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Prenatal developmental conditions have long-term effects on offspring fecundity

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Maternal effects, in which differences in parental state cause differences in offspring fitness, are important in trade-offs influencing an individual's optimal reproductive strategy. In zebra finches (*Taeniopygia guttata*) we manipulated the nutritional state for four weeks before the start of breeding through protein supplementation. Zebra finches were kept on identical diets during the rest of the experiment. We then tested the effects of maternal state on offspring size, survival and fecundity. In order to separate the effects of maternal state occurring through egg production, incubation and chick-rearing, we used a cross-fostering experiment. We show that a protein-rich diet prior to laying improved maternal body weight prior to breeding compared with birds on a protein-poor diet. Poorer maternal state prior to breeding gave rise to offspring with lower fecundity than offspring from birds in a better nutritional state. Maternal state is thought to affect the conditions developing offspring experience through the bird's ability to produce and incubate eggs. Male and female embryos differed in their responses to conditions at different developmental stages. This shows that embryonic developmental conditions and sex differences in vulnerability to these conditions need to be incorporated into future models of selection, life-history evolution and sex-ratio theory.

Keywords: maternal effects; egg quality; incubation; *Taeniopygia guttata*; prenatal development; offspring fitness

1. INTRODUCTION

Life-history theory predicts that individuals should invest in reproduction so as to maximize their lifetime fitness. Parental reproductive effort in current reproduction may affect the parent's future reproduction (intra-individual trade-off) and/or the fitness of their offspring (inter-generational trade-off) (Stearns 1992). In the past, intra-individual trade-offs received the most attention because of the historic emphasis on the flow of resources within individuals (Stearns 1992). More recently, maternal effects, where variation in the conditions experienced by the parents affects offspring phenotype in a non-genetic manner, have received increasing attention (Mousseau & Fox 1998). Maternal effects can be potentially important sources of variation in offspring fitness and therefore influence optimal reproductive strategies.

Maternal effects can act at any stage of development from conception to independence, and there is good evidence that postnatal development affects offspring phenotype (reviewed in Lindström 1999; Metcalfe & Monaghan 2001). For example, in birds, postnatal developmental conditions can affect offspring traits that are related to survival, fecundity and mate attractiveness (e.g. Gustafsson & Sutherland 1988; Haywood & Perrins 1992; Schluter & Gustafsson 1993; Blount *et al.* 2003). Although the offspring's resource demand is probably highest during postnatal development, even small adverse influences acting during prenatal development, before embryos have the capacity to respond to environmental influences, can also have large and irreversible effects on offspring phenotype and fitness (Rhind *et al.* 2001).

It has been shown that prenatal developmental conditions can influence embryo growth, organ development, predisposition to diseases and adult reproductive behaviour (e.g. Clark & Galef 1995; Desai & Hales 1997). For example, in birds, offspring phenotype can be affected by egg size (Williams 1994) or by aspects of egg composition, such as hormones (Schwabl 1997), carotenoids (e.g. Blount *et al.* 2002) and immunoglobulins (e.g. Gasparini *et al.* 2001). Incubation effort can affect embryo survival and hatchling weight (Reid *et al.* 2002; Larsen *et al.* 2003). In species where parents provide the nutritional and thermal environment in which the embryo develops, offspring phenotype is therefore likely to be affected by prenatal parental care.

The causal relationship between maternal effects arising during prenatal development and offspring fitness is often equivocal because many studies are correlative and/or followed offspring for only a limited period of time. Factors that influence parental reproductive effort during prenatal development may also affect parental reproductive effort during postnatal development (Metcalfe & Monaghan 2001). Correlative studies, therefore, cannot separate the effects acting at different times during development. Correlative studies also rarely control for genetic effects, and poor prenatal developmental conditions could be the result of poor genes rather than maternal effects. Birds offer a good opportunity to study such effects experimentally. They have several distinct developmental stages where parents modulate the environment in which their offspring develop, and conditions can be manipulated during one stage and the effects during that stage can be separated from effects acting in other stages by using cross-fostering experiments.

