Male attractiveness regulates daughter fecundity non-genetically via maternal investment

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Mothers can non-genetically influence offspring phenotype in response to environmental conditions, including mate attractiveness. If such ‘maternal effects’ influence the offspring’s reproduction and F2 generation, there is a mechanism for non-genetic trans-generational effects on phenotype, including epigenetic phenomena, with implications for evolution and population dynamics. We demonstrate in the zebra finch Taeniopygia guttata such non-genetic effects on offspring fecundity and the size of early stage F2 (eggs) in response to experimentally manipulated father’s attractiveness. Our experimental design allowed us to deduce that the mechanism for this non-genetic paternal effect was via maternal investment in eggs. This affected female offspring size and, consequently, fecundity and F2 (egg) size. This demonstrates that female perception of mate attractiveness can have non-genetic, trans-generational fitness consequences and this may have important implications for the evolution of sexually selected traits and population dynamics.

Keywords: maternal effects; paternal effects; egg resources; zebra finch; Taeniopygia guttata

1. INTRODUCTION

There is a great deal of current interest in how a mother’s phenotype can non-genetically influence offspring phenotype, often in response to different environmental conditions. Such non-genetic ‘maternal effects’ are wide-ranging, involve a large variety of environmental stimuli, a range of effects on offspring and have been recorded across taxa. Maternal effects may often be the result of a maternal strategy to optimize the fitness of her offspring and herself according to the environment the mother predicts her offspring will encounter [1]. For example, offspring of the monocarpic herb, Campanulastrum americanum, have 3.4 times better fitness if grown in their maternal light environment than otherwise [2] and female cichlid fish reared under poor conditions produce larger young that survive better than small individuals in a harsh environment [3].

If such non-genetic maternal effects last into offspring adulthood and affect their reproductive parameters, there is a mechanism for long-term trans-generational maternal effects. Through these influences on the distribution of phenotypes in the next generation, maternal effects can have strong implications for population dynamics [4] and for the gene flow, the strength and direction of evolution and speciation [5–8]. For example, red deer Cervus elaphus mothers that experienced low food conditions when young gave birth to calves later in the autumn, and these late-born calves had worse fitness prospects than early-born individuals [9]. Thus, the lifetime reproductive success of whole cohorts of individuals can be profoundly altered by conditions experienced by their grandparents.

Recent studies on arthropods have demonstrated long-term intergenerational impacts of maternal effects, in which the actions of the parental generation non-genetically affect the phenotype of the grand-offspring (F2 generation) [4,10]. A small number of studies of avian species have now shown effects in response to maternal parasitism or early developmental factors into the offspring (F1) and early F2 stages [11,12], and in response to maternal age and lifespan [13,14]. It would be particularly interesting to find such long-term maternal effects in response to a more subtle environmental factor that has no direct influence on maternal condition, eggs or chicks. One such factor could be mate attractiveness in species where males contribute no resources to the female (or eggs). Most, if not all, studies of non-genetic parental effects have been concerned with maternal effects. Interestingly, an effect of mate attractiveness on offspring fecundity or grand-offspring phenotype would constitute, ultimately, a paternal effect. Such a paternal effect, as part of the female’s environment, would necessarily operate indirectly via maternal effects.

Mate attractiveness is an interesting type of environmental factor that has been shown, in avian species, to influence maternal investment in chick provisioning [15,16], egg size [17], clutch size [18,19] and allocation of yolk resources such as androgens [20,21], antioxidants [22] and immunoglobulins [23]. Such differential investment is likely to affect offspring phenotype and, if this affects offspring reproductive parameters, may form a mechanism for trans-generational parental effects. Indeed, in zebra finches, Taeniopygia guttata, mate attractiveness has been shown to influence the early development of offspring non-genetically, through maternal effects acting via egg resources [24]. However, this study did not investigate maternal effects on the offspring’s adult or reproductive characteristics, which is an essential pre-requisite to inferring trans-generational maternal effects. It would be...
fascinating to investigate whether an environmental cue as subtle as a mate’s perceived attractiveness could influence offspring phenotype down several generations non-genetically via parental effects.

Here, we test for mate attractiveness-dependent maternal effects on offspring reproductive parameters (male attractiveness and female fecundity)—the prerequisites for inferring long-term trans-generational maternal effects. We test this using the zebra finch, a species where the male does not provision the female before or during egg laying.

