

Early Developmental Conditions and Male Attractiveness in Zebra Finches

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Abstract

Traits used in mate choice are often costly to produce or maintain, and thus can reflect an individual's current condition. Mate choice, however, might not only be influenced by the current condition of a potential partner, but also by the condition it had experienced during its early development which can have strong and long-lasting effects on various traits. Here we studied the effects of different early developmental conditions, imposed by brood size manipulations (small, medium and large broods), on male attractiveness as measured by female choice experiments in zebra finches (*Taeniopygia guttata*). In three different experiments, we allowed females to choose between males that had been raised in different experimental brood sizes. In none of the experiments, females showed a significant preference for males which had experienced better developmental conditions, i.e. were raised in the relatively smaller experimental broods. Song rate was higher in males coming from small than large broods, but females did not prefer males that sang more. These results suggest that sexual attractiveness either was not affected by our experimental treatment or that males subsequently had compensated in their overall attractiveness for negative effects of early developmental conditions.

Introduction

The environmental conditions that individuals experience during early development have marked effects on their development, behavior, reproduction, life history and survival (Lindström 1999; Metcalfe & Monaghan 2001; Lummaa & Clutton-Brock 2002). Such effects could result from nutritional stress (Nowicki et al. 1998; Buchanan 2000), maternal effects (Fox & Mousseau 1998; Groothuis et al. 2005), parasite infections (Fitze et al. 2004) or from social sibling competition (Neuenschwander et al. 2003). If these conditions have long-term negative effects on the offspring's phenotype, early developmental stress may result in reduced sexual

attractiveness, as the development of costly traits is expected to be traded-off with other costly energetic requirements. A number of studies in birds have shown that the expression of sexually selected traits, such as song (de Kogel & Pijls 1996; Nowicki et al. 2000, 2002; Spencer et al. 2003a,b; but see Gil et al. 2006) or plumage (Ohlsson et al. 2002; Naguib & Nemitz 2007) can be strongly influenced by the environmental condition during development. Given these effects, females that choose males on the basis of these traits would choose males with a better developmental history (Spencer et al. 2005; Naguib & Nemitz 2007), and thus presumably males of higher quality. A previous mate choice experiment on zebra finches (*Taeniopygia*

guttata) indeed showed that early developmental stress as imposed by brood size manipulations can affect male attractiveness (de Kogel & Prijs 1996).

Here, we allowed non-domesticated female zebra finches to choose between males that were raised and kept in different experimental brood sizes until day 35, i.e. until nutritional independence (Naguib et al. 2004). The experiment differs from that by de Kogel & Prijs (1996) in that in their experiments subjects remained in their experimental groups until day 50, i.e. until sexually selected plumage ornaments were well developed, whereas in our experiment treatments terminated at day 35, i.e. before development of plumage ornaments. Moreover, in our experimental set-up for the choice experiments, males were prevented from seeing each other and females were impaired from seeing the other male when being in the preference zone of the chosen male, whereas in de Kogel's experiment all birds were continuously in visual contact with each other (see Discussion for details). The subjects of our experiments stemmed from an experiment in which early development was manipulated by cross-fostering nestlings in small, medium and large brood sizes. This manipulation had strong effects on nestling and adult size and condition, nestling immunocompetence and testosterone levels (Naguib et al. 2004), female reproductive investment (Gil et al. 2004) affecting even the next generation (Naguib & Gil 2005; Naguib et al. 2006). However, this manipulation neither affected male song rate, song learning, song complexity nor its underlying neuronal structures (Gil et al. 2006), contrasting findings in other studies which used nutritional manipulations and application of high levels of corticosterone (Spencer et al. 2003a,b; Buchanan et al. 2004). Despite the lack of effect of our brood size manipulations on male song in a standardized context, all other findings raise the question whether the conditions during early development directly or indirectly influence male attractiveness in a binary choice situation. In the experiment presented here, females were exposed to pairs of males that differed in the experimental brood size in which they had been raised. In three different experiments, we allowed each female to choose between two males coming from the three possible combinations of different brood sizes. We predicted that, if experimental brood size affects male attractiveness, females should associate more with males coming from the relatively smaller brood sizes, i.e. with males that experienced better developmental conditions.

