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Experimental manipulation of the rearing environment influences adult female zebra finch song preferences

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A R T I C L E I N F O

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Keywords: brood size manipulation developmental stress female condition female song learning mating preference sensory learning sexual selection *Taeniopygia guttata* zebra finch In songbirds, sensory and social learning processes in juveniles contribute to variation in male song and female preferences. The developmental stress hypothesis proposes that suboptimal early development affects the costly brain structures involved in male song learning and, as a consequence, song quality. As an extension of this hypothesis we tested in this study whether developmental conditions also modulate female song preference acquisition. We tested song preferences in adult female zebra finches, Taeniopygia guttata, originating from a brood size manipulation experiment that had induced differences in mass, condition, immune response and levels of plasma testosterone at the early nestling stage. During the song-learning phase, juvenile birds were housed in small mixed-treatment groups with unrelated adult male song tutors. Adult females' song preferences were tested in an operant set-up where females could trigger different song playbacks by pecking different response keys. When females could choose between their own and an unfamiliar tutor's song they preferred their tutors' songs independent of experimental brood size. However, when females chose between two unfamiliar songs there was a significant effect of experimental brood size on preference strength: females from small broods showed significantly stronger preferences than those from medium and large broods. Hence, both females' rearing environment and sensory learning processes appear to contribute to variation in the direction and strength of female preference for male mating signals.

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Sexual selection by female mate choice is widely accepted as a powerful selection force (Andersson 1994), but how development and the early rearing environment might contribute to variation in female mating preferences remains poorly understood and has rarely been addressed experimentally (Jennions & Petrie 1997; Widemo & Saether 1999; Cotton et al. 2006). This is in strong contrast to the research effort invested in the now well-documented condition or state dependency of male signals (Andersson 1994). Little is known about how environmental and social conditions experienced during early development might affect receivers' behaviour, such as the perception and decoding of mating signals (Widemo & Saether 1999). Costs and benefits of mate choice behaviour and preferences might shift through suboptimal development (Badyaev & Qvarnström 2002; Cotton et al. 2006). Such effects could play an important role in the evolutionary dynamics of mating signals. Females' preference functions, that is, their ranked preferences for different male phenotypes can be affected by early sensory learning processes, such as sexual imprinting in the visual or auditory domain in birds (ten Cate & Vos 1999; Riebel 2003a) or olfactory imprinting in mammals (Owens et al. 1999) and via as yet unidentified sensory mechanisms in fish (Verzijden & ten Cate 2007). However, at our current state of knowledge, it is unclear how such sensory learning processes during development interact with other environmental factors. Deficits in early nutrition, for instance, could constrain development of central and peripheral nervous systems (e.g. Buchanan et al. 2004; MacDonald et al. 2006) and the nutritional stress hypothesis suggests that this could negatively affect sensory learning (Nowicki et al. 1998).

In songbirds, male song plays an important role in mate choice (Searcy & Yasukawa 1996). Signal development and expression depend critically on sensory learning processes and are limited by a wide array of costs (Gil & Gahr 2002). Consequently, suboptimal conditions at crucial phases of development could be reflected in the accuracy of male song learning ('developmental stress hypothesis' Nowicki et al. 1998; Buchanan et al. 2003; Spencer et al. 2003; Gil et al. 2006; Holveck & Riebel, in press; Zann & Cash 2008).

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Here, we tested the hypothesis that sensory learning processes in females (Riebel 2003a) may be similarly sensitive to conditions experienced during early development. We tested this hypothesis by investigating the effects of brood size manipulation on female song preference development in zebra finches, Taeniopygia guttata, a model species for studies of vocal learning and sexual selection (Zann 1996; Riebel 2009). Zebra finches do not fully compensate for the increased nutritional needs of larger broods: chicks from larger broods are smaller and have lower body mass than those from small broods, even under ad libitum feeding conditions in domesticated and wild stock birds (de Kogel & Prijs 1996; Naguib et al. 2004; Tschirren et al. 2009). Brood size manipulations keeping within the natural range thus provide an excellent experimental procedure of inducing ecologically relevant variation in early condition. The females tested in this study originated from a brood size manipulation experiment in which nestling growth, body condition and immune response had been found to be negatively affected by increasing experimental brood size while nestlings' plasma testosterone levels (indicating physiological adjustment to higher levels of competition) increased with number of siblings in the nest (Naguib et al. 2004). Because both male advertising song and female song preferences are influenced by subadult sensory learning processes in songbirds, next to our experimental manipulation of rearing conditions, we also experimentally controlled subjects' song experiences during the sensitive phase for song learning just after nutritional independence (see methods in Gil et al. 2006).

To test whether the experimentally induced variation in early rearing conditions had affected subadult preference learning, upon reaching sexual maturity, each female was given the choice between the specific tutor song she had heard earlier (at the age when song experiences lead to long-lasting preferences, Riebel 2003b) and an unfamiliar song ('tutor versus unfamiliar') in an operant set-up. This set-up allowed us to test female song preferences independently of variation in other male traits or behaviour (Riebel 2000) and song preferences measured this way predict preferences for live males in this species (Holveck & Riebel 2007). Second, to test whether the treatment had affected their preference for qualitatively different stimuli, females were given a choice between two unfamiliar songs of different duration ('short versus long') as female zebra finches have been reported to prefer long over short song (Clayton & Pröve 1989; but see Leadbeater et al. 2005; Holveck & Riebel 2007) and because male song duration can be affected by early nutritional condition in this species (Spencer et al. 2003; but see Gil et al. 2006; Holveck et al. 2008; Zann & Cash 2008).