2. METHODS
(a) Breeding experiment
Male attractiveness may affect offspring traits not only through maternal effects, but also through ‘good genes’ effects. Therefore, we designed an experiment to control for any over-riding effects of parental genetic quality on offspring traits. We experimentally manipulated perceived male attractiveness by randomly assigning a red- or pale green-coloured plastic ring to each leg. Studies of both captive-bred and wild-caught zebra finches have demonstrated that females prefer males with red leg rings, whereas males with pale green rings are the least attractive, and preference for ring colour appears to over-ride all other male secondary sexual characteristics [15,25,26]. We have confirmed this preference in the zebra finch population at St Andrews University used in this study [27]. We prevented assortative mating, i.e. mating between attractive males and high-quality females, by randomly allocating pairs and allowing them to produce eggs and chicks in individual cages. In order to test that the mechanism for any observed maternal effects on offspring traits was differential investment of egg resources, rather than nestling care, the link between the genetic parents and nestling care must be broken. To achieve this, we cross-fostered whole clutches between nests of similar clutch size (± 1 egg) and similar dates (± 3 days) of clutch initiation, and in equal numbers between- and within-attractiveness treatment groups. This avoided as much as possible any over-riding effects on offspring traits of parental differential investment in nestling care either in response to mate attractiveness or owing to parental quality.

A total of 70 breeding pairs were used. This was achieved in two rounds of breeding: initially, 36 breeding pairs were used (18 from each attractiveness treatment; see Gilbert et al. [24] for a study of chick early development from this experiment) but, owing to losses of eggs and chicks, which is normal in caged zebra finches, only 55 offspring survived to 100 days. This was not enough for meaningful analysis given that our experiments used males and females separately to compare those from red- and green-ringed fathers. Therefore, we set up a second breeding round using another 34 pairs using different individuals from the first round (17 from each attractiveness treatment) and these produced a further 59 offspring that survived to 100 days. Since female size and condition have been shown to affect egg resources in our population of zebra finches at St Andrews University [28], we recorded the mass of each female at pairing and confirmed there was no difference in mass between females allocated to red- or green-ringed males ($F_{1,31} = 2.70, p = 0.1104$).

Egg laying order was recorded by marking each egg on the morning on which it was laid. The second egg from each clutch was removed for use in a different study and replaced with a dummy egg to maintain natural clutch size. Chick hatching order was recorded by checking nests four times daily, and marking new hatchlings with a non-toxic marker.

All zebra finches were wild phenotype, sourced from captive-bred populations of UK universities. No birds had prior experience of red or green rings, and none had bred in the six months before the study. Before breeding, males were housed separately from females.

Birds were provided daily with mixed seed (foreign finch mix by Haith’s, Cleethorpes, Lincolnshire, UK), oystershell grit, cuttlebone and fresh drinking water containing calcium and vitamin supplements ad libitum. This was supplemented with reconstituted Haith’s egg biscuit and fresh spinach twice weekly. Birds were maintained on a 14 L:10 D cycle dark lighting schedule with full-spectrum artificial lights, since ultraviolet light is important for correct colour discrimination and mate choice in zebra finches [26,29].

(b) Offspring (F1) parameters
When the offspring were more than 100 days old (i.e. fully independent and sexually mature), we estimated their reproductive potential. For females, this was estimated using clutch size and egg mass. For males, this was estimated as attractiveness to females.

F1 females were paired with non-experimental stock males whose attractiveness had not been manipulated, i.e. they wore a single orange identification leg ring only. At pairing, we measured the mass, tarsus length and fat score (amount of fat in the furculum, on a scale of 0 to 5 [30] of each F1 female). Pairs were kept in individual breeding cages under standard breeding conditions (as described above) and allowed to produce eggs. All eggs were weighed on the morning of the day of laying.

Attractiveness of male offspring was estimated through female choice trials (see the electronic supplementary material).

(c) Statistical analysis
Analyses were conducted using the SAS System for Windows v. 9.1. F1 female fecundity (clutch size, clutch mass and egg mass) data were analysed using general linear mixed models (GLMMs). Initially, in order to test for ultimate effects such as parental effects (rather than proximate effects such as the individual’s own size and condition) on these response variables, we entered the following explanatory variables into these GLMMs: father’s ring colour, foster-father’s ring colour, mother’s mass, laying order of the egg from which the individual hatched and breeding round. Nest identification was entered as a random effect since a small number of F1 females originated from the same nest; this takes into account between- and within-nest variation, avoiding pseudo-replication [31]. We then explored potential mechanistic reasons for the ultimate effects we found by testing for proximate effects (individual’s size and condition). We entered F1 female body mass, tarsus length and fat score into models as proximate explanatory variables in models of F1 female reproductive output (response variables were clutch size, clutch mass and egg mass). We then explored the strength of any significant proximate effects by adding them into the final model of ultimate effects. If the significant proximate effect replaced the significant ultimate effect (in terms of its significance), it suggests that the proximate effect is a key mechanism through which the ultimate effect influences F1 fecundity. If the significant proximate effect does not replace the significant ultimate effect (in terms of its
significance), it suggests that other, untested, mechanisms also play an important role in explaining how the ultimate acts on F1 female fecundity.

