Maternal developmental stress reduces reproductive success of female offspring in zebra finches

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Environmental factors play a key role in the expression of phenotypic traits and life-history decisions, specifically when they act during early development. In birds, brood size is a main environmental factor affecting development. Experimental manipulation of brood sizes can result in reduced offspring condition, indicating that developmental deficits in enlarged broods have consequences within the affected generation. Yet, it is unclear whether stress during early development can have fitness consequences projecting into the next generation. To study such trans-generational fitness effects, we bred female zebra finches, *Taeniopygia guttata*, whose mothers had been raised in different experimental brood sizes. We found that adult females were increasingly smaller with increasing experimental brood size in which their mother had been raised. Furthermore, reproductive success at hatching and fledging covaried negatively with the experimental brood size in which their mothers were raised. These results illustrate that early developmental stress can have long-lasting effects affecting reproductive success of future generations. Such trans-generational effects can be life-history responses adapted to environmental conditions experienced early in life.

Keywords: early developmental stress; maternal effects; thrifty phenotype; trans-generational fitness effects; zebra finch

1. INTRODUCTION

A central goal of evolutionary biology is to understand the mechanisms underlying phenotypic traits and how they are affected by genetic and environmental factors (Stearns 1992; Mousseau *et al.* 2000). Studies in plants (Donohue & Schmitt 1998) and invertebrates (Fox & Mousseau 1998) show that the environment in which the individual develops can have sustained effects on phenotypes across several generations. Recent research focusing on maternal effects and nutritional programming in vertebrates has provided new insights into how the maternal environment determines the condition and life of the offspring. Thus, it is well documented that the conditions experienced during early development can significantly affect physiology, behaviour and reproductive performance later in life (Clark & Galef 1998; Lindström 1999; Metcalfe & Monaghan 2001; Qvarnström & Price 2001; Strasser & Schwabl 2004). In mammals (Huck *et al.* 1986, 1987; Albon *et al.* 1987), including humans (Lummaa & Clutton-Brock 2002; Bateson *et al.* 2004), the conditions of the mother during embryonic development and lactation can have strong and long-term effects on the offspring. In humans, studies have shown that a mother's condition, as determined by food deprivation (i.e. famine) during pregnancy and lactation, has strong long-term effects on offspring development and reproduction (Lummaa & Clutton-Brock 2002). Such maternal effects in mammals where the offspring develops within

the mother and subsequently depends on her milk can be explained at least in part by the direct physiological connection between mother and offspring. Such effects can be adaptive, if the offspring is programmed to cope as an adult with environments characterized by poor nutrition, for instance by reducing body size, metabolic rate and activity. This suite of modifications has been included within the term 'thrifty phenotype' (Hales & Barker 1992; Wells 2003; Bateson *et al.* 2004; Bateson 2005; Rhodes 2005).

Birds are well-established model systems to study the effects of environmental conditions during different developmental stages (Price 1998). In order to study the consequences of stress during post-embryonic development, i.e. after hatching, manipulations of brood size have been a powerful tool to manipulate nestling condition. Numerous studies have shown that birds being raised in enlarged broods have reduced growth, condition (Tinbergen & Boerlijst 1990; Birkhead *et al.* 1999; Brinkhof *et al.* 1999; Naguib *et al.* 2004), survival (Dijkstra *et al.* 1990; de Kogel 1997) and recruitment rates after migration (Gustafsson & Sutherland 1988; Smith *et al.* 1989). Yet the long-term fitness effects of the environmental conditions experienced during this early developmental period are not well known. We have shown previously in zebra finches, *Taeniopygia guttata*, that effects of developmental stress on offspring affect their reproductive investment. Females decreased their investment of testosterone into their eggs with increasing level of stress experienced

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as nestlings (Gil *et al.* 2004) and the body size of their offspring was negatively affected by the level of early developmental stress experienced (Naguib & Gil 2005). Specifically, female offspring were increasingly smaller with increasing experimental brood size in which their mothers were raised. This raises the question of whether or not these females after having reached sexual maturity differ in their reproductive success, or compensate in their reproduction for negative conditions experienced by their mother during early development.

