. I., & Kokko, H. (2010). Differential allocation and compensation: who deserves the silver spoon? *Behavioral Ecology*, 21(1), 195-200. doi: 10.1093/beheco/arp168
- Reid, J. M., Bignal, E. M., Bignal, S., McCracken, D. I., & Monaghan, P. (2003). Age-specific reproductive performance in red-billed choughs *Pyrrhocorax pyrrhocorax*: patterns and processes in a natural population. *Journal of Animal Ecology*, 72(5), 765-776.
- Rutstein, A. N., Gilbert, L., Slater, P. J. B., & Graves, J. A. (2004). Mate attractiveness and primary resource allocation in the zebra finch. *Animal Behaviour*, 68, 1087-1094.
- Rutstein, A. N., Gorman, H. E., Arnold, K. E., Gilbert, L., Orr, K. J., Adam, A., . . . Graves, J. A. (2005). Sex allocation in response to paternal attractiveness in the zebra finch. *Behavioral Ecology*, 16(4), 763-769. doi: 10.1093/beheco/ari052
- Saino, N., Bertacche, V., Ferrari, R. P., Martinelli, R., Møller, A. P., & Stradi, R. (2002). Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. *Proceedings of the Royal Society B-Biological Sciences*, 269, 1729-1734.
- Sanz, J. J. (2001). Experimentally reduced male attractiveness increases parental care in the pied flycatcher *Ficedula hypoleuca*. *Behavioral Ecology*, 12(2), 171-176. doi: 10.1093/beheco/12.2.171
- Sheldon, B. C. (2000). Differential allocation: tests, mechanisms and implications. *Trends in Ecology and Evolution*, 15, 397-402.
- Sheppard, J. L., Clark, R. G., Devries, J. H., & Brasher, M. G. (2013). Reproductive effort and success of wild female mallards: Does male quality matter? *Behavioural Processes*, 100, 82-90. doi: 10.1016/j.beproc.2013.07.024
- Spencer, K. A., Heidinger, B. J., D'Alba, L. B., Evans, N. P., & Monaghan, P. (2010). Then versus now: effect of developmental and current environmental conditions on incubation effort in birds. *Behavioral Ecology*, 21(5), 999-1004.
- Tobler, M., & Sandell, M. I. (2009). Sex-specific effects of prenatal testosterone on nestling plasma antioxidant capacity in the zebra finch. *Journal of Experimental Biology*, 212(1), 89-94.
- Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, 179, 90-92.
- Uller, T., Eklof, J., & Andersson, S. (2005). Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. *Behavioral Ecology and Sociobiology*, 57(6), 584-590. doi: 10.1007/s00265-004-0886-2
- Veasey, J. S., Houston, D. C., & Metcalfe, N. B. (2001). A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. *Journal of Animal Ecology*, 70(1), 20-24.
- Vezina, F., & Williams, T. D. (2005). The metabolic cost of egg production is repeatable. *Journal of Experimental Biology*, 208(13), 2533-2538. doi: 10.1242/jeb.01675
- von Engelhardt, N., Carere, C., Dijkstra, C., & Groothuis, T. G. G. (2006). Sex-specific effects of yolk testosterone on survival, begging and growth of zebra finches. *Proceedings of the Royal Society B-Biological Sciences*, 273(1582), 65-70.
- Witte, K. (1995). The differential-allocation hypothesis: Does the evidence support it? *Evolution*, 49(6), 1289-1290.
- Zann, R. A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. Oxford: Oxford University Press.

Figure 1. (a) Adult body mass at first breeding of birds raised by red-ringed foster parents (closed symbols) and by green-ringed foster parents (open symbols) in relation to laying order. **(b)** Mean (± 1 SE) adult tarsus length in relation to ring colour treatment of the biological father. Open bars show the tarsus length of birds raised by foster parents in the green-ring group and the shaded bars of birds raised by foster parents in the red-ring group. See table 2 for results of the statistical analysis. Numbers above the bars represent the numbers of offspring.

Figure 2. Mean (± 1 SE) clutch mass (number of eggs * mean egg mass) at first breeding of daughters that hatched from eggs laid by red-ringed biological parents (shaded bars) and by green-ringed biological parents (open bars) in relation to laying order. For presentation, daughters hatched from early-laid eggs (first two eggs) and later-laid eggs (eggs 3 to 5) are shown separately, but laying order was used as a continuous variable in the analysis (see Table 3 for results of the statistical analysis). Numbers above the bars indicate the numbers of daughters.

Table 1. Results of general linear mixed models on parental care behaviour at day 9 post-hatching including the ring colour of foster fathers and sex of foster parent as factors, latency to lay, total brood mass and ambient temperature at the day of the behavioural recording as covariates and identity of the ‘nest’ as a random factor.

	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)	$Z = 3.29, P = 0.001$	$Z = 0.19, P = 0.85$	$Z = 0.92, P = 0.36$

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

Table 2. Results for mixed models on the effect of the colour ring treatment on hatchling mass, nestling growth, and body mass and length of offspring tarsus at adulthood when breeding the first time.

