Original Article

Developmental stressors that impair song learning in males do not appear to affect female preferences for song complexity in the zebra finch

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A number of recent studies have provided evidence that the environmental factors experienced during development contribute to variation between females in the direction and strength of their mating preferences. The developmental stress hypothesis suggests that the complex male songs of many songbirds and female preferences for those complex songs have evolved because song quality reflects how well an individual was able to cope with suboptimal developmental conditions. In this study, we tested whether female preferences for song complexity are affected by developmental stress. Female zebra finches, *Taeniopygia guttata*, were raised under conditions of nutritional stress or control conditions. In adulthood, their song preferences were tested in an operant setup where females could trigger playback of song recordings by landing on different perches. The subjects could choose between pairs of songs that were digitally manipulated to ensure that they varied only in the number of syllables in the song. Across all subjects, there was a significant preference for the more complex song of the song pair, but there was no difference between the treatment groups in the direction or strength of their preference. These results suggest that adverse developmental conditions do not impair females' ability or motivation to discriminate between songs on the basis of complexity and thus to obtain information about potential mates' developmental history. *Key words:* bird song, developmental stress, early environment, female choice, female condition, mate choice, mating preferences, sexual selection, *Taeniopygia guttata*, zebra finch. *[Behav Ecol 22:566–573 (2011)]*

The importance of female choice as a powerful and widespread selective force is well documented (Andersson 1994). However, relatively little attention has been paid to the role individual variation in female preference plays in mate choice (Jennions and Petrie 1997; Widemo and Saether 1999; Qvarnstrom et al. 2000; Cotton et al. 2006). In particular, although there is good empirical support for the condition dependence of female preferences (Cotton et al. 2006), the contribution to female preference of variation in environmental factors that might influence growth has rarely been addressed. Hunt et al. (2005) found that the strength of the mating preferences of female black field crickets (*Teleogryllus commodus*) was affected by the quality of the diet on which they were reared, with those raised on a high protein diet showing stronger preferences and more sexual responsiveness in phonotaxis tests. Female *Schizocosa* wolf spiders raised on a high nutrition diet preferred males raised on a similar diet, whereas those raised on a diet of lower quality showed no preference (Hebets et al. 2008). In vertebrates, Holveck and Riebel (2010) found that the brood size in which female zebra finches (*Taeniopygia guttata*) were reared affected the direction of their preference for male songs in adulthood, whereas Riebel et al. (2009) found that female zebra finches reared in large or medium size broods exhibited weaker song preferences than those from small broods when offered a choice of 2 unfamiliar songs. Using the same model species, we have recently found that zebra finch females reared under conditions of nutritional stress were less active than controls during mate choice trials, suggesting a difference in mate choice strategies (Woodgate et al. 2010).

There are several mechanisms by which unfavorable conditions during development could affect female choice in adulthood. Detrimental effects on development might impair the ability of females to accurately assess male secondary sexual traits, for example via the well-documented deleterious effects of stress on neural development (McEwen and Sapolsky 1995;

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de Kloet 2000). Even in the absence of a direct effect on trait perception, adverse developmental conditions can lead to reduced phenotypic quality (e.g., Birkhead et al. 1999; Metcalfe and Monaghan 2001, 2003; Arnold et al. 2007). Low-quality females may prioritize mate choice cues differently to higher quality females (Fawcett and Johnstone 2003b) and are predicted to invest less time and resources sampling potential mates and to be less discriminating in their mate choice (Cotton et al. 2006). Finally, low-quality females may seek to limit the cost of competition for mates and minimize the risk of failing to mate, by expressing a preference for low-quality males (Fawcett and Johnstone 2003a; Hardling and Kokko 2005).

The potential effects of developmental stress on song preferences in songbirds are of particular interest because there is mounting evidence that the complexity of male songs may function as an honest signal of how an individual was able to cope with developmental stress. This was proposed as the nutritional stress hypothesis by Nowicki et al. (1998) and later extended to cover all potential environmental stressors as the developmental stress hypothesis (Buchanan et al. 2003). A number of studies over the past several years have provided evidence that developmental stress may have a detrimental effect on neural development in the HVC region of the songbird brain, a nucleus associated with the learning and production of song (Nowicki et al. 2002a; Buchanan et al. 2004; Spencer, Buchanan, et al. 2005; MacDonald et al. 2006). Stressors experienced during early development have been found to have a detrimental effect on song learning in the swamp sparrow, *Melospiza georgiana* (Nowicki et al. 2002a), and zebra finch (Holveck et al. 2008; Brumm et al. 2009); and on measures of song complexity in the European starling, *Sturnus vulgaris* (Spencer et al. 2004), canary, *Serinus canaria* (Spencer, Buchanan, et al. 2005), and zebra finch (Spencer et al. 2003; Zann and Cash 2008). Female zebra finches prefer complex songs (Clayton and Prove 1989), and the less complex songs of stressed male zebra finches are less attractive to females than those of control males (Spencer, Wimpenny, et al. 2005). In wild song sparrows, *M. melodia*, adult males displaying a larger stress response both sang less complex songs and were less likely to return to breed the following year (MacDougall-Shackleton et al. 2009). However, some studies have failed to demonstrate any effect of developmental stress on neural development, song structure or attractiveness, in male zebra finches (Gil et al. 2006; Naguib et al. 2008), and Bolund et al. (2010) found no evidence for a heightened effect of developmental conditions on sexually selected traits, such as song, compared with nonsexually selected traits, in zebra finches.