Here, we report a breeding experiment with zebra finches in which we followed effects of brood size manipulation within the natural level of variation over two generations. Through cross-fostering, we imposed different degrees of developmental stress on nestlings by creating broods ranging from two to six nestlings. We followed development of these nestlings into adulthood (Naguib *et al.* 2004) and subsequently allowed the female offspring to breed in order to assess trans-generational effects of early developmental stress on reproductive success.

2. MATERIAL AND METHODS

(a) *Breeding of the first generation*

We conducted the experiment on zebra finches of wild Australian origin at the University of Bielefeld, Germany, from 2001 to 2005. The daughters from females that originated from an experiment conducted in 2001 were used for breeding in the experiments reported here. In 2001, we conducted a cross-fostering experiment in which we raised zebra finches in different experimental brood sizes within the natural range. Experimental broods consisted of two to six cross-fostered chicks originating from one to four different broods. Thus, the experimental brood sizes were within the natural range of brood sizes in wild (Zann 1996) and laboratory populations (Naguib *et al.* 2004). Broods were divided into three experimental groups: (i) small broods (two to three nestlings); (ii) medium broods (four nestlings) and (iii) large broods (five to six nestlings). The methods are described in detail in a previous paper in which we show that experimental manipulation affected nestling growth, levels of plasma testosterone and components of immunocompetence, as well as adult body size and body condition (Naguib *et al.* 2004). The females raised in the different experimental brood sizes were allowed to breed in 2003 and we showed in a previous paper that the nestlings they produced were increasingly smaller with increasing experimental brood size in which the breeding females' mothers were raised (Naguib & Gil 2005).

(b) *Breeding of the second generation*

In the experiment reported here, we used the female offspring that hatched in 2003 as breeding stock, i.e. the daughters from the females that had been raised in different experimental brood sizes. The females that we used for breeding are the F₅ generation of wild zebra finches raised in our lab. Each of the mothers from these females was raised in a different experimental brood. The breeding experiment with this second generation was conducted from September to December in 2004. The females were paired with unrelated males that had been raised in non-manipulated broods. Females used for

breeding ($n=36$) originated from 18 different broods and were 18 months old when breeding commenced. Overall, we used 17 females whose mothers were raised in small experimental broods (eight small broods), 10 females whose mothers were raised in medium experimental broods (six medium broods) and 9 females whose mothers were raised in large experimental broods (four large broods). Pairs were kept in individual cages (83×30×40 cm) and were supplied daily with a variety of both dried and germinated seeds, egg and fresh water (plus additional vitamins three times a week). Each cage was provided with a wooden nest box attached to the side (12.5×12×14 cm) and with coconut fibres on the floor to be used as nesting material. Coconut fibres were supplied daily during nest building. Rooms had a temperature of 25 °C and a L/D regime of 16:8 h, with light on at 06.00 h. Egg laying and hatching dates were recorded daily between 11.00 and 13.00 h. The offspring were kept with their parents until day 35 (nutritional independence) when they were transferred in groups with song tutors in order to allow them to socialize, to learn song and to develop song preferences. Out of 36 breeding pairs, in seven pairs males did not build a nest and females laid no eggs. Zebra finch females rarely lay eggs when males do not build nests so that we excluded these pairs from the analysis. Pairs in which males built a nest but in which females did not lay eggs ($n=4$), however, were included in the analysis. Final sample size in the analysis consisted of 13 females whose mothers were raised in small experimental broods (seven small broods), 8 females whose mothers were raised in medium experimental broods (four medium broods) and 8 females whose mothers were raised in large experimental broods (four large broods).