Methods

General

We conducted the experiments on non-domesticated zebra finches of wild Australian origin (Forstmeier et al. 2007) at the University of Bielefeld, Germany, from 12 Jul to 28 Aug 2002. Males used for the mate choice experiments came from a cross-fostering experiment in which we raised zebra finches in different experimentally controlled brood sizes within the natural range (Naguib et al. 2004). We cross-fostered birds at the age of 2 ± 1 d and experimental broods consisted of two to six cross-fostered chicks coming from one to six different original broods. There was no correlation between original brood size of a female and her cross-fostered brood ($r = 0.13$, $p = 0.46$, $n = 34$). From nutritional independence (day 35) until sexual maturity (day 90), males were kept in mixed sex groups with a song tutor so that they could socialize and learn song from an adult male (Gil et al. 2006). After this period, males were kept in single sex groups. Thus, they had no breeding experience at the onset of the experiments reported here. We divided broods in three experimental groups: (1) small broods (two to three nestlings); (2) medium broods (four nestlings); and (3) large broods (five to six nestlings). Further details of the experimental procedure are given in Naguib et al. (2004) and Gil et al. (2006). On the day of the mate choice tests, males were 12 months old ($x \pm SD = 358 \pm 20$ d). Female subjects were randomly chosen from birds raised in natural broods that were at least 1 yr old and had breeding experience. For several weeks prior to the experiments, these females were kept in single-sex groups of 10–20 individuals in larger aviaries. All rooms had an air temperature of 22–25°C and an L/D regime of 16:8 h. Birds were kept under *ad libitum* feeding conditions and were supplied daily with both dried and germinated seeds and fresh water (with added vitamins three times a week).

Female Choice Experiments

To test for effects of early developmental stress on male attractiveness, we confronted females with pairs of males that differed in the brood size they were raised in. We ran three experiments, in each of which we confronted a female with two males, each in a separate cage side by side (Fig. 1). Each female was used only once and had not been in visual contact with any of the males before. Both males were