METHODS

Experimental Subjects

We used adult female offspring (\leq F4) of laboratory-kept, wildcaught Australian zebra finches at Bielefeld University which had been raised by foster parents in a brood size manipulation experiment (for details see Naguib et al. 2004). Chicks were crossfostered 1–3 days after hatching in experimental brood sizes varying from two to six nestlings (Table 1) which is within the natural range of this species (Zann 1996). A cross-fostered brood always consisted of siblings from more than one brood and original broods were never wholly cross-fostered. The brood size manipulation had various effects on juvenile morphology and condition: at age 10 days posthatching, body size, body weight, weight in relation to size (tarsus length, see Garcia-Berthou 2001) and immune response decreased with experimental brood size; nestling plasma testosterone levels increased with increasing brood size (for full details see Table 1 and Figures. 1 and 2 in Naguib et al. 2004). Table 1

Brood size	Ν		Number of females		Days to keypecking $(\overline{X}\pm SD)$	
	Broods	Females	SelfLearn*	Training	All SelfLearn†	All training‡
2	5	6	2	4	3.8±3.6	1.0±1.7§
3	5	8	5	3	$3.6{\pm}2.2$	1.3 ± 1.2
4	9	19	10	9	4.2±1.9	$1.7{\pm}1.6$
5	2	6	3	3	3.8±1.6	$1.7{\pm}2.1$
6	6	14	9	5	4.3±2.3	3.4±1.7
All	27	53	29	24	4.0±2.2	1.9±1.7

From the initial 56 adult females from the brood size manipulation experiment, two died before the preference tests started and one (from an experimental brood size of two) never learned to peck the keys, leaning a sample size of N = 53 females. Number of days to learn the operant task did not differ between experimental brood sizes (one-way ANOVA: all females: $F_{4,51} = 0.15$, P = 0.96; females with training: $F_{4,22} = 1.4$, P = 0.27; females without training: $F_{4,24} = 0.9$, P = 0.49). Note that the training schedule aimed to maximize the number of females learning and was not standardized with respect to the quantity and timing of training. Hence, the number of days to key pecking is in this context not a proper measure of interindividual differences in learning speed.

* SelfLearn: females learning to press the keys by autoshaping.

[†] From the day a female was moved into the experimental cage.

[‡] From the first day with training to key pecking.

 $\,^{\$}$ Data of one bird excluded because a technical problem required us to repeat the training.

Because effects of a brood size manipulation might also affect the father's song output (Verhulst 2003; Brumm et al. 2009) and perhaps also other performance aspects of his song, the young from the different cross-fostered broods were split up at 35 days (full nutritional independence) and assigned to 19 different unrelated adult male song tutors in mixed-sex, mixed-treatment tutor groups (3–9 chicks, mean 5.8) in 19 different indoor aviaries ($0.8 \times 2.2 \text{ m}$ and 1 m high or 0.9×1.9 m and 1.9 m high) in large indoor birdholding facilities with at least three aviaries per room until a mean age \pm SD of 89 \pm 2 days posthatching. If housed with a live tutor at this age, both males and females will learn the tutor song, even if other males are singing in the same room in cages close by (Riebel et al. 2002). However, we additionally controlled for possible effects of tutor group housing by including tutor group as a random factor in all statistical analyses and by always using songs from a different room as unfamiliar stimuli. At the beginning and at the end of the tutoring phase (at 35 and 90 days posthatching) birds were weighed and measured again. Birds had by now caught up on weight and body condition (weight corrected for size) which no longer differed between the groups while the differences in body size persisted (Naguib et al. 2004). Females were then housed in groups of 15 (from different treatments and different tutor groups) in four indoor aviaries (0.92×1.8 m and 1.85 m high or 1×3 m and 3.3 m high) in large bird rooms with other single- and mixed-sex aviaries. All aviaries were equipped with wooden perches and were additionally enriched with natural branches as well as a sand floor. At a mean \pm SD of 106 \pm 19 days, females (N = 56) were moved to Leiden University. Here, they were housed in groups of seven or eight birds per cage $(0.5 \times 1 \text{ m and } 0.8 \text{ m high})$ in the same bird room (lights on 0745-2145 hours 20-22 °C, and 40-60% humidity). Throughout, birds were maintained on an ad libitum food and water regime consisting of standard seed mix supplemented daily with germinated seeds and three times a week with vitamins (in Bielefeld) or with gisto-cal mineral-vitamin supplement and twice weekly with egg mix, fruit and greens (in Leiden).

Both Bielefeld and Leiden University have licences to keep and breed zebra finches; the experimental procedures we report here were reviewed and approved by Leiden University's animal experimentation ethical committee. After the song preference tests in Leiden, birds were returned to the Bielefeld colony where they were kept in large mixed-sex aviaries (1×3 m and 3.3 m high or 2×3 m and 3.3 m high) to monitor their and the subsequent generation's breeding behaviour. This revealed further evidence for long-term and trans-generational effects of the brood size manipulation: females that originated from large nests (and their offspring) had fewer and smaller offspring than those that had been raised in small nests (Gil et al. 2004; Naguib & Gil 2005; Naguib et al. 2006).

Preference Tests

In zebra finches, female preferences for male advertising song predict preferences for live males (Clayton 1990; Holveck & Riebel 2007; Riebel 2009). Because we wanted to test how females' rearing condition would affect their adult song preferences rather than their attractiveness to males (Jones et al. 2001), we chose an operant song preference test where females can express their song preferences without male interference. Song is played back as a reward for key pecking which allows females to control actively which of two songs they want to hear and how often. This method has excellent internal and external validity: female song preferences are repeatable within and between tests (Riebel 2000; Riebel et al. 2002) and song preferences measured this way predict females' preferences for live males in choice chambers (Holveck & Riebel 2007) and latency to first egg in the nest (Holveck & Riebel, in press).