(c) *Data collection and analysis*

Biometric measurements were taken when the females were introduced to the breeding cages. Body mass was taken with a Pesola scale to the nearest 0.5 g. Tarsus length was measured with callipers to the nearest 0.01 mm and wing length with a ruler (flattened wing) to the nearest 1 mm. Nests were then checked daily for egg laying and for presence of hatchlings. Hatchling body mass was taken with an electronic scale (Sartorius PT120) to the nearest 0.01 g.

We analysed the data using general linear models (GLM) in SPSS 12. Experimental brood size (small, medium or large brood) in which the breeding females' mothers were raised was declared as a fixed factor. Sibling females in all analyses were nested within their mother in order to correct for genetic effects and their shared early environment. Biometric traits of females were used as covariates in the initial model, but removed for the final model, as none of the variables was significant in the model. We corrected p values for the expected ordered heterogeneity of the experimental groups, but used two-tailed prediction tables (Rice & Gaines 1994). Number of fledglings was tested non-parametrically using a Kruskal–Wallis ANOVA as the data strongly deviated from a normal distribution even after transformation.

3. RESULTS

The results show that the biometry of adult females was significantly affected by the experimental brood size in which their mothers were raised ($F_{6,32}=2.97$, $p<0.0004$;

Figure 1. Effects of the experimental brood size in which females were raised on tarsus length and wing length (mean $\pm$ s.e.) of her adult daughters, i.e. the females that were used for breeding in the experiments reported here. The data on female biometry presented in a previous paper (Naguib & Gil 2005) showed biometry of the females when they reached nutritional independence so that the data shown here indicate that females subsequently did not compensate for reduced early growth.

nested multivariate GLM). Subsequent nested univariate analyses revealed that females had increasingly shorter tarsi, shorter wings and lower weight with increasing brood size in which their mother was raised (tarsus length, $F_{2,18}=2.65$, $p<0.04$; wing length, $F_{2,18}=2.61$, $p<0.04$; weight, $F_{2,18}=2.93$, $p<0.04$; GLM; figure 1).

In their first breeding opportunity, female reproduction was significantly affected by the experimental brood size in which their mother was raised ($F_{6,24}=2.53$, $p<0.001$; nested multivariate GLM). Subsequent nested univariate analyses revealed that females produced clutches with lower hatching success ($F_{2,14}=10.12$, $p<0.0001$; figure 2a) and produced significantly fewer hatchlings with increasing level of early developmental stress their mother had experienced ($F_{2,14}=6.85$, $p<0.0001$; figure 2b), although the number of eggs laid remained unaffected ($F_{2,14}=0.12$, $p>0.4$). Furthermore, the number of fledglings decreased significantly with increasing brood size in which the females' mother was raised (Kruskal–Wallis ANOVA, $\chi^2=6.118$, $n=29$, $p=0.047$; figure 2c). Overall, the effect of maternal brood size on reproductive success of daughters (number of hatchlings and fledglings) was most pronounced for females whose mother had been raised in large broods (figure 2b,c). No female whose mother had been raised in large experimental broods produced any surviving offspring in this first breeding attempt (figure 2c).

Figure 2. Relation between the experimental brood size in which females were raised and reproductive traits of their daughters in their first breeding attempt, (a) percentage of eggs that hatched, (b) number of hatchlings and (c) number of fledglings (mean $\pm$ s.e.).

4. DISCUSSION

The breeding success of females whose mothers had been raised in different experimental brood sizes show that early developmental stress can have long-term fitness consequences by affecting the reproductive success of the next generation. These results expand on previous findings in which we showed that early developmental stress can have trans-generational body size effects, i.e. female offspring being increasingly lighter with increasing experimental brood size in which their mother was raised (Naguib & Gil 2005). The non-genetically transmitted phenotypic effects are particularly interesting, as females apparently did not compensate for the stress their mothers had experienced during their own period of nutritional dependence in the first month of life. The lack of any reproductive success by the females whose mothers had been raised in large experimental broods, i.e. experienced the highest level of stress, appears unexpected given that the levels of stress were well within a natural range and the birds were under *ad libitum* food condition in captivity. However, this was the first breeding attempt by these females and songbirds

