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ORIGINAL ARTICLE

K. A. Spencer · J. H. Wimpenny · K. L. Buchanan · P. G. Lovell · A. R. Goldsmith · C. K. Catchpole

Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*)

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Abstract Developmental stress has recently been shown to have adverse effects upon adult male song structure in birds, which may well act as an honest signal of male quality to discriminating females. However, it still remains to be shown if females can discriminate between the songs of stressed and non-stressed males. Here we use a novel experimental design using an active choice paradigm to investigate preferences in captive female zebra finches (*Taeniopygia guttata*). Nine females were exposed to ten pairs of songs by previously stressed and non-stressed birds that had learned their song from the same tutor. Song pairs differed significantly in terms of song complexity, with songs of stressed males exhibiting lower numbers of syllables and fewer different syllables in a phrase. Song rate and peak frequency did not differ between stressed and non-stressed males. Females showed a significant preference for non-stressed songs in terms of *directed* perching activity and time spent on perches. Our results therefore indicate that developmental stress affects not only the structure of male song, but that such structural differences are biologically relevant to female mate choice decisions.

Keywords Bird song · Corticosterone · Developmental stress · Mate choice · Sexual selection

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K. A. Spencer (✉) · J. H. Wimpenny · A. R. Goldsmith
School of Biological Sciences, University of Bristol,
Woodland Road,
Bristol, BS8 1UG, UK
e-mail: karen.spencer@bristol.ac.uk
Tel.: 0117-954-5945
Fax: 0117-925-7374

K. L. Buchanan
Cardiff School of Biosciences, Cardiff University,
Park Place,
Cardiff, CF10 3TK, UK

P. G. Lovell
Department of Experimental Psychology, University of Bristol,
Woodland Road,
Bristol, BS8 1TN, UK

C. K. Catchpole
School of Biological Sciences, Royal Holloway, University of London,
Egham,
Surrey, TW20 0EX, UK

Introduction

It is widely accepted that female mating preferences can result in selection on male behaviour and morphology, with many studies showing that males with enhanced traits have improved mating success (Andersson 1994). The handicap principle specifically suggests that such exaggerated signals honestly reflect male quality and must be associated with a cost of signal production or development, to maintain signal honesty (Zahavi 1975). Bird song is a well-known example of such a sexually selected trait and song complexity has been related to female choice in many songbird species (Catchpole and Slater 1995; Searcy and Yasukawa 1996). The developmental stress hypothesis (Nowicki et al. 1998) proposed that song complexity is a reliable indicator of male quality, because the underlying neural pathway controlling song production and learning develops at an early stage of life (Kirn et al. 1999; Kittelberger and Mooney 1999; Brainard and Doupe 2002), when young birds are likely to be particularly at risk of developmental stress. Recent experimental evidence supports this hypothesis and has shown that developmental stress in the form of reduced food availability or increased stress hormones can play a significant role in mediating the honesty of the song signal, by affecting the ability of a male to learn and produce complex songs (Nowicki et al. 2002a; Buchanan et al. 2003; Spencer et al. 2003, 2004). It has thus been suggested that females, in preferring males with complex songs, are using developmental history to indicate male quality.

That developmental stress causes detectable differences in male song is fundamental to its role in influencing the evolution of complex acoustic signals. However there is a paucity of evidence that female birds can discriminate between the songs of males from differing rearing environments. Several studies have shown that females can discriminate between normal and abnormal forms of their own species songs (Searcy and Yasukawa 1996). For example, female song sparrows (*Melospiza melodia*) do not respond at all to song produced by males that had been deafened during song learning (Searcy and Marler 1987). One study that investigated female responses to male songs from stressed backgrounds found that female song sparrows assessed males on the basis of song learning ability, rather than song repertoire size or complexity (Nowicki et al. 2002a, b).

In this study, we employed a series of preference trials to test the hypothesis that stress experienced during development causes differences in the adult male song signal that are detectable by females. An active choice paradigm was used to determine the preferences of female zebra finches (*Taeniopygia guttata*) when given a choice between pairs of male songs. Each song pair consisted of one song from a male reared in stressed (elevated corticosterone levels or food restriction) conditions and another from control (*ad libitum* food) conditions (Spencer et al. 2003). We predicted that females would prefer the songs of non-stressed males. Active choice trials using perching activity have been used recently to good effect in bird song research to test female preferences (Riebel and Slater 1998; Gentner and Hulse 2000; Riebel 2000). We used pairs of songs from a population of male zebra finches bred in a previous laboratory experiment (Spencer et al. 2003) where we found significant negative effects of developmental stress on song complexity, i.e. a reduction in the number of syllables in a phrase (Fig. 1). Our experiment thus provides essential information about the form and function of female preferences, and the role of developmental stress in the evolution of complex signals.