Female songbirds of many species also possess the song-control nuclei of the brain, albeit often much reduced in volume (Nottebohm and Arnold 1976; Ball and MacDougall-Shackleton 2001), and a number of studies have provided evidence that these neural structures play a role in the perception of, and preference for, male song (Brenowitz 1991; Del Negro et al. 1998; Halle et al. 2002; Leitner and Catchpole 2002). MacDonald et al. (2006) found that nutritional stress during development led to reduced HVC volume in female as well as male song sparrows, so might affect female perception or preference for song. Several other brain regions, notably the caudomedial nidopallium (NCM) and caudomedial mesopallium, have been found to be involved in song recognition in birds of both sexes (Bolhuis et al. 2000; Terpstra et al. 2004; Gobes and Bolhuis 2007), and it has been suggested that the NCM forms the neural substrate for memory of the tutor song (Bolhuis and Gahr 2006). It is not known whether developmental stress would have similar deleterious effects on the development of these areas to those reported for the HVC.

If nutritional stress has a detrimental effect on females' ability to discriminate between male songs, this would have important implications for the developmental stress hypothesis because low-quality females would be unable to acquire information pertaining to male quality, but no studies have directly addressed this question to date. Two studies have, however, investigated the influence of developmental conditions on female song preferences: Riebel et al. (2009) allowed female zebra finches from small or large broods to choose between recordings of unmanipulated male songs in operant song preference tests and found that those from small broods exhibited stronger preferences than those from medium or large broods when choosing between unfamiliar songs. When forced to choose between an unfamiliar song and that of their tutor, females from all treatments preferred the tutor song, implying that any effect of developmental stress in song discrimination ability was not large enough to prevent recognition of the tutor song. In a similar study, Holveck and Riebel (2010) found that female zebra finches raised in small broods had a significant preference for the song of males raised in small broods while those from larger broods preferred the song of males raised in large broods, implying assortative preferences by phenotypic quality. Again, these results suggest that there was no effect of treatment on females' ability to discriminate between the stimulus songs.

The developmental stress hypothesis proposes that male song complexity has been selected for as a sexual signal because it reflects developmental conditions (Nowicki et al. 1998, 2002a). We sought to test the hypothesis that developmental stress experienced by females could alter the strength or direction of their preference when offered a choice between songs of differing complexity. Female zebra finches raised under stress or control conditions were able to trigger playback of song recordings that had been digitally edited to vary in complexity. Female preferences were evaluated using an operant song preference design similar to previous studies which allows us to measure female preference in isolation from other male traits and without the influence of male preferences (Riebel 2000). Similar choice tests predict preferences for live males (Holveck and Riebel 2007) and have been used in previous studies investigating the effects of developmental conditions on female preference (Riebel et al. 2009; Holveck and Riebel 2010).

MATERIALS AND METHODS

Experimental subjects

Subjects were 32 adult female zebra finches raised at the Max Planck Institute for Ornithology in Seewiesen, Germany under control conditions or nutritional stress. These females were the offspring of 21 pairs of laboratory raised finches and included 7 pairs and 3 trios of genetic sisters. The experimental manipulation of nestling condition was achieved by limiting the food intake rate of nestlings and so impairing growth rates, as in previous studies (Spencer et al. 2003; Buchanan et al. 2004; Woodgate et al. 2010). Briefly, each pair of nesting zebra finches raised 2 broods in succession under identical conditions. Pairs were allocated at random either to the control treatment, in which they were provided with ad libitum seed mix, or to the nutritional stress treatment in which a limited mass of seed was provided, mixed with husks in a 1:2 ratio by volume (Lemon 1993; Spencer et al. 2003). The mass of seed provided to nests under the stress treatment was adjusted for brood size and the age of the chicks. Half of the chicks in each brood were cross fostered, at random, to another brood of similar age within 3 days of hatching. These treatments are described in greater detail in Woodgate et al. (2010).

The female offspring were transferred to the University of Bristol as adults (mean age in days ± standard error [SE] =

189.7 ± 8.2) in September 2006, where they were maintained at 21 °C on a 12:12 light:dark photoperiod and provided with ad libitum seed mix and water. There were 8 possible combinations of the 3 treatment factors: control or stress treatment, cross fostered or not, and brood 1 or 2. Four females from each combination of treatments were chosen as subjects in the song preference trials, giving a total sample size of 32. If more than 4 females were available from a particular treatment combination, 4 subjects were selected at random. Twenty-eight of the subjects had previously been used in visual mate choice trials, described in Woodgate et al. (2010).

Song discrimination apparatus

We tested female song preferences using a song discrimination apparatus adapted from that used by Spencer, Buchanan, et al. (2005). This consists of an L-shaped wooden box with 2 "active perches," one in each arm of the apparatus. These perches were connected via microswitches to a computer (RM, Milton Park, UK), which logged the number of hops made by a female on each perch (PerchLog, P.G. Lovell, UK). On activation, PerchLog also played a prerecorded sound from a speaker (Sony, Weybridge, UK) mounted above the perch, with a maximum amplitude of 80.4 dB at the level of the perch (23 cm beneath the speaker). When a playback was in progress, further perches were logged but did not trigger another playback. Food and water were provided ad libitum in dishes mounted above the active perches. A third, central, perch was situated between the arms of the apparatus and was not electronically monitored. To encourage females to spend their time on the perches rather than the floor, the bottom of the apparatus was filled with water to a depth of 1 cm. This had the desired effect of encouraging females to sit on the perches, only rarely landing on the bottom of the apparatus. Birds spent a maximum of 1 h/day in the apparatus and spent a total of 7 h in the apparatus over the course of the experiment.

Song preference tests

During the experiment, each female underwent 3 acclimatization trials on consecutive days to familiarize them with the novel apparatus and with the experimental protocol. On the 2 days following the last acclimatization trial, they experienced 2 experimental trials, each with a different pair of stimulus songs. Approximately 2–5 weeks later, each female underwent a further 2 trials on consecutive days with the same 2 pairs of stimulus songs they had experienced in the initial trials. Thus, each subject experienced 3 acclimatization trials and 4 experimental trials: 2 trials with each of 2 pairs of stimulus songs. All trials, acclimatization and experimental, lasted 1 h each.