To test the hypothesis that females' rearing background might affect subadult song preference learning experimentally, it was paramount that females differed only in their rearing background and not in the amount and order of sensory experiences during not only the subadult phase but also the adult test phase (for a comparable design see Lauay et al. 2004). To this end all females were first tested with their respective tutor song versus an unfamiliar song (the tutor song of another tutor group) and subsequently with two unfamiliar songs that differed in song duration to test effects both on specific learned and more general aspects of song preferences. Song preference tests began a mean \pm SD of 145 ± 18 days posthatching, an age at which all individuals will have sexually matured (Zann 1996). We used eight identical experimental set-ups and tested females blindly with regard to their early treatment using our previously described protocol (e.g. Riebel 2000; Riebel et al. 2002; Holveck & Riebel 2007). Briefly, within a sound-attenuated chamber, females were housed in a cage $(70 \times 30 \text{ cm and } 45 \text{ cm high})$ with five equally spaced perches for the duration of the training and test phase. There were two red response keys (diameter: 1 cm) which could be illuminated by small LED lights fixed to the rear panel just above the first and fifth perches. When females pecked either of these keys they received one song playback as a reward. A mini-computer (with Oki MSM6388 sound chip, Tokyo, Japan) kept a data log: it recorded the keypecks and controlled the playbacks (70 dB re 20 µPa peak amplitude at 30 cm; CEL-231 sound level meter, fast response setting) via a loudspeaker (Quart 250, Blaupunkt, Hildesheim, Germany or JBL Control 1, Northridge, CA, U.S.A.) fitted behind a central opening (diameter: 9 cm) in the rear panel. Apart from the first and last 15 min of the lights-on period the keys were active throughout the whole training (several days) and test period (4 days). The familiar (tutor) song and an unfamiliar song (the tutor song of another group) were randomly assigned to one side and switched automatically between the two keys every night during the lights-off period to control for possible side preferences. We began training for all females by leaving them to explore the cage with the red LED lights of the pecking keys switched on. The continuous data log was at all times accessible for downloading or reading in a display from outside the experimental chamber, which allowed us to monitor females' learning progress in addition to

daily observation and/or training sessions. Females differed in activity and exploration levels, but once they discovered the keys and the associated rewards they showed learning curves typical of operant tasks. Females first showed no key pecking (this phase differed between females from several hours to several days). This was followed by a phase of occasional key pecking then eventually a brief phase with exponential increase of key-pecking activity levelling off to relatively stable activity levels. The day after this observation, preference testing would begin. Note that this is a preference, not a discrimination test: the sole inclusion criterion was thus that females had to learn the contingency between pressing the keys for song reward (which was ascertained as described above), not a 'discrimination criterion' (i.e. a minimum preference strength) because this would exclude females that found two songs equally attractive. Similarly, we had no preset minimum activity level, but specifically included all key-pecking females (52/53 females) in the analyses. This way we could avoid sampling biases towards a particular learning or sampling strategy (as these may covary with female rearing condition) and could test for effects of early rearing condition on activity levels and/or interactions between rearing condition, activity levels and preference strength.

After introduction to the experimental cage, females started pecking keys regularly after a mean \pm SD of 4 ± 3.3 days in the experimental cage and this did not differ between the females from different brood sizes (Table 1). Most females (N = 29/53) learned the operant task by autoshaping (i.e. during exploration of the cage the females pecked the keys by accident initially but then rapidly increased the number of keypecks). Those that had not learned to peck the keys started to do so within a mean \pm SD of 1.9 \pm 1.7 days after shaping began (N = 23/53 females 3.3 ± 1.4 days after females had been moved to the cage with the operant panel) which consisted of one or two training sessions of 20-30 min per day. During shaping, the experimenter first drew females' attention to the keys by flashing the LED lights before playing the song reward and then gradually rewarding all behaviour leading to closer approach and exploration of the keys, taking care to reinforce the keys on both sides of the cage. After an initial delay, female learning was often very rapid and went from zero keypecks/day up to several hundred times per day (average per female per day \pm SD = 285 \pm 155; range 43–736; Table 2). After this type of initial training, females' song preferences can be reliably measured: in our earlier studies using this method we showed that female preference strength and activity levels were repeatable between the first and the second 2-day block of a 4-day test (Riebel et al. 2002) and 1 and 6 months after training (Riebel 2000). From the initial sample of 56 females only one female never learned the association between the key pecking and song reward and another two died in their home cages before we could test them. All the other 53 females learned the task, yielding a sample size of N = 53 for test 1 and N = 51 for test 2 (because of a technical problem the data for test 2 for two females could not be recovered from the memory cards).

Table 2
Female activity level measured as total number of keypecks ($N = 53$ for test 1, $N = 51$
for test 2 and total)

Experimental brood size	Ν	Test 1 $(\overline{X} + SD)$	Test 2 $(\overline{X}+SD)$	$ \begin{array}{l} \mbox{Total (test 1 + test 2)} \\ (\overline{X} + \mbox{SD}) \end{array} $
2	6	787±443	270±184	1057±561
3	8	1112 ± 746	$340{\pm}297$	1582 ± 890
4	19	801±367	275±163	1072±432
5	6	752 ± 583	266 ± 176	1018 ± 754
6	14	$745{\pm}408$	$351{\pm}290$	$1096{\pm}634$
All	53	826±480	303±222	$1140{\pm}619$

The actual preference testing consisted of two sequential tests each lasting 2 days (1 day with one stimulus song of a set on either side to control for potential side preferences and diurnal effects). During test 1, females could choose between the tutor and an unfamiliar song (the tutor song of another group from a different room). On the morning of the third day, test 2 with two unfamiliar songs began ('long' versus 'short' song; see Stimulus songs). To control for sensory learning experiences during tests we opted to keep sensory experiences the same for all subjects and decided against varying the test order between subjects. This meant that we cannot compare preference strength between tests within females (because we did not control for possible order effects), but that our primary interest, that is, the comparisons between treatment groups within tests, will not be confounded by different sensory experiences prior to a particular test. All comparisons between treatment groups were thus strictly kept to within-test comparisons and analysed using separate general linear models per preference test (for details see below).