are often less successful when breeding the first time. Thus, at subsequent breeding attempts these females might eventually be more successful, and compensate for earlier reproductive failures. In natural conditions, however, in which environmental conditions and lifespan are more unpredictable, failing to breed at the first opportunity is likely to result in substantially reduced fitness. In addition to a possible different investment made by females of different quality, males may have also modified their investment in response to the quality of their mates and thus might have invested less in raising young when females were of lower quality (Jones *et al.* 2001).

The findings reported here are in line with previous studies on birds and mammals that have shown long-term fitness effects of early developmental stress. In pied flycatchers, *Ficedula albicollis*, experimental increases in brood size resulted in fewer recruits to the breeding population, i.e. fewer offspring returning to the breeding population after migration (Gustafsson & Sutherland 1988). Thus, the fitness effects of early developmental stress imposed by brood size manipulation appear to be mainly mediated by survival of the offspring. In our experiment, we show that fitness effects directly act on the surviving offsprings' reproductive performance, reflecting selection on fecundity rather than on viability. Thus, early developmental stress experienced by females seemed to have a direct effect on maternal investment in the first breeding attempt by their daughters. These effects appeared to be reflected on two different levels of reproductive investment. The reduced hatching success in broods by females whose mother had experienced early developmental stress may reflect lower investment into the egg or in incubation. The fact that those same females failed to produce any surviving offspring indicates also a deficit on a different level of maternal investment since these females (and their mates) presumably failed to provide sufficient brood care. Regardless of the cause of reproductive failure, in both cases females made an investment that involved zero fitness. Whether or not experience gained during such unsuccessful breeding attempts is beneficial in future effective reproduction is a possibility that remains to be shown.

In a previous study, we found that yolk testosterone levels decreased with increased brood size experienced by females (Gil *et al.* 2004). Since this maternal effect can play a role in the programming of phenotypes (Gil 2003; Groothuis *et al.* 2005), it is conceivable that the females we used for breeding in the experiment reported here may have been programmed differently in the pre-hatching environment by means of different levels of yolk androgens. A non-exclusive alternative is that females may have received different intensity of parental care, depending on the environment in which their mother was raised, so that the effects on reproduction shown here are causally linked to the parental care that females had received as nestlings.

Maternal effects can be long-lasting as shown also by those studies on humans and rodents that have revealed long-term physiological effects in offspring raised in poor conditions (Gluckmann & Manson 2004). This body of literature shows that individuals raised under nutritional shortages not only can be better adapted to poor environments at later stages in life (thrifty phenotypes) but also suffer when environmental conditions become

affluent. For instance, humans that live in affluent nutritional conditions are more likely to suffer from diabetes if they were previously raised under nutritionally poor conditions (Hales & Barker 1992; Rhodes 2005). In hamsters, *Mesocricetus auratus*, it has been shown that food restriction over the two first months of life (during and beyond lactation) leads to reduced reproduction (Huck *et al.* 1986) whereas restriction after lactation, i.e. after the physiological link has terminated, did not have such substantial effects. Our study on birds emphasizes that also those environmental conditions during early post-embryonic development that are not linked directly to the mother's physiology as in mammals are crucial in affecting phenotypic and life-history traits even across generations. A prediction from the thrifty hypothesis that remains to be tested is whether those females raised under high levels of developmental stress would have done better under restricted feeding conditions than under the *ad libitum* conditions of our experiment.

Such long-term trans-generational effects can be adaptive life-history responses. Females whose mothers had experienced poor conditions during development may increase fitness by either producing fewer offspring or by delaying reproduction when their current condition at the first breeding attempt turns out to be not sufficient to deal with increased costs of raising young.

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