Females were allocated at random to 1 of 6 groups, and the order in which each group underwent their trials was determined at random. Thus, all females in a group underwent 5 consecutive days of trials before the females of another group began their trials. Females remained in their groups for the final 2 trials and were used in the same order as before. Within groups, each female experienced all her trials at the same time of day, but time of day was balanced across females from the different combinations of treatments to control for any effect of time of day on behavior.

At the start of each trial, the female was induced by the experimenter (J.L.W.) to land several times on each of the active perches in order to familiarize them with the stimulus songs played back by each perch (mean number of songs played during this familiarization period ± 1 standard deviation: 25.8 ± 3.2). The 1-h trial began when the experimenter left the room. During acclimatization trials, one active perch triggered playback of a male zebra finch song, whereas the other triggered playback of a sample of guitar music of identical length and mean amplitude. These sounds were assigned to the left and right perches at random.

In the experimental trials, landing on one perch triggered playback of a zebra finch song, and the other played a simplified version of the same song. The allocation of these songs to the left and right perches was balanced within individual females, such that if the complex song was triggered by the left perch in the first experimental trial, it was triggered by the right perch in the second trial with that pair of stimulus songs. In each trial, we measured 3 response variables: 1) the total number of hops made on both active perches; 2) preference strength, defined as the proportion of total hops that was made on the perch that received the greater number of hops, regardless of whether that perch triggered playback of the control or simplified song; and 3) preference for the control (more complex) song, defined as the proportion of total hops that were made on the active perch that triggered playback of the control song.

Stimulus songs

In each experimental trial, the female could choose between a pair of stimulus songs that differed only in complexity, consisting of one control and one simplified song. Six pairs of stimulus songs were created from recordings of 6 male zebra finches. Each female experienced trials with 2 of the 6 pairs of stimulus songs. Song pairs were assigned in a balanced manner across treatment groups such that each song pair was used with equal numbers of control and stressed females. All the stimulus songs were unfamiliar to the subjects in this experiment.

Stimulus songs were constructed by editing the source recordings using Raven 1.2 sound analysis software (Cornell, USA). Control song files consisted of a single phrase excerpted from the source recording and repeated 5 times to form a song bout. The first repetition of the phrase was preceded by 4 introductory notes taken from the same source recording. Four song files were constructed for each song, each using a different recording of the song phrase, selected at random from the same source recording. During the experiment, each hop on the relevant perch triggered the playback of 1 of the 4 song files at random. This allowed us to control for subtle variation in song production.

Simplified songs were constructed by selectively deleting syllables from the control song files (Figure 1). In this way, we produced songs that mimicked the simplified song structure of males that have experienced developmental stress (Spencer et al. 2003) but were identical to the control songs in all other respects. From each song phrase, we removed 2 syllables, one that was repeated elsewhere in the song phrase and one that was unique. The first syllable of a phrase was never removed, and 2 consecutive syllables were never removed. Of the remaining syllables, which fulfilled these requirements, the 2 to be deleted were chosen at random.

Control songs were significantly longer than simplified songs (mean phrase length [s] ± 1 SE control = 0.54 ± 0.03; simplified = 0.40 ± 0.03; $t_9 = 3.34$, $P = 0.009$) and contained more syllables (mean number of syllables per phrase ± 1 SE control = 7.33 ± 0.49; simplified = 5.33 ± 0.49; $t_{10} = 2.86$, $P = 0.017$). The reduction in the number of different syllable types in the song phrase of the simplified stimulus songs fell short of statistical significance (mean number of different syllables per phrase ± 1 SE control = 5.83 ± 0.54; simplified = 4.83 ± 0.54; $t_{10} = 1.30$, $P = 0.222$). The characteristics exhibited by the manipulated songs are consistent with the songs of stressed males (Spencer et al. 2003).
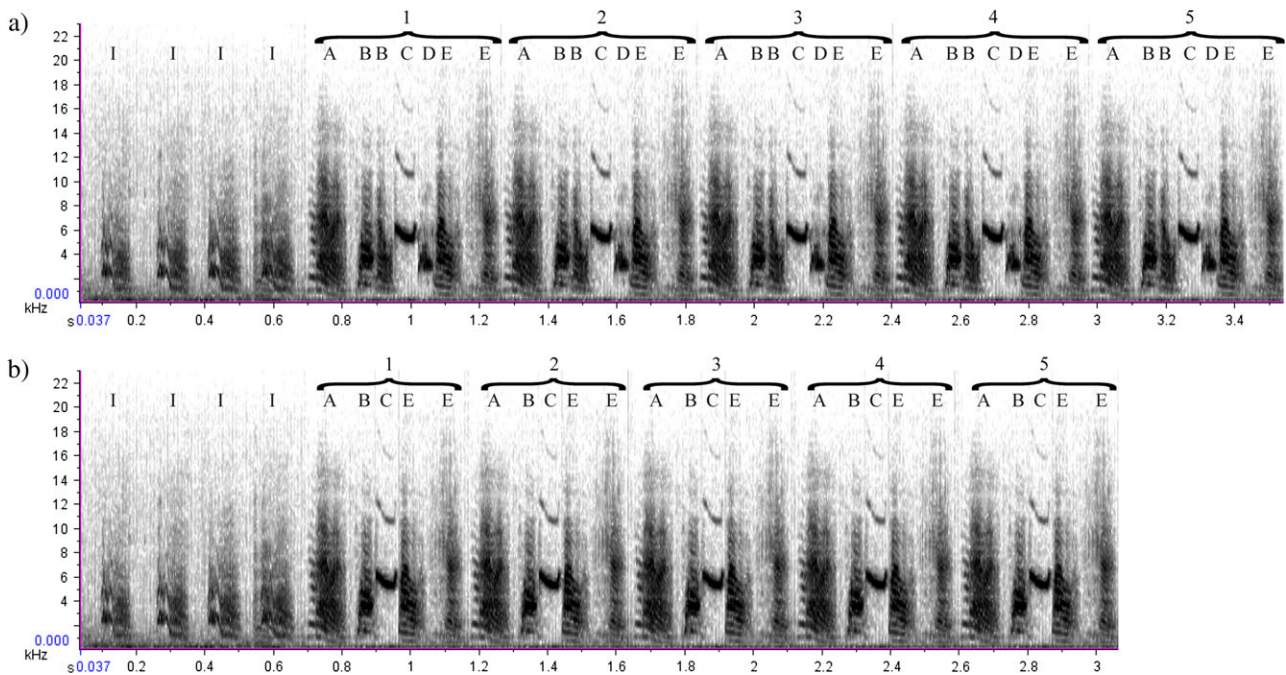
Figure 1
Example spectrograms of stimulus songs used in the song preference tests. Song a) is a control song bout consisting of 4 introductory syllables (I) followed by 5 repetitions of the song phrase. Song b) is the corresponding simplified song, created by deleting 2 syllables: D and the second repeat of syllable B.