Stimulus Songs

All males were recorded (Sennheiser ME66/K6microphone, SONY TCD 5M tape recorder) when caged individually adjacent to a female. Songs were digitized (Cool Edit software, Syntrillium software, Scottsdale, AZ, U.S.A., 32 kHz, 16 bits, PCSoundblaster Live! Soundcard) and further edited with SASLab Pro software (R. Specht, Berlin, Germany). For one tutor we could not obtain sufficiently good recordings for preference testing. For each of the remaining 18 tutors, a song lasting a mean \pm SD of 5.8 \pm 0.3 s was selected. These songs consisted on average of 5.8 \pm 0.8 repetitions of the individually distinctive song motif. The 18 tutor songs were assigned to nine sets of two songs each (from different males and housing rooms) such that each tutor song also served as an unfamiliar song for another tutor group. For the second test (test 2), 18 song motifs were selected from recordings of 29 males of the Bielefeld breeding colony (mean motif duration \pm SD = 824 \pm 201 ms, range 600–1404 ms, coefficient of variation = 24%) and, with motif duration as a criterion, assigned to nine long-short stimulus sets which we aimed to differ by 30% relative duration (short: 727 \pm 129 ms, range 600–1125 ms; long: 937 \pm 161 ms, range 750– 1404 ms; average difference \pm SD = 29 \pm 0.4%). Using Avisoft Soundlab Pro software (R. Specht, Berlin, Germany), we inserted a 180 ms pause after each motif before it was digitally multiplied into a four-motif repetition long song (mean total duration: short songs: 3626 ± 516 ms; long song: 4467 ± 645 ms). The nine stimuli sets were assigned to females in turn such that all females received test songs from unfamiliar males only.

Note that the stimuli differed qualitatively between tests 1 and 2 because they tested different aspects of song preferences: a learned preference (using natural songs of approximately the same duration but with some variation in total motif repetition number) and a directional preference for motif length (using edited song to keep the number of motif repetitions and intermotif interval and performance variability constant). It is possible that it was therefore easier for females to make choices in either test; however, we did not aim to compare females' preferences across tests. Instead, our intent was to test for two different aspects of song implied in mating preferences (Clayton & Pröve 1989; Neubauer 1999; Riebel 2003b) and to compare females' performances within tests. Stimuli in either set were within the range observed for motif and song duration for the Bielefeld laboratory-kept offspring of wild-caught Australian birds (Clayton et al. 1991; Gil et al. 2006; K. Riebel, unpublished data). Songs of four or more motif repetitions per playback provide more sensory input than zebra finches need to discriminate between two auditory stimuli (zebra finches can e.g.

learn to discriminate identical motifs differing in the position of only one element, Verzijden et al. 2007).

Statistical Analysis

Preference strength was calculated as the proportion of keypecks for the familiar song in test 1 (tutor versus unfamiliar song) and the preferred song (the song receiving the higher number of keypecks) in test 2 (two unfamiliar songs) in relation to the total number of keypecks in a given test. We fitted general linear mixed models using JMP v.7.0.1 (SAS Institute, Cary, NC, U.S.A.) with brood size as a covariate (and repeated all analyses also with brood size squared to check for nonlinearity of the data). Tutor identity was included as a random factor in all models for each of the response measures (arcsine-transformed preference, key-pecking activity) and pecking activity and standardized weight at day 10 were included as additional variables. Stimulus set, natal and rearing nest were also tested as random effects, but explained little of the variance without changing the results and were therefore excluded to simplify the analyses.

RESULTS

Preference for Tutor Song (Test 1)

The proportion of keypecks ($\overline{X} \pm SE = 70.9 \pm 1.4\%$) for the songs females had heard as juveniles (i.e. the 'tutor songs') was higher than for unfamiliar control songs (i.e. other females' tutor songs). At the individual level, 50 of 53 females pecked significantly more for their tutor song (*G* tests, in 50 of 53 females: $G_{adj} > 3.84$, P < 0.05). This learned preference was independent of brood size (or brood size squared, Table 3), meaning that across all groups, females had memorized their tutors' song and now as adults equally strongly preferred their tutor songs over the unfamiliar control songs (Fig. 1). While brood size affected neither the direction nor the strength of preference in test 1, tutor identity (i.e. the tutor or tutor group a female was exposed to from 35 to 89 days) as random effect explained 13.7% of the variation in preference strength (13.7% = 0.00166/0.0121 total variance, 95% confidence internal -0.0027-0.0061). The number of keypecks was not correlated with brood size, or brood size squared (all P > 0.3; Table 2). The relationship between key pecking and preference strength for tutor song was positive (Table 3): females with stronger preferences showed higher pecking activity but there were no interactions with brood size.

Preference for Unfamiliar Long versus Short Songs (Test 2)

The total number of keypecks per 2-day test was lower in the second test than in the first test (paired *t* test: $t_{50} = 9.0$, P < 0.001), but the change in number of keypecks from the first to the second test was independent of brood size (interaction: size×number of pecks in test 1: $F_{1,47} = 2.1$, P = 0.15). Females with high pecking rates in the first test also pecked at a higher rate in the second test (Pearson correlation: $r_{49} = 0.47$, P < 0.0005) and, like in test 1, brood size (and brood size squared) did not explain interfemale variation in key-pecking rates (all P > 0.3; Table 2).