Here, we investigated maternal effects during embryo development on offspring phenotype and fecundity in a

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captive population of zebra finches (*Taeniopygia guttata*). We aimed to test the effects of parental state at the start of breeding on offspring size, survival and fecundity. We manipulated the parents' nutritional state at the start of breeding by using diets of different protein content for a short time during the pre-breeding period only, and egg-production effort was experimentally standardized to ensure that differences in parental state persisted beyond egg laying. The diet manipulation stopped before breeding started, to ensure that maternal effects acting through parental state and direct environmental effects acting on the offspring were not confounded. It is possible that the manipulation may have affected several developmental stages, and, to separate maternal effects arising during the different developmental stages (egg production, incubation, nestling rearing), we cross-fostered fresh eggs and hatchlings between nests.

2. METHODS

(a) *Manipulation of parental state*

The experiment was carried out in the captive zebra finch colony at the University of Glasgow. To confirm and strengthen the results we repeated the experiment nine months later. Birds were kept on a standard diet of *ad libitum* mixed seed and water, with vitamin, protein and calcium supplements, greens, oyster grit and cuttlebone (for details of husbandry see Gorman & Nager 2003).

To manipulate the birds' pre-breeding state experimentally, we used experimental diets of different protein content fed to the parents for four weeks prior to breeding. Diets were given before breeding began in order that all birds bred in an identical environment and to exclude direct effects of the treatment on the offspring. Pairs were randomly assigned to one of two diet treatments. In the protein-poor supplement (LPS; 20 pairs in replicate 1, and 30 pairs in replicate 2) birds received a standard diet with a protein supplement once per week. In the protein-rich supplement (HPS; 16 pairs in replicate 1, and 26 pairs in replicate 2) birds received the protein supplement with added hens' egg five times per week. The protein-rich diet significantly improved the parental state at the start of breeding compared with the protein-poor diet. Between the last weight measurement before the manipulation and the body weight at pairing, females on HPS gained more weight ($n = 24$, $14.1 \pm 3.6\%$) than females on LPS ($n = 31$, $3.5 \pm 2.7\%$) (repeated-measures analysis, diet manipulation: $F_{1,31} = 6.63$, $p = 0.015$; replicate: $F_{1,21} = 2.25$, $p = 0.148$; body weight was not available for all birds). All males gained some body weight during the period of the diet manipulation ($n = 57$, $5.9 \pm 1.9\%$) (diet manipulation: $F_{1,32} = 0.06$, $p = 0.813$; replicate: $F_{1,31} = 0.85$, $p = 0.368$).

As females on HPS were in an improved state, they were expected to lay more and larger eggs than LPS birds. We therefore manipulated the females to lay similar-sized clutches. At nests of LPS pairs we removed the first three eggs within hours of being laid to induce the birds to increase the number of eggs they would lay. At nests of HPS pairs we added one false egg per day for the first 4 days after pairing to induce them to lay fewer eggs (modified from Veasey *et al.* 2001). This procedure resulted in the two treatment groups laying clutches of the same size (LPS: 5.7 ± 0.2, $n = 42$; HPS : 5.1 ± 0.3, $n = 34$; $F_{1,74} = 2.56$, $p = 0.114$) and eggs of similar mass (LPS: 1.20 ± 0.025 g, $n = 41$; HPS : 1.25 ± 0.045 g, $n = 33$; $F_{1,74} = 0.11$, $p = 0.740$).

(b) *Cross-fostering experiment*

Although the manipulation of parental state did not affect egg mass, it could have altered egg composition independently of egg mass. We therefore cross-fostered all eggs, allowing us to detect and separate any differences between the effects of eggs (egg environment) and incubation conditions (incubation environment) on offspring phenotype. Nests were checked each morning, and all eggs were removed from the nest on the morning of laying. They were then exchanged between nests so that all pairs incubated a clutch that contained eggs laid by both LPS and HPS parents. Eggs laid by pairs from the two pre-breeding diet treatments were equally distributed between incubating foster parents from the two diet treatments ($n = 56$ offspring that went on to breed at least once, $\chi^2_1 = 2.53$, $p = 0.112$). The clutch size that each pair incubated was standardized to five eggs, so all pairs incubated the same number of eggs. In order to avoid confounding effects acting during prenatal (egg and incubation environment) and postnatal (nestling environment) development, hatchlings were also cross-fostered. Towards the end of the incubation period nests were checked for hatching three times per day in order to assign each chick to the egg it hatched from. Neonates were individually marked on their down using non-toxic coloured pens and exchanged between nests in the same way as at laying. Eggs laid by pairs from the two pre-breeding diet treatments were equally distributed between chick-rearing foster parents from the two diet treatments ($n = 56$, $\chi^2_1 = 0.08$, $p = 0.782$) and brood sizes were held constant between treatment groups. The size of clutches that produced offspring that went on to breed ($n = 32$ clutches) did not differ between replicates ($F_{1,29} = 0.0006$, $p = 0.986$) or diet treatments ($F_{1,30} = 0.07$, $p = 0.788$). Pairs never incubated or reared their own offspring and did not differ in reproductive effort in any of the developmental stages, and there was no bias in the order in which surviving offspring were exposed to LPS and HPS parents.