Statistical analysis

All analyses were carried out using Minitab 15 statistical software (Minitab Inc., State College, PA). The data used in the analysis of the song preference tests were the mean activity level and preference ratios from the 2 trials each female experienced with each pair of stimulus songs. We used the mean activity level data to test whether there was a treatment effect on females' motivation to sample the song of potential mates, and the preference ratios to test whether stress affected the direction or strength of female preference. One female from the stress treatment group did not hop at all during any of her 4 trials so was excluded from the analysis, reducing the total sample size to 31. Hopping data were normalized by log transformation and the preference ratios by arcsine square root transformation. These data were analyzed with repeated measures general linear models. We started with a full factorial model with stress treatment, cross-fostering treatment, brood number and stimulus song pair as fixed factors, and female identity (nested within each combination of treatments) entered as a random effect. Nonsignificant interaction terms, and then factors, were sequentially deleted from the model, resulting in a final model that consisted of stress treatment and song pair as fixed factors and female identity as the random factor.

The mean number of hops made by control females had a greater SE than did the activity of females from the stress treatment group, due to the influence of 2 very active females in that group. To ensure that the influence of these birds did not drive the results of the activity levels analysis, we also performed a Kruskal–Wallis test to test whether stressed and control females differed in the total number of hops made across all 4 trials.

To test whether females showed an overall preference for complex songs, we calculated the proportion of each female's total hops, across all 4 trials, that were made on the perch triggering the more complex song of a pair. A Wilcoxon signed-rank test was used to test whether this differed from 0.5.

The subjects in this experiment included several sets of genetic sisters. Since activity levels in a different type of mate choice apparatus are known to have a heritable component (Forstmeier 2005), we used another set of general linear models to test for a genetic family effect on female behavior. The data used in this analysis were the mean hops and preference ratios across all 4 trials experienced by each female. Again, the hopping data were log transformed and the preference ratios arcsine square root transformed to normalize them. The model consisted of stress treatment as a fixed factor and genetic family as a random effect.

RESULTS

There was no treatment effect on the total number of hops made during preference trials, suggesting that females raised under control conditions or nutritional stress were equally motivated to hear male song (Figure 2; $F_{1,29} = 0.03$, $P = 0.875$; $H_1 = 0.08$, $P = 0.782$). Females from the control and stress treatments did not differ in overall strength of preference (Figure 3; $F_{1,29} = 0.54$, $P = 0.467$). The 6 pairs of stimulus songs did not differ in terms of the overall activity they elicited ($F_{1,26} = 0.52$, $P = 0.758$), or the strength of preference females displayed for their preferred song ($F_{1,25} = 1.44$, $P = 0.245$).

Control and stressed females did not differ in their degree of preference for the more complex song in a pair of stimulus songs (Figure 4; $F_{1,29} = 0.12$, $P = 0.732$) nor was their preference affected by which stimulus song pair was presented ($F_{1,25} = 0.37$, $P = 0.863$). Across all song pairs, females preferred the more complex song in the pair ($W = 331.5$, $N = 31$, $P = 0.01$).

There was no effect of the genetic family of origin on females' activity levels ($F_{20,9} = 0.83$, $P = 0.652$) nor on the strength of preference they showed for either their preferred song in each pair ($F_{20,9} = 1.61$, $P = 0.233$) or for the control songs ($F_{20,9} = 0.91$, $P = 0.597$).
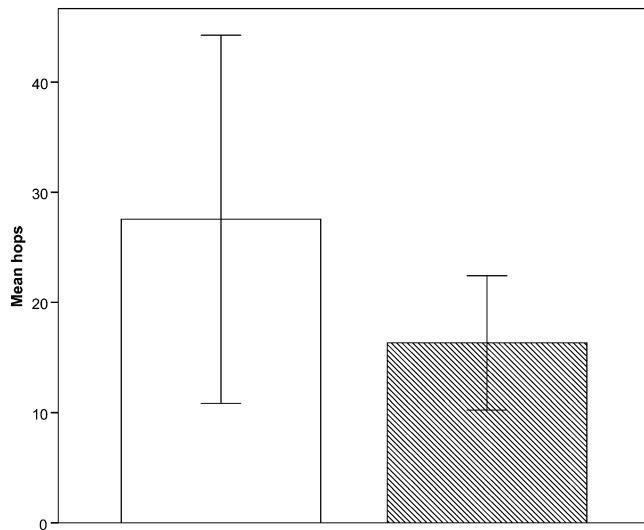
Figure 2

Mean number of hops (±1 SE) made on both active perches per 1-h trial by control (white bars) and stressed females (shaded bars).