There was no directional preference for the longer songs overall (preference strength: $48.5 \pm 15.1\%$; no deviation from 50% chance level: $t_{51} = 0.7$, P = 0.49) nor was it affected by experimental brood size or brood size squared (Table 3). Tutor identity explained a mere 5.9% of the variation (5.9% = 0.0016/0.0268 total variance, 95% confidence interval -0.0057-0.0088). However, individual females' choices were not random: most females clearly preferred one of the two unfamiliar songs (mean $62.9 \pm 1.2\%$; significant preferences in

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Table 3

Results of the GLMMs testing whether preference strength (for tutor song, for long song and preferred unfamiliar song) was affected by rearing brood size

	Response variable	Variables	Estimate (SE)	F (df)	Р
Test 1	Tutor song preference (R^2 =0.29)	Constant	0.93 (0.06)		
		Brood size	0.0021 (0.012)	0.03 (1,50)	0.9
		Keypecks	0.000075 (0.000032)	5.43 (1,47.5)	0.024
	Rejected terms	Brood size squared	0.0029 (0.0095)	0.096 (1,49.0)	0.8
		Weight day 10	-0.022 (0.026)	0.73 (1,47.2)	0.4
Test 2	Long song preference (R^2 =0.11)	Constant	0.82 (0.081)		
		Brood size	-0.014 (0.018)	0.63 (1,48.7)	0.4
	Rejected terms	Brood size squared	0.013 (0.14)	0.85 (1,47.2)	0.4
		Weight day 10	-0.015 (0.039)	0.14 (1,46.5)	0.7
	Preference strength* (R^2 =0.39)	Constant	1.00 (0.05)		
		Brood size	-0.2 (0.0099)	4.18 (1,48.5)	0.046
	Rejected terms	Keypecks	0.000025 (0.000058)	0.18 (1,44.1)	0.7
		Brood size squared	0.0067 (0.0077)	0.76 (1,47.8)	0.4
		Weight day 10	-0.0059 (0.022)	0.07 (1,46.9)	0.8

Numbers in bold indicate significant P values. The rejected terms are indicated in italics.

Preference strength for the preferred of the two test songs.

38 of 51 females: $G_{adj} > 3.84$, P < 0.05) and preference strength for these individually preferred songs was more pronounced when females had been reared in smaller broods (Fig. 2, Table 3). Tutor identity explained an additional 22.9% (22.9% = 0.0020/0.0088 total variance, 95% confidence interval -0.0008-0.0049) of the variance in preference strength. Standardized weight at day 10 did not explain the variation in female preference strength: adding this variable as a covariate did not improve the model (Table 3).

DISCUSSION

There is ample demonstration of how conditions experienced during early development affect adult phenotypes (West-Eberhard 2003) and an increasing interest in condition dependency of female mating preferences, but as yet little experimental study of these questions (reviewed in Jennions & Petrie 1997; Widemo & Saether 1999; Cotton et al. 2006). We here tested whether female zebra finches from a brood size manipulation experiment that had induced differences in juvenile and adult phenotypes (Naguib et al. 2004, 2006; Naguib & Gil 2005) would develop different sensory preferences for male advertising song in adulthood. We found that the rearing and social conditions experienced during different developmental stages were reflected in adult female song preferences. Experimental brood size covaried with preference strength



Figure 1. Preference strength (untransformed data) for the tutor song in adult females from different experimental brood sizes.

when females were given the choice between unfamiliar songs, and learning about song during the juvenile stage altered adult valuation of different song variants: for females from all brood sizes, there was a strong preference for the familiar tutor song over unfamiliar song. It is important to stress that this preference could not have arisen because the tutor songs were by accident in some way more attractive than the unfamiliar control songs: all unfamiliar songs were the tutor songs of females from other groups. All stimulus songs were attractive (i.e. the preferred song) for those females that had heard them as juveniles but were unpreferred by other females when pitted as unfamiliar songs against their familiar songs. Early familiarity was thus the criterion used above any other aspect of song quality by females choosing which song to hear in test 1. This learned preference was not affected by the experimental brood size confirming the high salience of songs learned early in life and the strong effects of early experiences on adult sensory preferences (Riebel 2003a, b).

Once the strong influence of familiarity was removed from the test situation in the second test with unfamiliar songs, female preference strength covaried with experimental brood size. Females from larger broods showed less pronounced preferences than those from smaller broods. An artefact arising from different forms of between-trial learning within this experiment for females from different brood sizes seems unlikely. Because all females had



Figure 2. Preference strength for the preferred of two unfamiliar songs. Data shown are untransformed preferences corrected for tutor identity, but note that analysis was carried out using arcsine-transformed raw data.

expressed equally strong and significant preferences for the tutor song in the first test, the differences in preference strength in test 2 between females from different brood sizes could not have arisen because the females from the larger brood sizes had not learned the operant task properly. Although all females were tested in the same order, the decrease in pecking activity in the second test occurred independently of experimental brood size and pecking was highly correlated within individuals between the two tests. The decrease in pecking activity was surprising given that earlier studies using this method showed no such systematic change in activity level (no habituation within or between tests in e.g. Collins 1999; Riebel 2000; Riebel et al. 2002). It is conceivable that the differences in stimulus quality between tests 1 and 2 (unfamiliar or edited songs might be less salient than the familiar/unedited songs or four stereotyped repetitions might be less attractive than more variable song, see Riebel 2009) or the lack of a break between the different types of stimuli (the earlier tests without habituation over a 4-day period had not introduced new stimuli mid-test) caused the decrease in pecking activity. Whatever the cause of this unexpected decrease in pecking activity it was independent of experimental brood size, the variable with the highest explanatory power for the differences in preference strength.

