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Transgenerational body size effects caused by early developmental stress in zebra finches

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The nutritional and social conditions that individuals experience during early development can have profound effects on their morphology, physiology, behaviour and life history. Experimental increases in brood size in birds can result in reduced offspring condition and survival, indicating that developmental deficits in enlarged broods have negative fitness consequences within the affected generation. To study long-term effects (i.e. transgenerational effects of developmental stress), we conducted a two-step breeding experiment in which we manipulated early developmental conditions in zebra finches *Taeniopygia guttata*. We raised zebra finches by manipulating brood sizes and controlled for maternal and genetic effects by cross-fostering. In a previous study, we showed that offspring condition and body size decreased with increasing brood size. Here we show that this effect was carried over to the next generation. Body size in nestlings and at nutritional independence was affected by the brood size in which the mothers were raised. Female offspring did significantly worse than male offspring when the mother had been raised in large broods, suggesting sex-specific influences of maternal effects. These findings directly link early developmental stress in females via maternal effects to the phenotype of the next generation.

Keywords: cross-fostering; early developmental stress; life history; maternal effects; zebra finch

1. INTRODUCTION

Conditions experienced during early development have been shown to affect reproductive performance later in life (Lindström 1999; Metcalfe & Monaghan 2001). Experimental manipulations of brood size in birds have been a powerful tool to manipulate nestling condition through developmental stress as birds being raised in enlarged broods have reduced growth, condition (Tinbergen & Boerlijst 1990; Brinkhof *et al.* 1999; Naguib *et al.* 2004), survival (de Kogel 1997) and recruitment rates after migration (Gustafsson & Sutherland 1988; Smith *et al.* 1989). However, in contrast to mammals (Huck *et al.* 1987) and insects (Fox & Mousseau 1998), in birds the extent to which these effects of developmental stress on offspring are projected into future generations is still not known. Given that effects of nutritional stress have been shown to be sex specific, with females being affected more than males (Kilner 1998; Martins 2004), we were also interested in determining whether or not these sex-specific effects would project into the next generation. Here we report a two-step breeding experiment in which we followed effects of brood size manipulation 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 the development of these nestlings into adulthood (Naguib *et al.* 2004) and allowed the females that were raised in these broods to breed in order to assess long-term effects of the early developmental conditions of females on their own offspring.

2. METHODS

(a) *Material and initial breeding methods*

We conducted the experiment on zebra finches of wild Australian origin at the University Bielefeld in 2003. Females that originated from a cross-fostering experiment in which we raised zebra finches in different brood sizes (Naguib *et al.* 2004) were used for breeding. Experimental broods were divided into three groups: small (two to three nestlings), medium (four nestlings) and large broods (five to six nestlings). The methods are described in detail in a previous paper (Naguib *et al.* 2004) in which we show that experimental manipulation affected nestling growth, testosterone and immunocompetence levels, as well as adult body size and body condition, irrespective of nestling sex.

(b) *Breeding of the second generation*

Female offspring from the above experiment were randomly assigned for breeding to unrelated males that had been raised in non-manipulated broods (12 females from small manipulated broods; 19 females from medium broods; 16 females from large broods). Pairs were supplied daily with dried and germinated seeds and fresh water (plus vitamins). Air temperature was 23 °C and the light/dark regime of 16 : 8 h. Offspring were kept with their parents until nutritional independence (day 40). Out of 47 females, 13 females did not reproduce (five females laid no egg; eight females produced no hatchling), and 13 did not produce surviving hatchlings. Although some females do not breed, partly owing to males failing to build a nest, the proportions of unsuccessful broods were higher than in previous experiments using the same conditions. However, the proportions of non-reproducing females were not affected by the brood size in which the females were raised (logistic regressions, both $\chi^2_1 < 0.12$, both $p > 0.5$), and thus do not bias the results reported here. The resulting sample sizes with respect to brood sizes in which the successfully breeding females were raised were: small brood ($n=6$), medium brood ($n=8$), and large brood ($n=7$). The sex ratio of offspring originating from these females was: small broods (5 males and 6 females in total), medium broods (12 males and 8 females in total), large broods (12 males and 8 females in total).

Biometric measurements were taken at hatching (day 1), 15 days post-hatching (shortly before fledging), and at 40 days (feeding independence).