DISCUSSION

Within the oscine group, complex songs are thought to have evolved as a result of female preferences (Catchpole and Slater 2008), and song complexity is hypothesized to honestly reflect how well the singer fared with stressors in the rearing environment (Nowicki et al. 1998, 2002a). There is evidence that female zebra finches have an inherent bias toward more complex songs (Clayton and Prove 1989; Collins 1999; Vyas et al. 2009). However, female preferences in many species have found to be influenced by treatments that manipulated early growth during development (Hunt et al. 2005; Hebets et al. 2008; Riebel et al. 2009; Holveck and Riebel 2010; Woodgate et al. 2010), and female birds are subject to the same variability
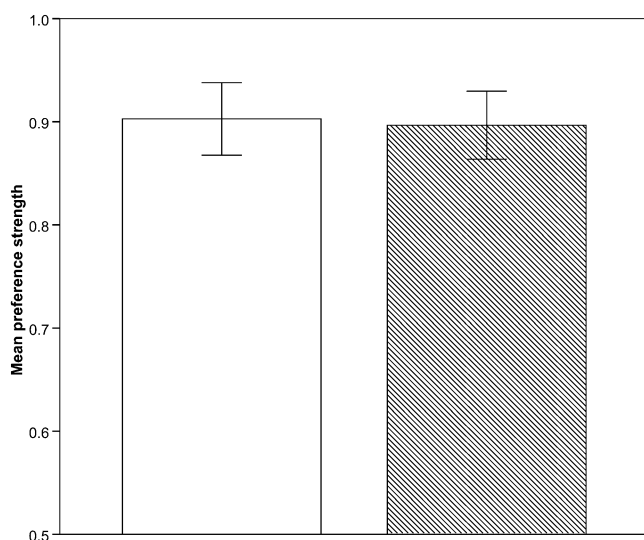

Figure 3

Mean preference strength (±1 SE) of control (white bars) and stressed females (shaded bars), for preferred song. Preference strength is calculated as: number of hops on the perch that received the greatest number of hops/total number of hops across both active perches. The scale starts at 0.5 because it is not possible for the preferred song to receive less than 50% of the total hops.
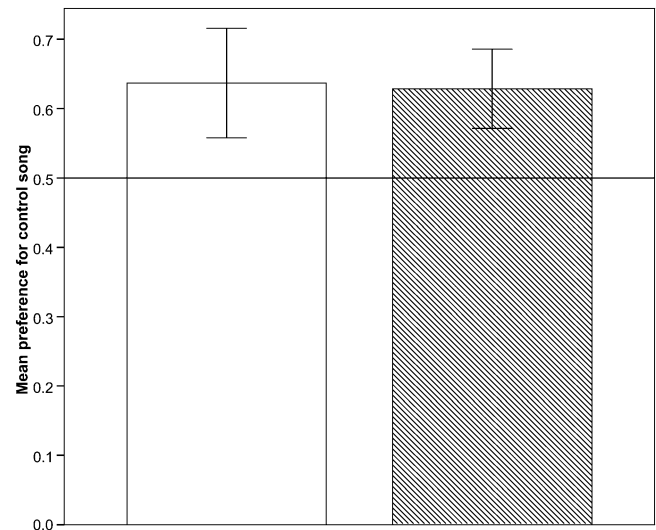

Figure 4

Mean preference (±1 SE) for the control song in a pair of stimulus songs, by control (white bars) and stressed females (shaded bars). Preference is calculated as: number of hops on the perch that triggered playback of the control song/total number of hops across both active perches. A ratio greater than 0.5 indicates a preference for the control song.

in developmental conditions as males. We tested whether female preferences for song complexity are affected by developmental stress, which would have implications for the potential of song complexity to serve as an honest signal. When control females or females that had experienced nutritional stress during development were allowed to choose between songs of differing complexity, we found that females from both treatments preferred the more complex song in a pair, but there was no difference in the strength of this preference between the treatment groups.

There are several possible explanations for the observed lack of a treatment effect on female preference. Firstly, is it possible that the experimental paradigm we used to investigate song preferences is not capable of detecting real differences in female preferences? Previous studies suggest this is unlikely: Operant song choice setups similar to ours, in which females can choose to trigger song playbacks by landing on a perch or pecking a button, have frequently been used to investigate song preferences (Riebel and Slater 1998; Collins 1999; Gentner and Hulse 2000; Spencer, Wimpenny, et al. 2005; Holveck and Riebel 2007), and preferences expressed in such experiments are consistent with choices made between live males (Holveck and Riebel 2007). Although fewer studies have used this paradigm to investigate differences in preferences between groups of females, it has been used successfully to detect differences in the strength or direction of preferences between groups that differed in previous exposure to male song (Riebel 2000), or the brood size in which they were reared (Riebel et al. 2009; Holveck and Riebel 2010). Secondly, perhaps the stimulus songs, which were created by editing the same source files, were not sufficiently different from one another to elicit different responses from the subjects. This is unlikely for 2 reasons: In terms of duration and complexity, the simplified stimulus songs resemble those of stressed males recorded by Spencer, Wimpenny, et al. (2005), which were shown to be less attractive to females than those of control males using the same song discrimination apparatus as in our study. In addition, across both groups of females in this study, we found a significant preference for

control songs, strongly suggesting that females were able to differentiate between the control and simplified songs, and they elicited different levels of preference. A third possibility is that the nutritional stress treatment used in this experiment might not have been sufficiently severe to induce differences in female development but that more extreme levels of stress might have greater effects on female choice behavior. The stressor applied in this study was designed to mimic levels of nutritional stress that are known to deleteriously affect survivorship and reproduction in zebra finches (Lemon 1993). We have previously shown that females reared under the stress treatment in this study had reduced nestling growth rates and were less active than controls in a mate choice context (Woodgate et al. 2010), confirming that the treatment did induce stress in the subjects. We cannot, however, rule out the possibility that more drastic levels of developmental stress might have greater effects. Likewise, it is possible that the timing of the nutritional stress treatment was inappropriate for manipulating female neural development and that stressors experienced later in the juvenile stage might have different effects. Nonetheless, as female neural development is thought to occur synchronously with that of males, and similar treatments have been found to affect male neural development in previous studies (Buchanan et al. 2004), we think this is unlikely. Perhaps the most parsimonious explanation for our results, then, is that adverse rearing conditions did not affect the strength or direction of female preferences for songs of differing complexity.