This suggests that the brood size manipulation indeed showed long-term effects on choice (for corroborating evidence see Holveck & Riebel, in press). From the combined evidence of recent studies testing the developmental stress hypotheses in zebra finches, a picture emerges that suggests that both cultural transmission processes and rearing conditions contribute not only to variation in male song traits but also to variation in female song preferences (for discussion see Riebel 2009). For the males of this brood size manipulation experiment, we found no effect on traditional measures of song learning such as the number of learned elements and percentage of elements shared with the tutor (Gil et al. 2006); neither did the subsequent studies in male zebra finches that showed effects on song performance and syntax accuracy (Holveck & Riebel, in press; Zann & Cash 2008; Brumm et al. 2009), parameters that unfortunately Gil et al. (2006) had not measured. This illustrates an important challenge for future work that ought to try to integrate how male traits and female preferences respond to environmental variation (Riebel 2009). For now, the combined results suggest, however, that in males and females, memorization of song is not as easily affected by the conditions experienced during early development as song production might be, as neither the males of this (Gil et al. 2006) nor of another brood size manipulation experiment (Holveck et al. 2008) or a food quality (Zann & Cash 2008) or availability (Brumm et al. 2009) manipulation were affected in how many elements they had copied from their tutor even though other song parameters were (for review and discussion see Brumm et al. 2009; Riebel 2009).

While the juvenile environment explained some variation in preference strength in test 2, remarkably, the direction and strength of preference in this test were not predicted by specific song types. The lack of directional preference for long songs in test 2 in particular seems contrary to expectations and needs some discussion, because female zebra finches in earlier studies showed directional preference for long versus short songs (Clayton & Pröve 1989; Neubauer 1999). However, these two earlier studies are not fully conclusive on the issue: one used a very limited set of stimuli (Clayton & Pröve 1989), while the other worked with artificially edited supernormal songs (Neubauer 1999). Our and other recent studies (Leadbeater et al. 2005; Holveck & Riebel 2007; Vyas et al. 2009) suggest that variation in song length per se within the natural range is less important in determining female preferences in zebra finches than previously thought. The results of Vyas et al.

(2009) show that an aspect of song complexity, that is, the number of different syllables, is more attractive than long songs per se and this has been shown experimentally in another species, the chaffinch, Fringilla coelebs, too (Leitão et al. 2006). Moreover, previously not-measured parameters relating to the amount of singing versus silence within a song seem to go some way to explaining variation in female preference in two recent studies that appeared after we had conducted these tests (Leadbeater et al. 2005; Holveck & Riebel 2007) and the consistency of this parameter seems also to signal early male condition (Holveck et al. 2008). Unfortunately, we cannot test for a similar effect in this data set as our song stimuli were randomly sampled from males of unknown rearing condition from across the colony. Two recent studies suggest that females pick up on differences between stressed (food restriction or corticosteroid administration) and nonstressed males and males from small versus large broods (Spencer et al. 2005; Holveck & Riebel, in press). However, the question whether females base their choices on the song parameters found to covary with juvenile condition or exposure to stressors in zebra finch males needs experimental studies with edited songs systematically varying these parameters. Nevertheless, even at this stage, a picture emerges that suggests that the question of what makes a male song attractive is a complex interplay between male and female quality and their respective developmental histories (Holveck & Riebel, in press).

Current theory of state-dependent mate choice states that lowquality/state females should invest less time and effort in choosing a mate (McNamara & Houston 1996; McNamara et al. 1999; Fawcett & Johnstone 2003; Härdling & Kokko 2005). Reduced preference strength has been suggested both as a mechanism to achieve this and as a measurable behavioural correlate or proxy for choosiness in experiments manipulating condition (e.g. McGlothlin et al. 2004; Burley & Foster 2006; Cotton et al. 2006). Recent evidence from different taxa shows plasticity of female mate choice because of context-specific benefits (Widemo & Saether 1999; Qvarnström 2001) or condition (Bakker et al. 1999; Syriatowicz & Brooks 2004; Hunt et al. 2005). The observation that brood size manipulation had long-term effects on adult mating preferences suggests that female zebra finches show state-dependent mating behaviour (McNamara & Houston 1996; Fawcett & Johnstone 2003; Burley & Foster 2006). While condition dependency of male advertising traits is well documented, the effect of condition on female mating behaviour is poorly understood and rarely addressed experimentally (Cotton et al. 2006). In zebra finches, individuals in low body conditon are less attractive to the opposite sex (Jones et al. 2001; Blount et al. 2003a, b) and zebra finches raised in large experimental broods differ not only in their attractiveness to the opposite sex (de Kogel & Prijs 1996) but also in longevity (de Kogel 1997) and standard metabolic rate (Verhulst et al. 2006) suggesting that they are both more time and energy limited. Reduced choosiness is one form of plasticity that is likely to save an individual time and energy when competing for mates (Badyaev & Qvarnström 2002; Fawcett & Johnstone 2003; Härdling & Kokko 2005). Our results are in line with the conclusion that female preference strength was contingent on conditions experienced during early development and ensuing phenotypic variation. Moreover, they expand recent findings of effects of manipulation of adult condition on mating preferences in this species (Burley & Foster 2006) by showing longterm effects of juvenile rearing conditions on the one hand and stability of learned preferences on the other (see also Riebel 2000). Although at this stage we cannot disentangle whether females' juvenile or adult condition might have been responsible for the observed differences, adult phenotypic differences covaried with the rearing environment (data in Naguib et al. 2004, 2006; Naguib & Gil 2005), suggesting that the brood size manipulation affects adult condition via as yet unidentified developmental pathways.