F1 male attractiveness data were in the form of proportions of time spent by the female with each male, i.e. bounded by 0 and 1. The response distribution was thus binomial and we used GLMMs with a logit link function using the Glimmix macro in SAS. We used data from males with red-ringed fathers, since they were tested with those with green-ringed fathers, so using both would be replicating data within the model. Thus, the null hypothesis was that the intercept of the model was 0.5, such that males from red-ringed fathers would be more attractive than those from green-ringed fathers if the intercept was significantly greater than 50 per cent. Similarly to the female offspring fecundity data, the explanatory variables initially entered into the model were foster-father’s ring colour, mother’s mass, laying order and breeding round.

For all analyses, a backwards step-wise procedure was carried out, such that variables with \( p > 0.1 \) were sequentially dropped from the model, with the least-significant interaction terms removed first. Hence, we do not present the values of test statistics for insignificant variables that were absent from the final models. While significance was assumed when \( p < 0.05 \), we used the threshold of \( p > 0.1 \) for inclusion in the model because marginally significant terms can influence the model and may be of biological interest.

3. RESULTS

(a) F1 female fecundity

Thirty five wild phenotype F1 females survived and were in condition for the fecundity breeding experiment (a further 19 were fawn or white phenotype and not used). The clutch size, both in terms of the number of eggs per clutch and total clutch mass, laid by F1 females varied with the attractiveness treatment of both their fathers and their foster-fathers (figure 1). F1 females laid heavier clutches if they had attractive rather than unattractive fathers \( (F_{1,29} = 6.24, p = 0.018) \) and if they had attractive rather than unattractive foster-fathers \( (F_{1,29} = 6.13, p = 0.019) \). A similar result was found for the numbers of eggs per clutch (fathers \( F_{1,29} = 4.05, p = 0.053 \); foster-fathers \( F_{1,29} = 8.32, p = 0.007 \)). The mean mass of eggs laid by female offspring of attractive fathers was also significantly greater than those laid by offspring of unattractive fathers \( (F_{1,30} = 8.98, p = 0.005) \), but there was no effect of foster-father’s attractiveness treatment (figure 1). Other variables initially entered into all the F1 female fecundity models (breeding round, mother’s mass and laying order of the egg from which the offspring hatched) were eliminated from the models owing to \( p > 0.1 \). For full statistical details see electronic supplementary material, table S1.

To further explore the possibility that F1 female tarsus length was a proximate mechanism through which father’s attractiveness operated on F1 fecundity, we re-ran the final ultimate effects model with the addition of F1 female tarsus length as a fixed factor. We predicted that, if F1 female tarsus does not replace father’s attractiveness (in terms of its significance), it suggests that other, untested, mechanisms also play an important role in explaining how father’s attractiveness acts on F1 female fecundity. However, for all F1 fecundity parameters (clutch size, clutch mass and egg mass) F1 tarsus replaced father’s attractiveness (but not foster-father’s attractiveness), suggesting that father’s attractiveness does indeed affect F1 fecundity via an effect on F1 body size (see electronic supplementary material, table S1).

(b) F1 male attractiveness

Of the 21 F1 males that were preferred by females, 12 had attractive (red-ringed) fathers, while nine had unattractive
and egg laying order) were eliminated from the models ing round, foster-father’s attractiveness, mother’s mass (green-ringed) fathers. All explanatory variables (breed-
ing tarsus length) on (c) mean egg mass. Raw data are shown.

(green-ringed) fathers. All explanatory variables (breed-
ing round, foster-father’s attractiveness, mother’s mass and egg laying order) were eliminated from the models owing to $p > 0.1$ and the intercept was not significantly different from 0.5: back-transformed mean ± s.e. (upper and lower confidence intervals) = 0.603 ± 0.615 (0.79, 0.38). In other words, females spent 60.3 per cent of their time with males of attractive fathers and 39.7 per cent with males of unattractive fathers but, because the confidence interval encompasses 50 per cent, this difference was not statistically significant.

4. DISCUSSION
We tested for long term, non-genetic paternal attractiveness effects, acting indirectly via maternal effects, on the reproductive potential of F1 offspring in the zebra finch. We found that F1 females of attractive fathers laid larger eggs and clutches than those of unattractive fathers, and those raised by attractive foster-fathers laid larger clutches (but not larger eggs). There was not sufficient evidence for any significant parental effects on F1 male attractiveness.