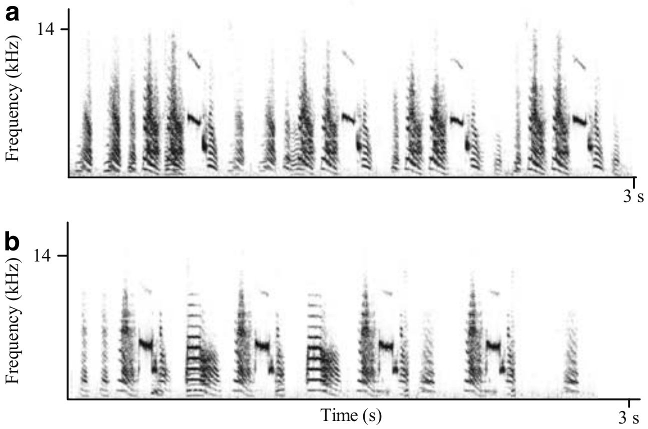

Fig. 1 Example sonograms of stimulus song used in song discrimination experiments using zebra finches (*Taeniopygia guttata*). Sonogram **a** is from a control-reared male, whilst **b** is from a stress-reared male. Both of these males learned their song from the same tutor

Methods

Mate choice apparatus and training period

Twenty adult female zebra finches that had previously been reared under *ad libitum* feeding conditions (Spencer et al. 2003) were placed in a custom-built song discrimination apparatus to habituate them to the novel surroundings and familiarise them with the experimental protocol (Fig. 2). Females were 497±45 days (mean±SD) old and had not had previous breeding experience when they began their training, which lasted approximately one month. When not in the apparatus the birds were kept communally in cages (118 × 50 × 50 cm) where they received ad libitum supplies of Foreign Finch Seed Mixture (Country Wide, Bristol, UK), water, grit and cuttlefish bone. The birds were maintained at a temperature of 20–24°C and a photoperiod of 14L:10D during training and experimental periods. After this they experienced a photoperiod of 8L:16D to facilitate molting.

The song discrimination apparatus (Fig. 2) consisted of an L-shaped box with a central wooden perch, an additional perch near the water supply and *ad libitum* food supplies. An ‘active perch’ was situated within each arm of the apparatus. These perches were connected via micro-switches (Microcontact, Petercem, France) to a laptop computer (Dell Latitude LS), which logged the position and duration of each perch made by a female (PerchLog, P.G. Lovell, UK). Upon activation PerchLog would also play an appro-

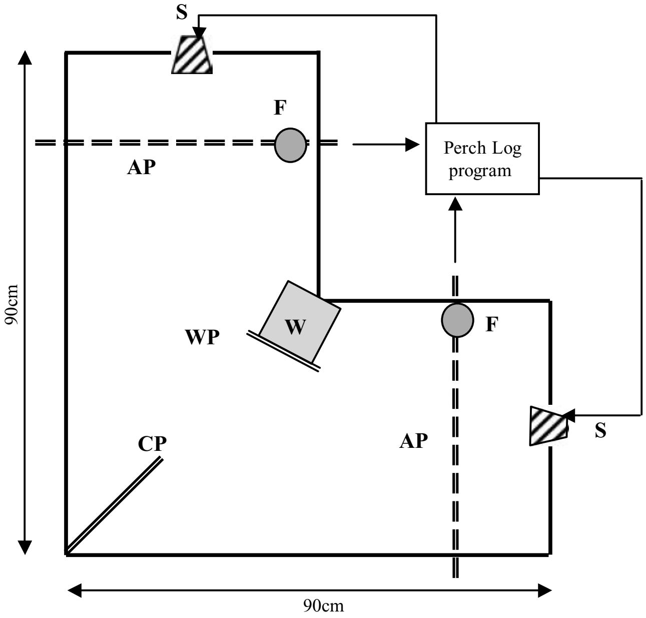

Fig. 2 Aerial view of the song discrimination apparatus used in this experiment. *AP* Active perch; *CP* central perch; *WP* water perch; *S* speaker; *W* water; *F* food. Dimensions: 90 cm long × 45 cm high. When a bird perches on an active perch the PerchLog (P.G. Lovell, UK) program plays the appropriate sound from the speaker, PerchLog records the number of activations of each perch and the duration of each activation

priate pre-recorded sound from a loudspeaker (Sony, UK) at the end of the arm where the perch had been depressed.