Female mating preferences might be subject to similar trade-offs between life history traits as are male sexual traits (Hunt et al. 2005). The hitherto neglected environmental contributions and context specificity of female mating preferences may hold an important key to understanding the as yet unexplained variation in mating preferences observed within populations.

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References

- Andersson, M. 1994. Sexual Selection. Princeton, New Jersey: Princeton University Press
- Badvaev, A. V. & Ovarnström, A. 2002. Putting sexual traits into the context of an organism: a life-history perspective in studies of sexual selection. Auk, 119. 301-310.
- Bakker, T. C. M., Künzler, R. & Mazzi, D. 1999. Sexual selection: condition-related mate choice in sticklebacks. Nature, 401, 234.
- Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., Devevey, G. L. & Monaghan, P. 2003a. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. Proceedings of the Royal Society B, 270, 1691-1696.
- Blount, J. D., Metcalfe, N. B., Birkhead, T. R. & Surai, P. F. 2003b. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science, 300, 125-127.
- Brumm, H., Zollinger, S. A. & Slater, P. J. B. 2009. Developmental stress affects song learning but not song complexity and vocal amplitude in zebra finches. Behavioral Ecology and Sociobiology, **63**, 1387–1395.
- Buchanan, K. L., Spencer, K. A., Goldsmith, A. R. & Catchpole, C. K. 2003. Song as an honest signal of past developmental stress in the European starling (Sturnus vulgaris). Proceedings of the Royal Society B, 270, 1149-1156.
- Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A. R. & Catchpole, C. K. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proceedings of the Royal Society B*, **271**, 2381–2386. **Burley, N. T. & Foster, V. S.** 2006. Variation in female choice of mates: condition
- influences selectivity. Animal Behaviour, 72, 713-719.
- Clayton, N. S. 1990. Mate choice and pair formation in Timor and Australian mainland zebra finches. Animal Behaviour, 39, 474-480.
- Clayton, N. S. & Pröve, E. 1989. Song discrimination in female zebra finches and Bengalese finches. Animal Behaviour, **38**, 352–354. Clayton, N. S., Hodson, D. & Zann, R. A. 1991. Geographic variation in zebra finch
- subspecies. Emu, 91, 2-11.
- Collins, S. A. 1999. Is female preference for male repertoires due to sensory bias? Proceedings of the Royal Society B, 266, 2309-2314.
- Cotton, S., Small, J. & Pomiankowski, A. 2006. Sexual selection and conditiondependent mate preferences. Current Biology, 16, R755-R765.
- Fawcett, T. W. & Johnstone, R. A. 2003. Mate choice in the face of costly compe-tition. Behavioral Ecology, 14, 771–779.
- Garcia-Berthou, E. 2001. On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. Journal of Animal Ecology, 70, 708-711. Gil, D. & Gahr, M. 2002. The honesty of bird song: multiple constraints for multiple
- traits. Trends in Ecology & Evolution, 17, 133–141. Gil, D., Heim, C., Bulmer, E., Rocha, M., Puerta, M. & Naguib, M. 2004. Negative effects of early developmental stress on volk testosterone levels in a passerine
- bird. Journal of Experimental Biology, 207, 2215-2220. Gil, D., Naguib, M., Riebel, K., Rutstein, A. N. & Gahr, M. 2006. Early condition,
- song learning and the volume of song brain nuclei in the zebra finch (Taeniopygia guttata). Journal of Neurobiology, **66**, 1602–1612.
- Härdling, R. & Kokko, H. 2005. The evolution of prudent choice. Evolutionary Ecology Research, 7, 697–715.
- Holveck, M.-J. & Riebel, K. In press. Low-quality females prefer low-quality males when choosing a mate. Proceedings of the Royal Society B, published online 7 October 2009, doi:10.1098/rspb.2009.1222.
- Holveck, M.-J. & Riebel, K. 2007. Preferred songs predict preferred males: consistency and repeatability of zebra finch females across three test contexts. Animal Behaviour, 74, 297-309.