Firstly, and most importantly, these results show strong evidence for long term, non-genetic paternal effects. Because we manipulated male attractiveness experimentally (in order to disassociate attractiveness from genetic quality), and males do not contribute resources to females (or, therefore, eggs), these paternal effects must have been acting indirectly via maternal effects. Owing to the experimental design, we can conclude that the differences in reproductive potential between female offspring of attractive and unattractive fathers were most probably owing to differences in how the mother allocated resources to her eggs in response to the attractiveness of her mate. Since the experiment did not allow assortative mating between high-quality males and females, and males were allocated colour rings and females randomly, the results were unlikely to be due to any over-riding genetic differences. Also, because we cross-fostered clutches and included the foster-father’s attractiveness in the models, the observed effects of father’s attractiveness on offspring reproductive potential were upheld even after allowing for any differences in post-hatching nestling care by the foster-parents.

Secondly, the result that female clutch size was influ-
eanced by the attractiveness of the foster-father suggests that nestling care (as well as egg resources) has long-term effects on offspring characteristics. Foster-fathers whose attractiveness has been experimentally enhanced (with red rings) may provide better nestling care than unattractive foster-fathers. Alternatively, and perhaps more likely, the female partners of attractive foster-fathers may respond to his perceived attractiveness by providing better nestling care than females paired with unattractive foster-males, as reported in several studies of avian species [13,14].

F1 females that had attractive fathers laid larger eggs and clutches, and we have argued that, owing to the experimental design, this must have been manifested via differential investment of egg resources by the mother in response to her mate’s attractiveness. But what proximate phenotypic trait of F1 females was influenced by maternal investment of egg resources to cause this effect? Investigating the proximate reasons for this showed that larger eggs and clutches were laid by larger F1 females. In addition, Gilbert et al. [24], using the same birds as in the first breeding round of this study, found that F1 females grew to be larger if they had attractive, rather than unattractive, fathers. Furthermore, when we entered F1 female body size into final ultimate effects models (those with parent’s attractiveness), F1 female body size replaced father’s attractiveness as a significant correlate of F1 fecundity for all fecundity parameters. In other words, females paired with attractive males allocated egg resources such that their female offspring grew larger, which enabled them to lay larger eggs and clutches. It is interesting to note that the significance of foster-father’s attractiveness was unaffected by the addition of F1 female body size in the model. This suggests that the mechanism for the effect of foster-father’s attractiveness on F1 female fecundity differed from that of father’s attractiveness, i.e. not acting via F1 body size or egg resources (which makes sense since foster-fathers could act only at the nestling stage).

Another potential mechanism for the effect of father’s attractiveness on F1 female fecundity instead of maternally allocated egg resources could be that, if attractive fathers had smaller clutches or smaller broods, there would be less intra-brood sibling competition, resulting in larger F1 females. However, Gilbert et al. [24], using the same birds as in the first breeding round of this study, found no effect of father’s attractiveness on clutch size, brood size or chick mortality, nor any effect of brood size on F1 female tarsus length. Therefore, in this case, the most likely mechanism is via maternally allocated egg resources affecting F1 female size.
Many other studies show that larger females of many avian species lay larger eggs, including for zebra finches [21,32,33]. This is likely to continue the parental effect into the offspring hatching from these eggs because larger eggs usually correlate with improved chick growth, mass and survival, both in zebra finches [33] and more generally in other avian species [34–36]. The implications for clutch size are more complex. Although laying more eggs necessarily means the potential for higher numbers of surviving offspring, it can also affect offspring performance through increased sibling competition, which can lead to variance in offspring fecundity, a phenomenon found across taxa [10,37]. Hence, the paternal and maternal effects on the F1’s clutch size that we have found are also likely to continue the parental effect into the hatching F2 offspring and beyond.

Our experimental design allowed us to conclude that the paternal effects on F1 female fecundity were via maternal allocation of egg resources. Which egg resources could produce the effects observed in this study? One candidate is yolk androgens such as testosterone since female zebra finches have been shown to invest more yolk testosterone when paired with attractive (red-ringed) males [20]. Several studies on avian species have found that experimentally increased yolk androgen levels can affect offspring phenotype in a number of ways, including growth rates, dominance, size and survival [38]. Other hormones or other egg resources can also play a role in shaping offspring phenotype, for example, the patterns of investment of maternally derived yolk antioxidants and immunoglobulins can vary with mate attractiveness and could affect offspring development, immune function and survival [22,23]. Another possible form of investment in eggs could be incubation effort, which can vary with male attractiveness in zebra finches [11].

Our findings demonstrate non-genetic paternal effects of male attractiveness (of both fathers and foster-fathers) on F1 fecundity and the size of early stage F2 (eggs) in a vertebrate system where father’s attractiveness was manipulated and thus did not imply genetic quality, and fathers do not contribute to maternal condition. The mechanism for this non-genetic paternal effect was manifested by nestling care and also via maternally allocated egg resources, one proximate effect of which was to influence the size of F1 females. Such effects on F1 reproductive parameters and the size and number of F2s are likely to have strong influences on population dynamics and the evolution of, in this case, sexually selected phenotypic traits.

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