(c) *Measuring performance of the offspring generation*

Hatching mass was used as a general index for prenatal developmental conditions, as birth mass is commonly used as a measure for prenatal conditions (e.g. Desai & Hales 1997). Within hours of hatching, chicks were weighed to the nearest 0.1 g. As soon as the chicks were big enough, the colour mark was replaced with an individually numbered orange leg ring. We measured the body mass and skeletal size (tarsus length) of all surviving offspring at independence (two weeks after fledging), when they reached their adult size (Zann 1996). The offspring could easily be sexed on reaching independence owing to adult plumage dimorphism (Zann 1996) and those that died prior to maturity were sexed using molecular techniques (Griffiths *et al.* 1998). Offspring were kept in single-sex groups until sexual maturity (Zann 1996). To test for maternal effects on the offspring's reproductive performance, all surviving offspring ($n = 65$) were introduced to randomly assigned non-experimental partners from our breeding stock, given access to a nest-box and allowed to lay eggs. Experimental birds were paired up at four months old, after reaching sexual maturity at three months of age (Zann 1996), and again in their second year of life (with a different partner). Lifetime reproductive success in birds is mainly the product of clutch size and longevity. Although the actual lifespan of zebra finches in the wild is very short, the ability to breed at a relatively older age should be strongly selected for (Zann 1996). Reproductive performance is not thought to differ with age in zebra finches (Williams &

Table 1. Offspring mass and size at independence, as well as survival, in relation to egg, incubation and nestling environments. (Mixed model analysis on offspring survival (from hatching to independence and as an adult), body mass and tarsus length (at independence). Identity of the biological parents was included as a random factor, and there were significant differences between sibling groups for growth, but not survival. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Only significant interactions are shown; all other interactions $p > 0.074$.)

factor	nestling survival to independence ($n = 97$)	body weight (g) ($n = 70$)	tarsus length (mm) ($n = 70$)	survival as adult ($n = 67$)
identity of biological parents	$Z = 0.17$	$Z = 5.52^{***}$	$Z = 5.39^{***}$	$Z = 0.77$
replicate	$F_{1,86.5} = 9.40^{**}$	$F_{1,61} = 4.15^{*}$	$F_{1,58} = 5.32^{**}$	$F_{1,59.4} = 1.32$
egg environment	$F_{1,69.9} = 2.56$	$F_{1,59} = 1.19$	$F_{1,58} = 2.30$	$F_{1,62} = 1.04$
incubation environment	$F_{1,83} = 0.913$	$F_{1,60} = 1.70$	$F_{1,57} = 1.17$	$F_{1,55.8} = 0.96$
nestling environment	$F_{1,87} = 5.00^{*}$	$F_{1,57} = 0.14$	$F_{1,55} = 0.04$	$F_{1,58} = 0.10$
hatching mass	$F_{1,81.8} = 13.84^{***}$	$F_{1,58} = 1.05$	$F_{1,56} = 0.72$	$F_{1,55} = 0.14$
offspring sex	$F_{1,84.4} = 0.53$	$F_{1,56} = 0.00$	$F_{1,58} = 3.95$	$F_{1,63} = 6.01^{*}$
egg environment × offspring sex	—	—	$F_{1,58} = 6.32^{*}$	—

Christians 2003), so the timing of breeding in our study is unlikely to bias the results. We therefore recorded clutch size and survival over 2 years (one breeding attempt per year) to give us a measure of offspring fecundity, providing an approximation of their fitness.