- Holveck, M.-J., Vieira de Castro, A. C., Lachlan, R. F., ten Cate, C. & Riebel, K. 2008. Accuracy of song syntax learning and singing consistency signal early condition in zebra finches. Behavioral Ecology, 19, 1267-1281.
- Hunt, J., Brooks, R. & Jennions, M. D. 2005. Female mate choice as a conditiondependent life-history trait. American Naturalist, 166, 79-92.
- Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biological Reviews of the Cambridge Philosophical Society, 72, 283-327.
- Jones, K. M., Monaghan, P. & Nager, R. G. 2001. Male mate choice and female fecundity in zebra finches. Animal Behaviour, 62, 1021-1026.
- de Kogel, C. H. 1997. Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. Journal of Animal Ecology, 66, 167-178.
- de Kogel, C. H. & Prijs, H. J. 1996. Effects of brood size manipulations on sexual attractiveness of offspring in the zebra finch. Animal Behaviour, 51, 699-708.
- Lauay, C., Gerlach, N. M., Adkins-Regan, E. & Devoogd, T. J. 2004. Female zebra finches require early song exposure to prefer high-quality song as adults. Animal Behaviour, **68**, 1249–1255.
- Leadbeater, E., Goller, F. & Riebel, K. 2005. Unusual phonation, covarying song characteristics and song preferences in female zebra finches. Animal Behaviour, 70. 909–919.
- Leitão, A., ten Cate, C. & Riebel, K. 2006. Within song complexity in a songbird is meaningful both to male and female receivers. Animal Behaviour, 71, 1289-1296
- MacDonald, I. F., Kempster, B., Zanette, L. & MacDougall-Shackleton, S. A. 2006. Early nutritional stress impairs development of a song-control brain region in both male and female juvenile song sparrows (Melospiza melodia) at the onset of song learning. Proceedings of the Royal Society B, 273, 2559-2564
- McGlothlin, J. W., Neudorf, D. L. H., Casto, M. Jr., V.N. & Ketterson, E. D. 2004. Elevated testosterone reduces choosiness in female dark-eyed juncos (Junco hyemalis): evidence for a hormonal constraint on sexual selection? Proceedings of the Royal Society B, **271**, 1377–1384.
- McNamara, J. M. & Houston, A. I. 1996. State-dependent life histories. Nature, 380, 215-221
- McNamara, J. M., Forslund, P. & Lang, A. 1999. An ESS model for divorce strategies in birds. Philosophical Transactions of the Royal Society B, 354, 223-236
- Naguib, M. & Gil, D. 2005. Transgeneration effects on body size caused by early developmental stress in zebra finches. Biology Letters, 1, 95-97.
- Naguib, M., Riebel, K., Marzal, A. & Gil, D. 2004. Nestling immunocompetence and testosterone covary with brood size in a songbird. Proceedings of the Royal Society B, **271**, 833–838.
- Naguib, M., Nemitz, A. & Gil, D. 2006. Maternal developmental stress reduces reproductive success of female offspring in zebra finches. Proceedings of the Royal Society B, 273, 1901-1905.
- Neubauer, R. L. 1999. Super-normal length song preferences of female zebra finches (Taeniopygia guttata) and a theory of the evolution of bird song. Evolutionary Ecology, **13**, 365–380.
- Nowicki, S., Peters, S. & Podos, J. 1998. Song learning, early nutrition and sexual selection in songbirds. American Zoologist, 18, 179-190.
- Owens, I. P. F., Rowe, C. & Thomas, A. L. R. 1999. Sexual selection, speciation and imprinting: separating the sheep from the goats. Trends in Ecology & Evolution. 14, 131-132.
- Qvarnström, A. 2001. Context-dependent genetic benefits from mate choice. Trends in Ecology & Evolution, 16, 5-7
- Riebel, K. 2000. Early experience leads to repeatable preferences for male song in female zebra finches. *Proceedings of the Royal Society B*, **267**, 2553–2558. **Riebel, K.** 2003a. The 'mute' sex revisited: vocal production and perception learning
- in female songbirds. Advances in the Study of Behavior, 33, 49-86.
- Riebel, K. 2003b. Developmental influences on auditory perception in female zebra finches: is there a sensitive phase for song preference learning? Animal Biology, **53**, 73–87
- **Riebel. K.** 2009. Song and female mate choice in zebra finches: a review. Advances in the Study of Behavior. 40, 197-238
- Riebel, K., Smallegange, I. M., Terpstra, N. J. & Bolhuis, J. J. 2002. Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. Proceedings of the Royal Society B, 269, 729-733.
- Searcy, W. A. & Yasukawa, K. 1996. Song and female choice. In: Ecology and Evolution of Acoustic Communication in Birds (Ed. by D. E. Kroodsma & E. H. Miller), pp. 454–473. Ithaca, New York: Comstock Publishing Associates.
- Spencer, K. A., Buchanan, K. L., Goldsmith, A. R. & Catchpole, C. K. 2003. Song as an honest signal of developmental stress in the zebra finch (Taeniopygia guttata). Hormones and Behavior, 44, 132-139.
- Spencer, K. A., Wimpenny, J. H., Buchanan, K. L., Lovell, P. G., Goldsmith, A. R. & Catchpole, C. K. 2005. Developmental stress affects the attractiveness of male song and female choice in the zebra finch (Taeniopygia guttata). Behavioral Ecology and Sociobiology, 58, 423-428.
- Syriatowicz, A. & Brooks, R. 2004. Sexual responsiveness is condition-dependent in female guppies, but preference functions are not. BMC Ecology, 4, 5.
- Cate, C. & Vos, D. R. 1999. Sexual imprinting and evolutionary processes in birds: a reassessment. Advances in the Study of Behavior, 28, 1-31
- Tschirren, B., Rutstein, A. N., Postma, E., Mariette, M. & Griffith, S. C. 2009. Shortand long-term consequences of early developmental conditions: a case study on wild and domesticated zebra finches. Journal of Evolutionary Biology, 22, 387-395.
- Verhulst, S. 2003. Song as a signal to negotiate a sexual conflict? Animal Biology, 53, 159-171.

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- Verhulst, S., Holveck, M.-J. & Riebel, K. 2006. Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. Biology Letters, 2, 478-480. Verzijden, M. N. & ten Cate, C. 2007. Early learning influences species assortative
- Werzijden, M. N. & Cher, C. 2007. Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. *Biology Letters*, **3**, 134–136.
 Verzijden, M. N., Etman, E., van Heijningen, C., van der Linden, M. & ten Cate, C. 2007. Song discrimination learning in zebra finches induces highly divergent responses to novel songs. *Proceedings of the Royal Society B*, **274**, 295–301.
- Vyas, A., Harding, C., Borg, L. & Bogdan, D. 2009. Acoustic characteristics, early experience, and endocrine status interact to modulate female zebra finches' behavioral responses to songs. Hormones and Behavior, 55, 50-59.
- West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. New York: Oxford University Press.
- Widemo, F. & Saether, S. A. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. Trends in Ecology & Evolution, **14**, 26–31. **Zann, R. A.** 1996. The Zebra Finch: a Synthesis of Field and Laboratory Studies. Oxford:
- Oxford University Press.
- Zann, R. & Cash, E. 2008. Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (Taeniopygia guttata). Behavioral Ecology and Sociobiology, 62, 391-400.