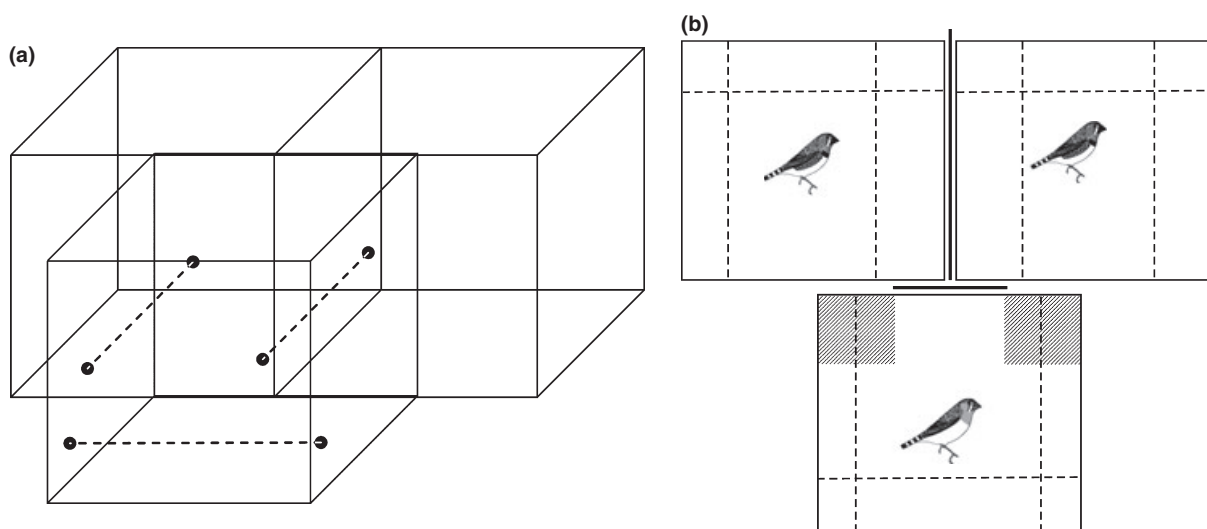


Fig. 1: Experimental set-up for the female choice experiments. The cage containing the female was centered in front of two cages containing males originating from the two different treatment groups respectively. (a) Three-dimensional view of the set-up. For clarity, perches of the male cages are not shown. (b) Top view. The area of the female's cage that was scored as a choice of one male (preference zone) is shaded in gray. Females within this zone were prevented by a small central card board barrier [not shown in (a); bold black line in (b)] from seeing the male in the cage on the opposite side. Males also were prevented from seeing each other by a card board barrier. Hatched lines within the cages indicate perches.

kept in different song tutoring groups during the period of song acquisition, so that they had learned a different song (Böhner 1990). We conducted 35 female choice experiments which were assigned to three different treatment groups: (1) males from a small brood were paired with males from a medium brood ($n = 15$ pairs); (2) males from a small brood were paired with males from a large brood ($n = 9$ pairs); and (3) males from a medium brood were paired with males from a large brood ($n = 11$ pairs). Overall, we used 16 males from small broods, 15 males from medium broods and 13 males from large broods. Thus, most males were used in two experiments, but no male was used twice within the same experiment. All birds to be tested on a given day were transferred from their aviaries to the test cages the day prior to the experiment and transported to a room adjacent to the experimental room. One hour prior to the experiments, the cages with the birds to be tested were transferred to the experimental room from which they had no auditory or visual contact to other birds. After the experiments were completed, the subjects were transferred in their cages back to the adjacent room and the next set of birds was transferred to the experimental room. At the end of each experimental day, subjects were returned to their original larger aviaries.

Test cages ($49 \times 43 \times 50 \text{ cm}^3$) were made of wire mesh with wooden frames and were equipped with

three perches and small dishes of food and water (Fig. 1). Food and water was supplied on the side of the cage that was opposite to the cage of the opposite sex. The cages with the two males were set side by side with a card board partition between them to prevent visual contact. The cage with the female was centered in front of the two cages with the males. When females were perched in front of the cage of one male, they were prevented from seeing the male in the other cage by a small visual barrier that blocked the view towards the other cage (Fig. 1).

Experimental Procedure and Measures of Response

Each trial consisted of two 20-min periods. To control for possible side preferences, initially males were randomly assigned to one side. After 20 min, we exchanged the position of the two cages with the males. After an acclimatization period of another 10 min, we then tested the female again during 20 min with the same two males. During the trials, we recorded every 5 s the position of the female and if a female switched sides within an interval we assigned 2.5 s to each male. As choice variable, we measured the time a female spent in front of each male. We counted a female as associating with a male only when she was perched in front of a given male at a position at

which she could not see the other male (shaded area in Fig. 1). In addition, we recorded all vocal activities using a Sennheiser ME66/K6 microphone (Sennheiser Electronic GmbH, Wedemark, Germany) and a SONY TCD5M (Sony Ltd., Tokyo, Japan) tape recorder. Songs were sampled at 20 500 Hz using Cool Edit 2000 Pro (Syntrillium Software Cooperation, Phoenix, Arizona, USA). Using SASLAB PRO 3.95 (R. Specht, Berlin, Germany), we counted the number of motives (Leadbeater et al. 2005) and the number of songs. A song was defined as a series of motives that was separated by less than 10 s. Analyses using of number of songs produced identical results to those using number of motives, and thus we only report the results on the latter. Statistic analyses were carried out with SPSS 14.0 (SPSS Inc., Chicago, Illinois, USA). All tests reported are two-tailed Wilcoxon matched-pairs signed rank tests using exact p-values.