During training sessions, female activity was logged in this fashion and each time a female landed on an ‘active perch’ this triggered an 8-s sound file of familiar female contact calls. These calls had been recorded from a group of adult females in a sound attenuated room, using Avisoft SAS-Lab Pro (R. Specht, Germany) via a Sennheiser K6 microphone and a laptop computer (Dell Latitude LS, UK). All birds experienced the same training sounds. Sound pressure levels 1 m from the speaker were 62 dB. During these sessions females responded to the contact calls by vocalization and increased perch hopping activity. Male song was not used in training sessions to avoid potential biases in the experimental testing period. Female zebra finches have been shown to prefer familiar songs (Riebel 2000; Riebel and Smallegange 2003); the use of female calls excluded the possibility that females may become accustomed to certain male songs or song features that could not be controlled for in the experiment. Each female experienced 15–20 training sessions, each lasting 50 min. Each bird was placed in the apparatus only once in any day with training sessions taking place every other day. Experimental trials were initiated less than 1 week after cessation of training procedures. Certain criteria were used to determine if a female could be used in the experimental procedure. First, females were only used in song experiments if they showed no significant side bias in their perching activity; quantified using paired *t*-tests (Minitab, PA, USA: P range=0.30–0.80). Ten birds showed significant side biases and were therefore excluded. Of the remaining birds one had very low mean activity rates (4 perches per hour) and was also excluded. The remaining nine birds maintained a relatively high mean activity rate of at least 8 perches h^{-1} over the training period (range 8–35.8) and these birds progressed to the experimental procedure. This elimination process took place before any experimental procedures were undertaken.

Experimental procedure

Each experimental trial consisted of a choice between two conspecific songs learned from the same tutor, one from a control-reared bird, and the other from a stress-reared bird. Each female (n=9) was exposed to all ten pairs of stimulus songs. Once females were placed in the mate choice apparatus they were induced to move from the central perch to the left and right active perches 4 times each, to familiarize them with the song to be played. PerchLog was then used to log their subsequent movements over a 50-min period, after which the female was returned to the communal cage. PerchLog was set up to play at random one of three different song files as described above from the same male upon depression of the active perch and to record the number and duration of active perches made in each arm. The order in which the female was exposed to each song pair and the speaker (left or right arm) from which the control song was played was counterbalanced across trials.

Stimulus song

Male songs for the experimental procedure were gathered from recordings from a previous experiment where male zebra finches had undergone stress during development (for details, Spencer et al. 2003). Pairs of songs were available where two males had learned their song from the same tutor (foster father) but one had experienced a stressful rearing environment whilst the other had experienced control rearing conditions. The details of the stress treatment during early male development are outlined by Spencer et al. (2003) and comprised either administration of corticosterone (CORT) or restriction of food (FOOD). Both of these stressors had very similar effects on song composition and timing (Spencer et al. 2003), therefore for the purpose of this experiment the two forms of stress were both used to generate ‘stressed’ male songs. In total, 10 pairs of songs were prepared and presented to each female (CORT n=5, FOOD n=5). Songs were recorded from male zebra finches between 100–200 days of age (when adult song has crystallized) in a sound attenuated room, using Avisoft SAS-Lab Pro (R. Specht, Germany) via a Sennheiser K6 microphone and a laptop computer (Dell Latitude LS, UK). The directed song of each bird was recorded twice for 10 min on different days immediately after the introduction of a novel female. Three different song files (.wav format) were made using Avisoft SASLab Pro for each male, each containing four sequential phrases selected from the original recordings (mean duration of stimulus files 3.9 s). Selection of the song files was done blind to treatment group. There were no differences in stimulus song file duration or sound pressure measurements between control and stressed treatments (paired t test: duration: t_9=0.84, P=0.425; amplitude: t_9 =−0.34, P=0.742). Although male zebra finches tend to sing a very stereotyped song, which rarely changes, syllable additions and deletions can occur (Helekar et al. 2000) and the use of three different song files from each male as stimuli allowed for this variation.