To date, there is weak support for an effect of developmental stress on female preferences. Two other studies have examined the effects of developmental stress on female song preferences in zebra finches: Riebel et al. (2009) found that females stressed by a brood manipulation differed in preference strength (those from small broods having a stronger preference) when choosing between unfamiliar songs, although when one song was that of their tutor, there was no difference in preference. Holveck and Riebel (2010) reported assortative preference for songs with females preferring the songs of males raised in a similar brood size. By contrast, Schielzeth et al. (2010) found no evidence for early rearing effects on female preference in an unmanipulated population of zebra finches. In terms of the underlying mechanism, MacDonald et al. (2006) found that nutritional stress had a deleterious effect on HVC growth in juvenile female song sparrows, but no studies have looked at the effects of developmental stress on female brain development in zebra finches or whether the differences in HVC volume found by MacDonald et al. extended into adulthood. Our results, however, suggest that any effect of stress on brain development is insufficient to prevent females discriminating between biologically relevant levels of variation in song complexity.

Theory suggests that low-quality females might preferentially mate with low-quality males under certain conditions, for example, if they cannot bear the costs of a prolonged search for a high-quality mate or are likely to lose out in competition for mates with higher quality females (Fawcett and Johnstone 2003a; Hardling and Kokko 2005). This would lead to assortative mating by phenotypic quality. Holveck and Riebel (2010) found that female zebra finches preferred the songs of males that were raised in similarly sized broods to themselves and argue that this is evidence that low-quality females prefer low-quality males. Preference strength is expected to reflect the costs and benefits of expressing a preference (regardless of the direction of preference), so that preferences will be weaker when costly and stronger if the benefits of being discriminatory are large (Cotton et al. 2006). The difference between the ability of high-and

low-quality females to bear the costs of choice is predicted to lead to reduced preference strength in low-quality females. In this study, stressed and control females did not differ in the strength or direction of their preferences suggesting that the potential benefits of using song complexity in mate choice decisions are large enough to counteract the costs of strong preference. One such benefit of attending to differences in song complexity is that complexity may accurately signal information about the male's developmental history (Nowicki et al. 1998; Buchanan et al. 2003; Spencer et al. 2003). Riebel et al. (2009) found that females originating from smaller broods expressed stronger preferences than those from large broods only when choosing between 2 unfamiliar songs. Females showed no preference for longer songs suggesting that there was little variation in the attractiveness of the stimulus songs. When the benefits of discriminating between songs are relatively small, as when Riebel et al. (2009) presented a choice between unfamiliar males, females that experienced adverse developmental conditions may be less able to bear the costs of choosiness and display weaker preferences than females raised under more benign conditions. By contrast, it is likely that when the benefits of choice are greater, such as when songs differ in a way that might signal differences in male fitness or when females have learned preferences for a song they were exposed to early in life (Miller 1979; Clayton 1988; Riebel et al. 2002), even females of low quality can maximize their fitness by expressing strong preferences.

The stimulus songs used by Holveck and Riebel (2010) were recorded from males raised in large or small broods. Interestingly, these songs did not differ in length or complexity, although both the phrase duration and the duration of sound production within the phrase (excluding silences between syllables) were found to be more variable in males from large broods (Holveck et al. 2008). Males from large broods also showed less accuracy in syntax learning from their tutor's song but since Holveck and Riebel (2010) only found evidence of assortative preferences when females were tested with unfamiliar songs it seems most likely that they used differences in singing consistency to distinguish between males from small and large broods. Rather than present song recordings from males raised under different conditions, in this study, we digitally edited recordings of zebra finch songs to create stimulus sets that differed only in complexity and in phrase duration (shorter songs being an inevitable consequence of removing sections of the original phrase). Thus, we were able to control for variation in all other respects (e.g., variation in singing performance, peak frequency, amplitude, rate of syllable production, types of syllables present in the song, syllable order etc.). This raises the question of whether the effects of brood size on song preferences so far documented (Riebel et al. 2009; Holveck and Riebel 2010) are due to the effects of developmental stress or represent preferences for characteristics that are intrinsically linked to brood size.

Female zebra finches respond to a number of auditory, visual, morphological, and behavioral traits when choosing a mate (Burley and Coopersmith 1987; Swaddle and Cuthill 1994; Bennett et al. 1996; Zann 1996; Williams 2001; Roberts et al. 2007; Riebel 2009). The strength and direction of preferences for some or many of these cues are likely to be condition dependent (Fawcett and Johnstone 2003a; Hardling and Kokko 2005; Cotton et al. 2006), and females of differing quality might prioritize cues differently (Fawcett and Johnstone 2003b), so the outcome of mate choice may differ between stressed and control females despite their preferences for song complexity being unaffected.

A final way in which developmental stress could affect female choice is by reducing the time and resources they invest in sampling potential mates. In this study, we found no significant

effect of the stress treatment on the total number of hops made on active perches. The mean activity level of control females had a greater SE than that of stressed females (see Figure 2), which could make it difficult to detect a small but real stress effect on hopping behavior in this experiment. However, a Kruskal–Wallis test confirmed that females from the stress and control treatments did not differ in their median activity, lending support to the conclusion that stress did not affect females' motivation to trigger song playbacks. Previous studies found no effect of brood size manipulations on the number of key pecks in similar song choice experiments (Riebel et al. 2009; Holveck and Riebel 2010). This suggests that, in this situation, developmental conditions have little or no effect on motivation to sample male songs, perhaps because the costs of sampling (i.e., flying to the relevant perch and hopping on it or pecking a key) are too low to have a measurable effect on behavior.