(d) *Statistical analysis*

We analysed offspring phenotype in relation to the diet of the biological parent that produced the egg (egg environment), the diet of the foster parent that incubated them (incubation environment) and the diet of the foster parents that raised them (nestling environment) using general linear mixed models with the identity of the biological parents and the identity of the individual offspring (in analyses where individuals contributed more than one measure) as random effects. Because the experiment was replicated, we included replicate in the statistical analysis. Where there was a significant effect of replicate it was included in the final model for analysis. Initial models also tested for the effect of parental clutch size on offspring phenotype, but the effect of clutch size was not significant and was therefore not included in the final model. Analyses were carried out in SAS v. 8.2 using PROC MIXED for variables with a normal error distribution and GLIMMIX for those with a binomial distribution (survival and breeding propensity). Both models used the Satterthwaite correction for degrees of freedom. The structure of the full dataset on male reproductive output allowed for only the interaction between diet treatment and breeding attempt to be tested using a log likelihood test (Littell *et al.* 1996). All two-way interactions and main factors were included in the model and excluded stepwise where possible, and therefore degrees of freedom vary with the number of factors included in the model.

3. RESULTS

Hatchling mass was not affected by prenatal developmental conditions or offspring sex ($n = 68$ fledglings, identity of biological parent: $Z = 1.13$, $p = 0.129$; replicate: $F_{1,59.5} = 0.64$, $p = 0.429$; sex: $F_{1,55.4} = 0.07$, $p = 0.797$; incubation environment: $F_{1,62.6} = 0.18$, $p = 0.674$), but hatchlings from eggs laid by HPS parents tended to be heavier (1.00 ± 0.04 g, $n = 47$) than hatchlings from eggs laid by LPS parents (0.91 ± 0.05 g, $n = 21$; egg environment: $F_{1,23} = 3.29$, $p = 0.083$). In the second replicate, offspring were more likely to survive to independence, but were lighter and smaller at independence than in the first replicate (table 1). Nestling survival to independence did not depend on conditions during embryo development, but heavier hatchlings were more likely to reach independence ($p < 0.001$; see table 1). However, chicks raised by HPS foster parents during the nestling stage had a higher survival to independence (0.76 ± 0.05, $n = 46$) than chicks raised by LPS parents (0.59 ± 0.07, $n = 51$) ($p = 0.028$; table 1).

Offspring body weight at independence was not affected by conditions at any stage of development (table 1). The sexual size dimorphism in tarsus length, however, was affected by the egg environment. Among fully grown offspring that hatched from eggs laid by HPS parents there was no difference in tarsus length between the sexes, whereas among offspring hatching from eggs laid by LPS parents, daughters had 4.8% longer tarsi at independence than sons (egg environment × sex: $p = 0.015$; see table 1).

Conditions during prenatal development influenced the reproductive performances of male and female offspring differently in adult life. The probability of breeding ($70.8 \pm 4.4\%$, $n = 65$) did not differ between treatment groups ($p > 0.312$). The pre-breeding diet of the parent that laid the egg influenced the reproductive output of male offspring, irrespective of incubation and nestling environments. The parental pre-breeding diet affected the reproductive output of male offspring in their first year of breeding, but not in their second year (mixed model likelihood-ratio test, egg environment × breeding attempt: $\chi^2_1 = 4.5$, $p = 0.034$; figure 1). In the first breeding attempt, standard females who were mated to experimental male offspring who had hatched from eggs laid by HPS parents produced larger clutches in the first and second replicates (5.5 ± 0.5 eggs, $n = 4$and 5.5 ± 0.3 eggs, n = 4, respectively) than those whose mates had hatched from eggs laid by LPS parents (4.5 ± 0.5 eggs, $n = 2$ and 3.8 ± 0.5 eggs, $n = 4$) ($p = 0.010$). The fecundity of female offspring, by contrast, was affected by the pre-breeding diet of the incubating foster parent and this effect persisted for at least 2 years (figure 2). Female offspring incubated by HPS parents laid larger clutches in replicates 1 and 2 (6.2 ± 0.5 eggs, $n = 5$ and 5.2 ± 0.3 eggs, $n = 10$, respectively) than female offspring incubated by LPS parents (3.5 ± 0.6 eggs, $n = 6$ and 4.3 ± 0.4 eggs, $n = 9$) ($p = 0.004$).