	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	$Z=0.51$ $P=0.61$	$Z= 2.40$ $P=0.02$	$Z=2.27$ $P=0.02$	$Z=2.35$ $P=0.02$

These models contained the ring colour of biological and foster parents, sex of the offspring and from what laying order it hatched (laying order) and the latency to lay with identity of the biological 'nest' and the foster 'nest' as random factors. All other interactions $P > 0.23$.

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	$Z=2.34$ $P=0.02$	$Z=0.07$ $P=0.95$	$Z=0.23$ $P=0.82$	$Z=2.35$ $P=0.02$

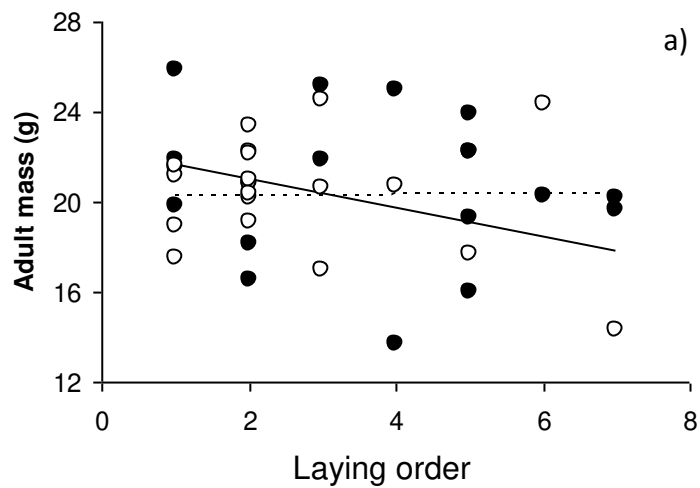
Independent variables were latency to lay (number of days between pairing and laying the first egg), clutch mass (number of eggs laid * mean egg mass), egg composition (ratio between wet yolk mass and wet albumen mass) and mass loss between pairing and clutch completion. These models contained the ring 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.

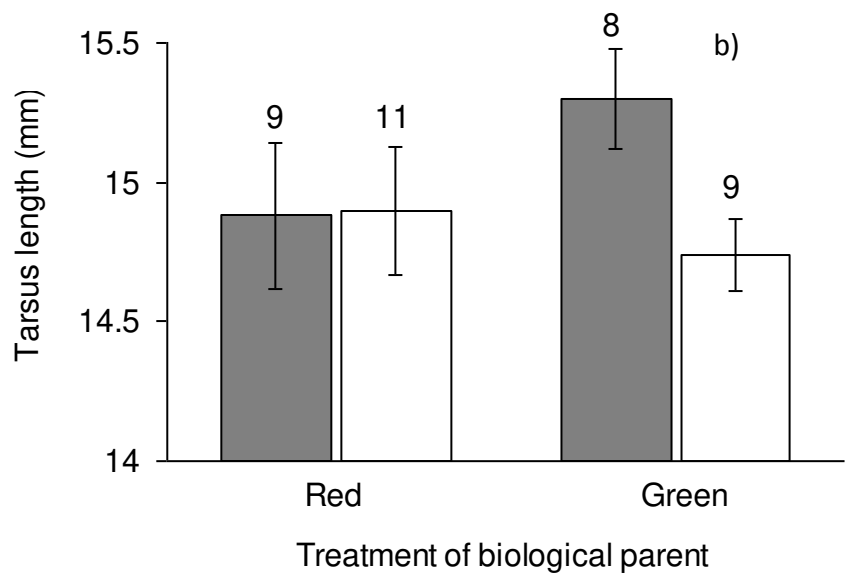
854



855

856

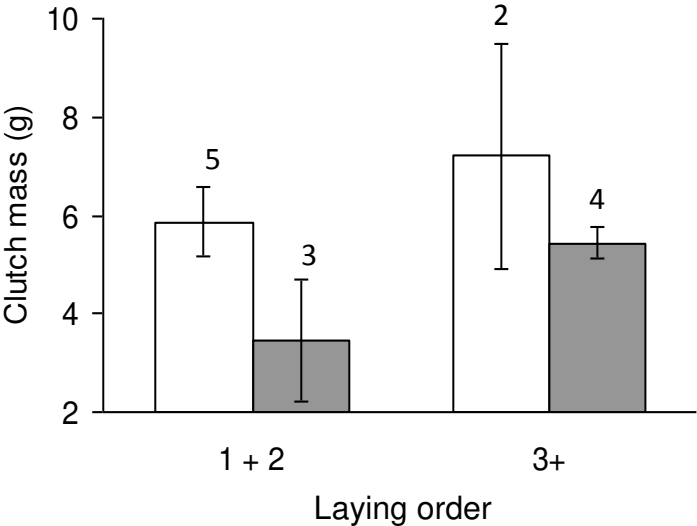
857 (b)



858

859 Fig. 2.

860



861

862