Results

In none of the three experiments did females spend significantly more time with the male coming from the relatively smaller brood size, even though on average they associated more with these males (small vs. medium, $z = -0.85$, $p = 0.239$, $n = 15$; small vs. large, $z = -0.3$, $p = 0.77$, $n = 9$; medium vs. large, $z = -0.62$, $p = 0.53$, $n = 11$; Fig. 2). Females did not spend more time with males that sang more or males with a larger repertoire in any of the three experiments (all $z > -1.35$, all $p > 0.38$). Males from small broods sang significantly more than males raised in large broods ($z = -2.1$, $p = 0.036$, $n = 9$), but not significantly more than males raised in medium-sized broods ($z = -1.38$, $p = 0.17$, $n = 15$). However, these differences disappeared after Bonferroni correction. Song rates in the experiment with males from medium and large broods did not differ significantly ($z = -0.41$, $p = 0.69$, $n = 11$).

Discussion

The female choice experiments did not reveal a significant effect of early developmental conditions experienced by males on their subsequent sexual attractiveness. Despite tendencies in the predicted directions, females did not significantly prefer males that were raised in smaller experimental broods over males that were raised in larger experimental broods. Within the constraints of drawing strong conclusions from such non-significant findings, the results

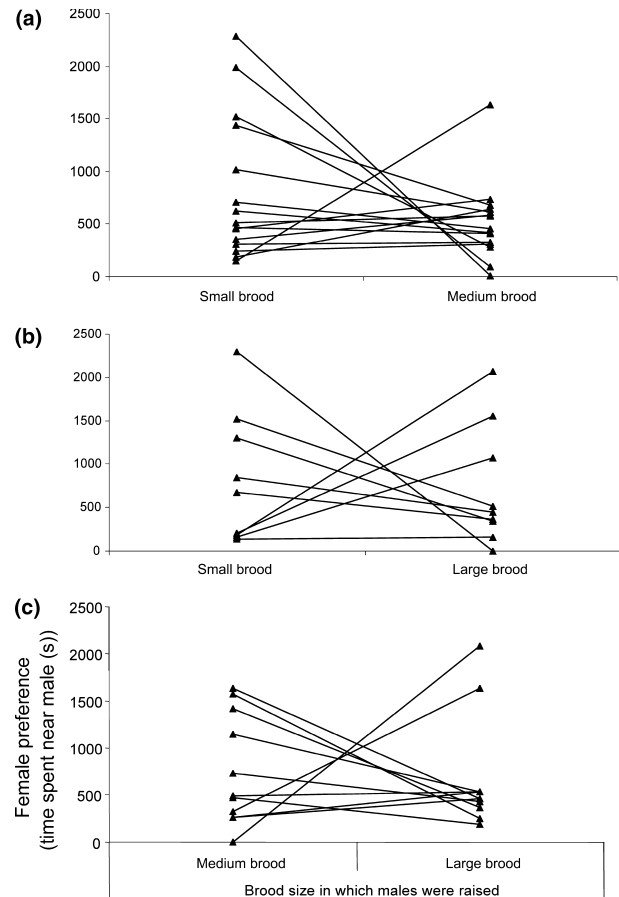


Fig. 2: Time females spent within the preference zone in the three different experiments. (a) Expt 1 in which females were tested with males originating from small and medium experimental broods ($n = 15$). (b) Expt 2 in which females were tested with males originating from small and large experimental broods ($n = 9$). (c) Expt 3 in which females were tested with males originating from medium experimental broods and large experimental broods ($n = 11$).

suggest that males were either not affected sufficiently in their traits determining sexual attractiveness or that they had compensated in attractiveness for deficits experienced during early development. Such a lack of an effect on attractiveness by a comparatively weak stressor, such as our brood size manipulation, is not completely unexpected (Metcalf & Monaghan 2001). Limitations in resources during some stages in development are a component of evolution in stochastic environments, so that animals are likely to have evolved mechanisms to subsequently compensate for stress experienced as young. However, there exist a number of other mutually non-exclusive different causes that could have contributed to the lack of an effect of early environment on male attractiveness in these experiments, as discussed below.