(c) *Statistical analysis*

We analysed the data using mixed lineal models using Proc Mixed (SAS v. 8.1). Experimental brood size in which the breeding females were raised was declared as a fixed factor. To control for random effects of females sharing a common environment as nestlings or being genetically related, data were first cross-classified by entering as random factors the original and experimental broods where females had been raised. Using this procedure, we controlled the effect of females sharing a common rearing environment (i.e. were raised in the same nest). None of these factors explained a significant part of the variance and thus were removed from the models. Individual data were cross-classified within females, and the effect in this case was always highly statistically significant (all $p < 0.001$). Therefore, we conserved female as a random factor. This method effectively removes the problem of non-independence of individual data by calculating their similarity and removing its

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effect in the model. Degrees of freedom were calculated using Satterthwaite's formula (Littell *et al.* 1996). The GLM does not compare sexes within each brood but compares the averages for the sexes within each category of brood size. We corrected p values for the expected ordered heterogeneity of the experimental groups but used two-tail prediction tables (Rice & Gaines 1994).

3. RESULTS

The experimental brood size in which a breeding female was raised had no effect on latency to lay the first egg ($F_{2,44}=0.09$, $p=0.90$) and on measures of quantity such as clutch size, brood size or number of fledglings (all $F_{2,44}<0.5$, all $p>0.6$). Brood size was 3.3±0.3 (mean±s.e.) with 52 fledglings (29 males and 23 females). Tarsus length of breeding females had a positive effect on offspring weight at the day of hatching but not thereafter (i.e. once parental care had commenced; table 1). After hatching, the experimental brood size in which a female was raised had significant and sex-dependent effects on offspring biometry (table 1). Interactions between the brood size in which the mother was raised and offspring sex were significant in measures taken before fledging (day 15) and at nutritional independence (day 40), showing that females were specially negatively affected with increasing experimental brood size in which their mother was raised (table 1, figure 1). In other words, female offspring grew less than males when their mothers were raised in a large experimental brood, but more than males when their mother was raised in a small brood. Measures of offspring biometry at days 15 and 40, except wing length, correlated significantly with each other (tarsus length: $r=0.51$, $n=68$, $p<0.001$; weight: $r=0.69$, $n=68$, $p<0.001$; wing length: $r=0.06$, $n=66$).

Table 1. Results from GLM analysis on factors affecting biometric variables of the offspring in first broods of females that were cross-fostered and raised in different experimental brood sizes. (Table shows *F*-values and significance values $^{\dagger}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; n.s. is given for variables that were not significant in the initial analysis and thus dropped for the final model; degrees of freedom were calculated by the Satterthwaite method to correct for the random factor of brood.)

	body mass	tarsus	wing length
day 1			
brood size in which mother was raised	$0.5_{2,19.2}$	not measured	not measured
female tarsus length	$6.01_{1,17}{}^{*}$		
day 15 (shortly before fledging)			
brood size in which mother was raised	$0.09_{2,17.9}$	$1.13_{2,17.8}$	$0.98_{2,15}$
sex	n.s.	$1.38_{1,41.7}$	n.s.
mother brood size × sex	n.s.	$3.97_{2,41}{}^{*}$	n.s.
day 40 (independence)			
brood size in which mother was raised	$0.83_{2,15}$	$8.56_{2,14.4}{}^{**}$	$1.28_{2,14.3}$
sex	n.s.	$0.63_{1,45}$	$4.70_{1,41.3}{}^{*}$
mother brood size × sex	n.s.	$7.73_{2,44.6}{}^{**}$	$10.53_{2,40.2}{}^{***}$