Our results suggest that there was no effect of developmental stress on the ability of female zebra finches to discriminate between male songs that differ in complexity. Females are under selection pressure to respond only to male signals that contain reliable and valuable information, when choosing a mate (Dawkins and Krebs 1978). If male song accurately signals information pertaining to the male's own developmental history then all females, even those that experienced developmental stress themselves, may be under selection to maintain the ability to access this information and to utilize it in mate selection. Our results are consistent with the developmental stress hypothesis (Nowicki et al. 1998, 2002b; Buchanan et al. 2003) because the value of an honest signal rests on the ability of the intended recipient to access the information it encodes.

In this study, nutritional stress did not affect the direction or strength of female preferences for song complexity. Although we did not directly address females' ability to discriminate between songs of differing complexity, our results strongly imply that it was not affected by the stress treatment. It is increasingly clear that environmental factors experienced during early life can affect female mate choice behavior (Hunt et al. 2005; Hebets et al. 2008; Riebel et al. 2009; Holveck and Riebel 2010; Woodgate et al. 2010), but preferences for complex song may be robust to developmental stress, perhaps owing to an inherent sensory bias toward complex songs (Collins 1999), potentially providing a strong selective force in the evolution of complex song.

REFERENCES

Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.

Arnold KE, Blount JD, Metcalfe NB, Orr KJ, Adam A, Houston D, Monaghan P. 2007. Sex-specific differences in compensation for poor neonatal nutrition in the zebra finch, Taeniopygia guttata. J Avian Biol. 38:356–366.

Ball GF, MacDougall-Shackleton SA. 2001. Sex differences in songbirds 25 years later: what have we learned and where do we go? Microsc Res Tech. 54:327–334.

Bennett ATD, Cuthill IC, Partridge JC, Maier EJ. 1996. Ultraviolet vision and mate choice in zebra finches. Nature. 380:433–435.

Birkhead TR, Fletcher F, Pellatt EJ. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. Proc R Soc Lond Ser B Biol Sci. 266:385–390.

Bolhuis JJ, Gahr M. 2006. Neural mechanisms of birdsong memory. Nat Rev Neurosci. 7:347–357.

Bolhuis JJ, Zijlstra GGO, den Boer-Visser AM, Van der Zee EA. 2000. Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Natl Acad Sci U S A. 97: 2282–2285.

Bolund E, Schielzeth H, Forstmeier W. 2010. No heightened condition dependence of zebra finch ornaments—a quantitative genetic approach. J Evol Biol. 23:586–597.

Brenowitz EA. 1991. Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. Science. 251: 303–305.

Brumm H, Zollinger SA, Slater PJB. 2009. Developmental stress affects song learning but not song complexity and vocal amplitude in zebra finches. Behav Ecol Sociobiol. 63:1387–1395.

Buchanan KL, Leitner S, Spencer KA, Goldsmith AR, Catchpole CK. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Proc R Soc Lond Ser B Biol Sci. 271:2381–2386.

Buchanan KL, Spencer KA, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of past developmental stress in the European starling (Sturnus vulgaris). Proc R Soc Lond Ser B Biol Sci. 270: 1149–1156.

Burley N, Coopersmith CB. 1987. Bill color preferences of zebra finches. Ethology. 76:133–151.

Catchpole CK, Slater PJB. 2008. Bird song: biological themes and variations. New York: Cambridge University Press.

Clayton NS. 1988. Song discrimination learning in zebra finches. Anim Behav. 36:1016–1024.

Clayton NS, Prove E. 1989. Song discrimination in female zebra finches and Bengalese finches. Anim Behav. 38:352–354.

Collins SA. 1999. Is female preference for male repertoires due to sensory bias? Proc R Soc Lond Ser B Biol Sci. 266:2309–2314.

Cotton S, Small J, Pomiankowski A. 2006. Sexual selection and condition-dependent mate preferences. Curr Biol. 16:R755–R765.

Dawkins R, Krebs JR. 1978. Animal signals: information or manipulation? In: Krebs JR, Davies NB, editors. Behavioural ecology. Sunderland (MA): Sinauer. p. 282–309.

de Kloet ER. 2000. Stress in the brain. Eur J Pharmacol. 405:187–198.

Del Negro C, Gahr M, Leboucher G, Kreutzer M. 1998. The selectivity of sexual responses to song displays: effects of partial chemical lesion of the HVC in female canaries. Behav Brain Res. 96:151–159.

Fawcett TW, Johnstone RA. 2003a. Mate choice in the face of costly competition. Behav Ecol. 14:771–779.

Fawcett TW, Johnstone RA. 2003b. Optimal assessment of multiple cues. Proc R Soc Lond Ser B Biol Sci. 270:1637–1643.

Forstmeier W. 2005. Quantitative genetics and behavioural correlates of digit ratio in the zebra finch. Proc R Soc Lond Ser B Biol Sci. 272:2641–2649.

Gentner TQ, Hulse SH. 2000. Female European starling preference and choice for variation in conspecific male song. Anim Behav. 59:443–458.

Gil D, Naguib M, Riebel K, Rutstein A, Gahr M. 2006. Early condition, song learning, and the volume of song brain nuclei in the zebra finch (Taeniopygia guttata). J Neurobiol. 66:1602–1612.

Gobes SMH, Bolhuis JJ. 2007. Birdsong memory: a neural dissociation between song recognition and production. Curr Biol. 17:789–793.