The survival from independence to 2 years of age, however, was not influenced by the conditions the birds experienced during early development, but males had a higher survival (0.83 ± 0.06, $n = 35$) than females (0.56 ± 0.09, $n = 32$) ($p = 0.017$; table 1).

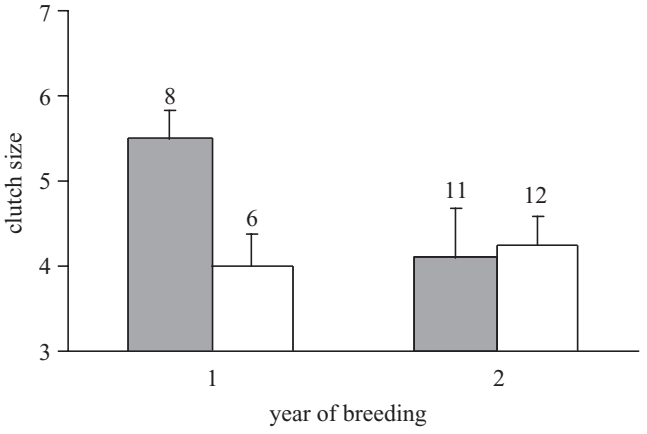

Figure 1. The reproductive performance (mean + s.e.) of male zebra finches was affected by the pre-breeding diet of the parent that produced the egg they hatched from. In the first year, females paired to males that hatched from eggs laid by parents on an HPS diet (shaded bars) produced larger clutches than females paired to males hatched from eggs laid by parents on an LPS diet (open bars) (egg environment: $F_{1,12} = 9.26$, $p = 0.010$; identity of male: $Z = 1.23$, $p = 0.108$; identity of biological parents: $Z = 0.33$, $p = 0.371$; replicate: $F_{1,8.6} = 0.06$, $p = 0.814$; incubation environment: $F_{1,11} = 2.50$, $p = 0.142$; nestling environment: $F_{1,11} = 2.25$, $p = 0.375$). The reproductive performance in the second year was not affected by developmental conditions ($p > 0.513$). All interactions, $p > 0.348$. The sample sizes are given above the error bars, and vary because not all birds bred in both years.

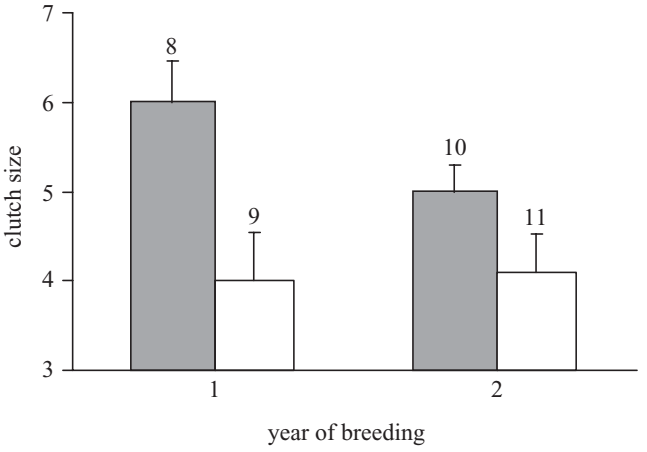

Figure 2. The reproductive performance (mean + s.e.) of female zebra finches was affected by the pre-breeding diet of the incubating foster parent. Females that were incubated by HPS parents (shaded bars) laid larger clutches in both breeding attempts than females incubated by LPS parents (open bars) (mixed model, incubation environment: $F_{1,25.7} = 10.39$, $p = 0.004$; identity of female: $Z = 0.47$, $p = 0.321$; identity of biological parents: $Z = 0.94$, $p = 0.175$; replicate: $F_{1,24.7} = 0.48$, $p = 0.496$; breeding year: $F_{1,14.6} = 1.97$, $p = 0.181$; egg environment: $F_{1,17.5} = 1.56$, $p = 0.228$; nestling environment: $F_{1,16.3} = 0.20$, $p = 0.662$). All interactions, $p > 0.103$. The sample sizes are given above the error bars, and vary because not all birds bred in both years.