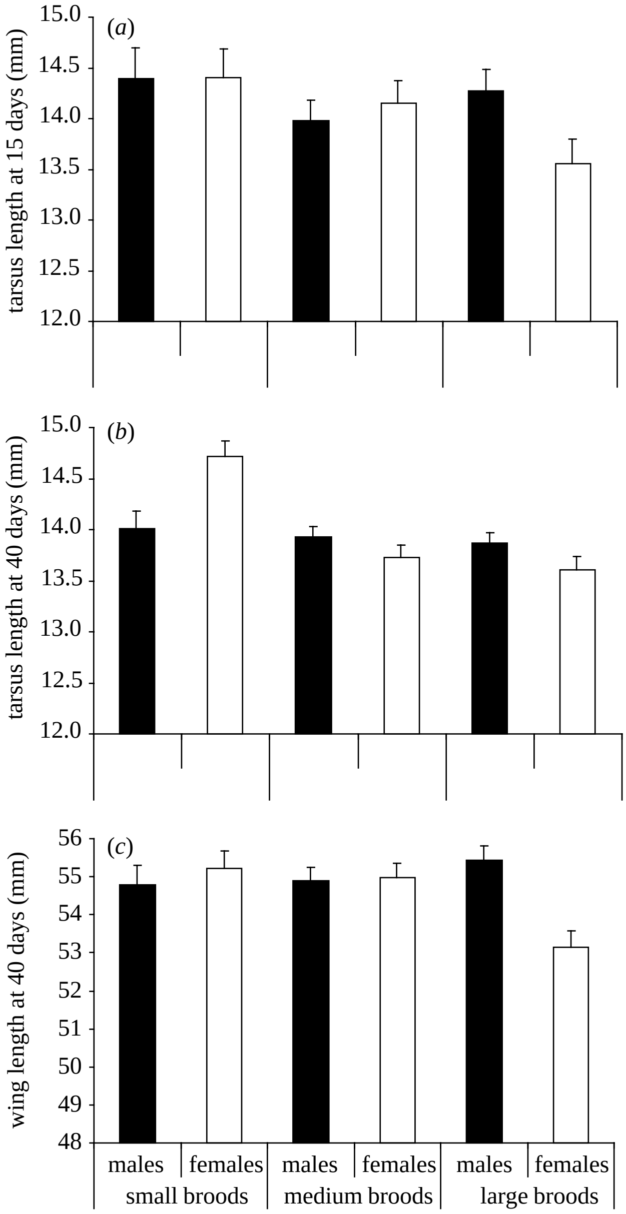

Figure 1. Relation between brood size in which the mother was raised and biometric traits of her male and female offspring, (*a*) tarsus length at day 15 (shortly before fledging), (*b*) tarsus length at day 40 (nutritional independence), (*c*) wing length at day 40. Shown are means±s.e. Only the females tend to do worse with increasing brood size.

4. DISCUSSION

The experiments show that effects of early developmental stress experienced by females project into the subsequent generation, i.e. their offspring. Previous studies of birds showed effects of such early developmental stress on an individual's phenotype (de Kogel & Prijs 1996; Nowicki *et al.* 2000; Spencer *et al.* 2003; Naguib *et al.* 2004) and survival (de Kogel 1997), but it has remained unclear whether or not such effects have further consequences on the subsequent offspring. Experiments in collared flycatchers *Ficedula albicollis* (Gustafsson & Sutherland 1988) and great tits *Parus major* (Smith *et al.* 1989) so far showed that individuals had lower return rates when being raised in enlarged broods, indicating that early development stress can affect reproductive potential. Our findings that offspring were increasingly lighter with increasing early developmental stress experienced by the mother expands on these findings and show transgenerational long-term consequences. Although females compensated for early developmental stress in several reproductive parameters such as time to egg laying, clutch size and brood size, the compensation was not complete once they started to provide parental care. Raising offspring with reduced body size may be advantageous for females coming from enlarged broods if this represents a reduction in the cost of parental care. Reduced body size, on the other hand, has been shown to be strongly selected against in field studies (Boag & Grant 1981; Alatalo *et al.* 1990) so that reduced parental effort may directly benefit the female with costs being paid by the young. It remains to be seen if these effects on offspring have sustained fitness effects. Interestingly, effects on body mass and size were strongly sex biased, with females doing worse than males when their mothers were reared in large experimental broods. The findings are consistent with previous evidence that female nestlings are more vulnerable to nutritional stress (Bradbury & Blakey 1998; Kilner 1998; Martins 2004; Rutstein *et al.* 2004) and show that such effects can even last into the next generation (also see Gorman & Nager 2004). As females raised in enlarged broods produce eggs with lower testosterone levels (Gil *et al.* 2004), it may be that our present findings were mediated through this mechanism, together with a possibly differential effect of yolk androgen in male and female embryos (Gil 2003). Future research needs to address whether the effects projecting into the generation of the offspring's offspring, are adaptations by low quality females to invest more in the sex with larger reproductive potential.

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