Halle F, Gahr M, Pieneman AW, Kreutzer M. 2002. Recovery of song preferences after excitotoxic HVC lesion in female canaries. J Neurobiol. 52:1–13.

Hardling R, Kokko H. 2005. The evolution of prudent choice. Evol Ecol Res. 7:697–715.

Hebets EA, Wesson J, Shamble PS. 2008. Diet influences mate choice selectivity in adult female wolf spiders. Anim Behav. 76:355–363.

Holveck MJ, de Castro ACV, Lachlan RF, ten Cate C, Riebel K. 2008. Accuracy of song syntax learning and singing consistency signal early condition in zebra finches. Behav Ecol. 19:1267–1281.

Holveck MJ, Riebel K. 2007. Preferred songs predict preferred males: consistency and repeatability of zebra finch females across three test contexts. Anim Behav. 74:297–309.

Holveck MJ, Riebel K. 2010. Low-quality females prefer low-quality males when choosing a mate. Proc R Soc B Biol Sci. 277:153–160.

Hunt J, Brooks R, Jennions MD. 2005. Female mate choice as a condition-dependent life-history trait. Am Nat. 166:79–92.

Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol Rev Camb Philos Soc. 72:283–327.

Leitner S, Catchpole CK. 2002. Female canaries that respond and discriminate more between male songs of different quality have a larger song control nucleus (HVC) in the brain. J Neurobiol. 52:294–301.

Lemon WC. 1993. The energetics of lifetime reproductive success in the zebra finch, *Taeniopygia guttata*. Physiol Zool. 66:946–963.

MacDonald IF, Kempster B, Zanette L, MacDougall-Shackleton SA. 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. Proc R Soc Lond Ser B Biol Sci. 273:2559–2564.

MacDougall-Shackleton SA, Dindia L, Newman AEM, Potvin DA, Stewart KA, MacDougall-Shackleton EA. 2009. Stress, song and survival in sparrows. Biol Lett. 5:746–748.

McEwen BS, Sapolsky RM. 1995. Stress and cognitive function. Curr Opin Neurobiol. 5:205–216.

Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol. 16:254–260.

Metcalfe NB, Monaghan P. 2003. Growth versus lifespan: perspectives from evolutionary ecology. Exp Gerontol. 38:935–940.

Miller DB. 1979. Long-term recognition of father's song by female zebra finches. Nature. 280:389–391.

Naguib M, Heim C, Gil D. 2008. Early developmental conditions and male attractiveness in zebra finches. Ethology. 114:255–261.

Nottebohm F, Arnold AP. 1976. Sexual dimorphism in vocal control areas of the songbird brain. Science. 194:211–213.

Nowicki S, Peters S, Podos J. 1998. Song learning, early nutrition and sexual selection in songbirds. Am Zool. 38:179–190.

Nowicki S, Searcy WA, Peters S. 2002a. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 188:1003–1014.

Nowicki S, Searcy WA, Peters S. 2002b. Quality of song learning affects female response to male bird song. Proc R Soc Lond Ser B Biol Sci. 269:1949–1954.

Qvarnstrom A, Part T, Sheldon BC. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature. 405:344–347.

Riebel K. 2000. Early exposure leads to repeatable preferences for male song in female zebra finches. Proc R Soc Lond Ser B Biol Sci. 267:2553–2558.

Riebel K. 2009. Song and female mate choice in zebra finches: a review. Adv Study Behav. 40:197–238.

Riebel K, Naguib M, Gil D. 2009. Experimental manipulation of the rearing environment influences adult female zebra finch song preferences. Anim Behav. 78:1397–1404.

Riebel K, Slater PJB. 1998. Testing female chaffinch song preferences by operant conditioning. Anim Behav. 56:1443–1453.

Riebel K, Smallegange IM, Terpstra NJ, Bolhuis JJ. 2002. Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. Proc R Soc Lond Ser B Biol Sci. 269:729–733.

Roberts ML, Buchanan KL, Bennett ATD, Evans MR. 2007. Mate choice in zebra finches: does corticosterone play a role? Anim Behav. 74:921–929.

Schielzeth H, Bolund E, Forstmeier W. 2010. Heritability of and early environment effects on variation in mating preferences. Evolution. 64:998–1006.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). Horm Behav. 44:132–139.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2004. Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*). Proc R Soc Lond Ser B Biol Sci. 271:S121–S123.

Spencer KA, Buchanan KL, Leitner S, Goldsmith AR, Catchpole CK. 2005. Parasites affect song complexity and neural development in a songbird. Proc R Soc Lond Ser B Biol Sci. 272:2037–2043.

Spencer KA, Wimpenny JH, Buchanan KL, Lovell PG, Goldsmith AR, Catchpole CK. 2005. Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). Behav Ecol Sociobiol. 58:423–428.

Swaddle JP, Cuthill IC. 1994. Preference for symmetrical males by female zebra finches. Nature. 367:165–166.

Terpstra NJ, Bolhuis JJ, den Boer-Visser AM. 2004. An analysis of the neural representation of birdsong memory. J Neurosci. 24: 4971–4977.

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. Horm Behav. 55: 50–59.

Widemo F, Saether SA. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. Trends Ecol Evol. 14:26–31.

Williams H. 2001. Choreography of song, dance and beak movements in the zebra finch (*Taeniopygia guttata*). J Exp Biol. 204: 3497–3506.

Woodgate JL, Bennett ATD, Leitner S, Catchpole CK, Buchanan KL. 2010. Developmental stress and female mate choice behaviour in the zebra finch. Anim Behav. 79:1381–1390.

Zann RA. 1996. The zebra finch: a synthesis of field and laboratory studies. Oxford: Oxford University Press.

Zann RA, Cash E. 2008. Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (*Taeniopygia guttata*). Behav Ecol Sociobiol. 62:391–400.