4. DISCUSSION

The aim of the experiment presented here was to study the effects of parental state at the start of breeding on fitness-related variation in offspring phenotype. Provision of a high-quality protein supplement during a short period prior to breeding increased maternal body weight compared with provision of a protein-poor diet. As all birds were kept in an identical environment from the start of breeding onwards, any differences among offspring have to be a result of differences in maternal state at the start of breeding. Our results demonstrate a maternal effect, where differences in pre-breeding maternal nutritional state caused significant differences in offspring fecundity. We believe this to be a good approximation of fitness, as we found no differences in adult survival between offspring experiencing different developmental conditions. Cross-fostering allowed us to separate the effects of prenatal (egg and incubation) and postnatal developmental conditions on offspring phenotype. Poor maternal state at the start of breeding affected the females' ability to produce and incubate eggs, giving rise to offspring with low fecundity. It also affected their ability to rear chicks up to fledging. We found sex differences in the effects of developmental conditions on body size and fecundity, and therefore the sexes differed in their vulnerability to poor prenatal developmental conditions. These results were evident in the first replicate and were confirmed by the second replicate. Although we tried to keep everything as standardized as possible, fledging success and fledgling size differed between replicates. All interactions between developmental conditions and replicate, however, were non-significant, suggesting that in all cases the effects of diet treatment did not differ between replicates.

The parents' nutritional state is likely to have influenced their ability and/or willingness to provide parental care, affecting developmental conditions for the offspring. Although the diet treatment significantly affected only female body mass, it is the female who invests in egg production, and the female invests more heavily than the male in incubation (Zann 1996; Gorman & Nager 2003). Manipulation of the female's nutritional state during egg production can affect resource allocation into egg production in zebra finches (reviewed in Houston 1998). We found no differences in egg size between diet treatments, possibly because we manipulated the clutch size. Because several egg components that can affect offspring performance vary independently of egg size (Nager *et al.* 2000; Blount *et al.* 2002; Royle *et al.* 2003), egg size may not be a good indicator of egg quality, i.e. the probability that eggs give rise to surviving and fecund offspring. The results from our experiment suggest that maternal state influenced a bird's ability to produce high-quality eggs, irrespective of egg size. Zebra finches are size monomorphic (Zann 1996), but parents on the protein-poor diet laid eggs that gave rise to males that were skeletally smaller (tarsus length) at independence than females. This suggests that the skeletal size of male zebra finches is more vulnerable to poor prenatal developmental conditions than is the female skeletal size, whereas females are usually more vulnerable than males to poor postnatal developmental conditions (Martins 2004).

Parents on the protein-poor diet laid eggs that also produced male offspring with a lower reproductive output than male offspring produced by parents on the protein-rich diet. As a possible explanation we suggest that prenatal developmental conditions might have affected male offspring's sexual attractiveness, and female zebra finches paired to less attractive males lay smaller clutches (Balzer & Williams

1998). Male sexual attractiveness in zebra finches is a multicomponent signal (Collins *et al.* 1994) that can be affected by postnatal developmental conditions (de Kogel & Prijs 1996; Blount *et al.* 2003; but see Birkhead *et al.* 1999). If sexual attractiveness is influenced by both current and past conditions (Scheuber *et al.* 2003), then the standard maintenance conditions could, with time, override the effect of prenatal developmental conditions and this could explain why the maternal effect on male fecundity did not persist beyond the first breeding attempt. Future tests will need to assess directly the effect of egg quality on male attractiveness using mate-choice tests starting early in the male's life.