Our findings contrast with those reported by de Kogel & Prijs (1996) who found that males reared in small experimental broods were sexually more attractive than males reared in large experimental broods. One possible cause for these diverging results may have been methodological differences in the treatments under which subjects were raised and in the experimental choice set-up. Subjects in de Kogel and Prijs' experiment remained in the experimental conditions until day 50, i.e. until sexually selected plumage ornaments were well developed, whereas our treatments ended on day 35, i.e. before plumage ornaments had developed. Even though it is not clear if group composition and size continues to play a role in development after nutritional independence from the parents around day 35, nutritional and social factors in general are well known to affect, for instance, development of sexually selected ornaments (McGraw et al. 2003; Leadbeater et al. 2005; Hill 2006; Naguib & Nemitz 2007). Indeed, in de Kogel's experiment, birds from smaller broods at day 50 were more advanced in beak coloration than those from larger broods even though such a difference was not present in a qualitative assessment of plumage ornaments. This experimental difference might have been relevant also as nutritional stress between day 35 and day 60, i.e. the period when sexually selected plumage ornaments in male zebra finches develop, significantly affects the size of orange check patches and subsequent male attractiveness in a similar choice situations as we used here (Naguib & Nemitz 2007). These findings together thus raise the question as to whether the period when sexual characters develop is more important than earlier developmental periods in affecting attractiveness.

A further possibly relevant difference between our study and the mate choice experiment by de Kogel & Prijs (1996) is a difference in social choice situation. Females here were allowed to choose between males presented at opposite sides of the female cages, allowing all three birds to be in social contact during the choice trials, whereas in our experiment males could not see each other. As it has been discussed for effects of male symmetry on mate choice (Waas & Wordsworth 1999), choice experiments in which direct male–male interactions provide additional cues to females, as they here may prefer or avoid males based on their performance during intra-sexual interactions rather than directly choosing males based on their direct inter-sexual attractiveness (Wong & Candolin 2005). Recently, it has been shown in zebra finches that the social context

such as the presence of other birds strongly affects social discrimination (Vignal et al. 2004) and thus also may affect whether or not an existing preference is displayed. Another possibility is that interactions of individual traits we did not record including personality-dependent preferences in females or female prior experience (Riebel 2003; Witte & Sawka 2003; Forstmeier & Birkhead 2004) may have masked the potential (but presumably weaker) effects of earlier developmental conditions on male attractiveness.

The lack of pronounced effects of early condition on song rate also contrasts with previous reports (Nowicki et al. 2002; Spencer et al. 2003a,b; Zann & Cash 2008), but are in line with our own findings when the same males as used here were recorded singly with a female, i.e. in a non-competitive set-up (Gil et al. 2006). In a separate analysis of several song variables including song rates towards a female recorded at an earlier age, we also did not find effects of experimental brood size on song rate (Gil et al. 2006). This suite of results suggests that early developmental effects on such a flexible trait are expressed in neither social context in which we tested the males. Even though we hesitate to draw strong conclusions from our non-significant results, the experimental differences raise the question as to whether traits important in sexual attractiveness are buffered against early developmental stress to a larger extent than other traits (Gil et al. 2006). Interestingly, the brood size manipulation we had applied affected a range of female reproductive traits (Gil et al. 2004; Naguib & Gil 2005; Naguib et al. 2006), but apparently had no effect on any male trait we measured (Gil et al. 2006; this experiment). Future research needs to address in more detail whether specific traits are affected by early developmental stress during specific developmental stages in a repeatable manner and to determine how pronounced individual phenotypic plasticity is with respect to which traits are affected and /or compensated for and in which contexts.

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