The results also showed that the nutritional state of the incubating foster parent affected the fecundity of female offspring for at least 2 years. We have previously shown that, in zebra finches, parental state influences their incubation behaviour (Gorman & Nager 2003). Females do most of the incubation, and all females increased the length of their incubation bouts as incubation progressed, but this change occurred earlier in HPS females than in LPS females. We measured incubation behaviour only in the first replicate (Gorman & Nager 2003), and the effect of the nutritional state of the incubating foster parent on the fecundity of daughters was significant (albeit with a small sample size) in the first replicate alone. Because there was no statistically significant interaction between replicate and incubation environment, this suggests that the observed effects of incubation conditions on female fecundity did not differ in the second replicate. It is possible that these differences in incubation behaviour might have lead to differences in the thermal environment experienced by the embryo. It is not clear whether the thermal environment has a direct effect or acts on the embryo indirectly by altering nutrient allocation to the developing embryo. Differences in incubation conditions can have consequences for embryo survival and development (Webb 1987). In Japanese quail (*Coturnix coturnix japonica*), variation in incubation conditions resulted in differences in ovary size (Callebaut 1991), which could explain differences in reproductive capacity.

In our experimental design, owing to the time elapsed since the diet treatment ended, differences in maternal state during chick rearing might have been small, and body weight at the end of incubation converged in the two treatment groups (Gorman & Nager 2003). Pre-breeding diet treatment, however, affected the parent's ability to raise young to independence. Young raised by a foster parent that had been on a protein-rich pre-breeding diet had higher nestling survival than young raised by parents that had been on a protein-poor diet before breeding, independent of the higher nestling survival in the second replicate. This supports the suggestion that, in zebra finches, differences in parental state can influence breeding effort for several weeks after the end of the treatment (Williams 1996).

The physiological mechanisms through which developmental conditions affect the developing offspring are complex and still poorly understood (Waterland & Garza 1999; Harding 2001; Rhind *et al.* 2001). Desai & Hales (1997) suggested that poor prenatal developmental conditions lead to a reduction in the growth of the embryo with consequences for the organism later in life. It has been suggested that postnatal growth also affects fecundity in zebra finches (Haywood & Perrins 1992; but see Williams & Christians 2003). We found a non-significant trend in which parents on a protein-rich diet laid eggs that produced offspring that were 9.9% heavier at hatching than offspring produced by parents on a protein-poor diet. However, because of the large measurement intervals relative to the range of data, small differences in hatching mass were difficult to detect. Lower hatching weight per se, or compensatory growth after hatching (Metcalfe & Monaghan 2001) to catch up with offspring produced by parents on a protein-rich diet, could have affected the development of skeletal size and structures involved in signalling male sexual attractiveness. The incubation environment, however, did not influence hatchling weight and hence hatching weight cannot explain the effects of incubation environment on female fecundity. Variation in developmental conditions could instead have acted through changes in the offspring's hypothalamic–pituitary–gonadal axis (Davies & Norman 2002) to cause a permanent modification of the female's reproductive organs during development. Our results also suggest that sex-specific vulnerability to poor developmental conditions may differ between stages of development. In our particular experimental design, however, we potentially affected the fitness of both sexes, which might explain the absence of a biased offspring sex ratio in this study (H. E. Gorman, unpublished data).

Previous studies focused on trade-offs between current chick-rearing effort, future reproduction and offspring fitness, but neglected the costs of egg production and incubation (Monaghan & Nager 1997). More recent evidence also demonstrates that reproductive effort during egg formation and incubation can cause intra-individual trade-offs (Nager *et al.* 2001; Visser & Lessells 2001) and inter-generational trade-offs (e.g. Nager *et al.* 2000). While incubation has been shown to be energetically costly (Tinbergen & Williams 2002), and variation in incubation effort can have effects on hatchlings (Reid *et al.* 2002; Larsen *et al.* 2003), here we also show that changes in incubation effort can have substantial effects on the potential fitness of the offspring. Such inter-generational maternal effects represent another cost that may influence parental-care decisions: the production of poor-quality offspring is costly both to the parents and to the offspring themselves.

It is clear from our observations that we need to include variation in pre- and postnatal developmental conditions into future models of natural and sexual selection to understand the observed variation in life-history traits. As the latency period of these effects is long, we require studies across the entire life course of an individual. As the mechanisms by which developmental conditions affect offspring quality and the stage of development at which they act can differ between the sexes, the fitness benefits of producing male and female offspring differ under specific developmental conditions. Therefore sex-difference in vulnerability to poor pre- and postnatal developmental conditions also needs to be considered in the understanding of adjustments of offspring sex ratio.

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