Statistical analysis

Females showed a high degree of variation in the total activity per trial on active perches. To control for this large variation we used a mean preference ratio (no. control or stressed perches/total no. perches in each experimental trial) for each female, rather than absolute numbers of perches, as the dependent variable. In addition, the proportion of time spent on stressed-song and control-song perches was calculated. To determine the preferences of females for stress-reared and control-reared songs paired *t*-tests were carried out on the mean preference ratio (across all song pairs) for each female. Differences in the proportion of time spent on each active perch were analyzed in a similar way. To determine the effects of rearing environment on song structure a series of paired *t*-tests were carried out on the following dependent variables: song rate (no. phrases per minute), the total number of syllables per phrase, the number of different syllables per phrase

Table 1 Differences between control and stressed song pairs used in song discrimination experiments. Significance level was calculated using paired t-tests. Effect size was calculated using the equation: $\frac{(\text{control}\,\mu - \text{stressed}\,\mu)}{\text{population}\,\sigma}$. Significant characteristics are indicated in bold

Song characteristic	Control (mean±SD)	Stressed (mean±SD)	Effect size	Significance level
No. syllables	**7.29±1.46**	**5.63+0.95**	**1.13**	T=**3.26**, P=**0.010**
No. different syllables	**5.83±0.094**	**4.82±1.07**	**0.91**	T=**2.75**, P=**0.022**
Song rate (phrases s^{-1})	1.06±0.26	1.21±0.29	−0.53	T=−1.08, P=0.307
Peak frequency (kHz)	17.06±3.66	16.24±2.75	0.23	T=0.66, P=0.526

and peak frequency of the phrase (kHz). The mean values for each of these parameters across all three stimulus files were used to characterize the differences between stimulus song pairs (Table 1). All data were checked for normality. Bonferroni corrections were not made to control for multiple testing as only relevant variables were chosen for analysis and such corrections would only serve to reduce statistical power and increase the probability of a type II error (Rice 1989). Where appropriate standardized effect sizes have been calculated for analyses (Nakagawa 2004).

Results

Male song pairs showed significant differences in song complexity, with songs from developmentally stressed males exhibiting reduced numbers of syllables and fewer different syllables within a phrase (Table 1). There was no effect of rearing environment on song rate (number of phrases s^{-1}) or the peak frequency within a phrase (kHz) in the stimulus songs. Both corticosterone stressed and food stressed songs shared similar characteristics in terms of number of syllables (T=0.69, P=0.512, df=8), number of different syllables (T=0.04, P=0.968, df=8), song rate (T=−1.60, P=0.160, df=8) and peak frequency (T=0.39, P=0.705, df=8). Female zebra finches exhibited a significant preference for control male songs over songs of developmentally stressed males during the test trials, in terms of perching preference ratio (paired T=5.37, P=0.001, n=9) with 59% (mean) of active perches per trial being used to play control songs. When each stress treatment was analyzed separately the same pattern appeared (Fig. 3a; CORT: T=2.83, P=0.022, n=9; FOOD: T=3.44, P=0.009, n=9). Females also spent a significantly larger proportion of time on the active perches that played control songs compared

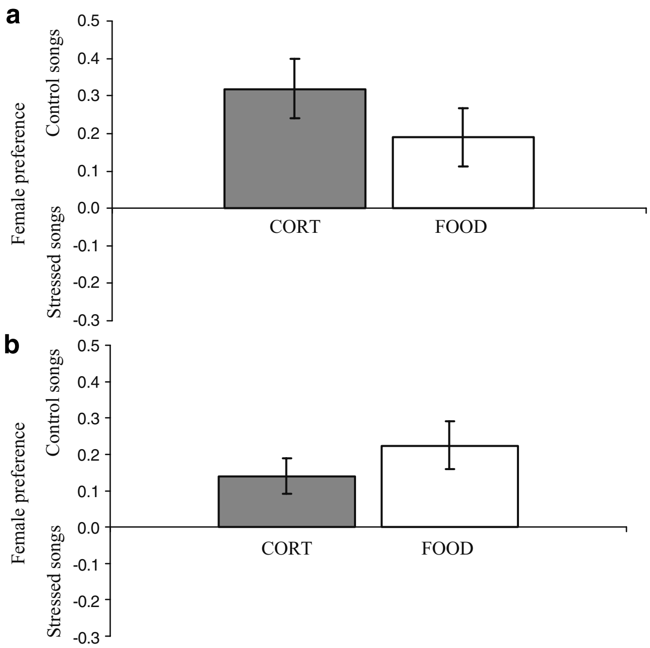

Fig. 3 **a** Female zebra finches show significantly higher preference ratios for control songs over both corticosterone and food stressed songs. **b** Female zebra finches also spent more time on perches that played control songs, rather than corticosterone or food stressed songs. Graphs show mean differences (±standard error) in preference ratios (control–stressed). In each case a positive value represents a preference for control songs

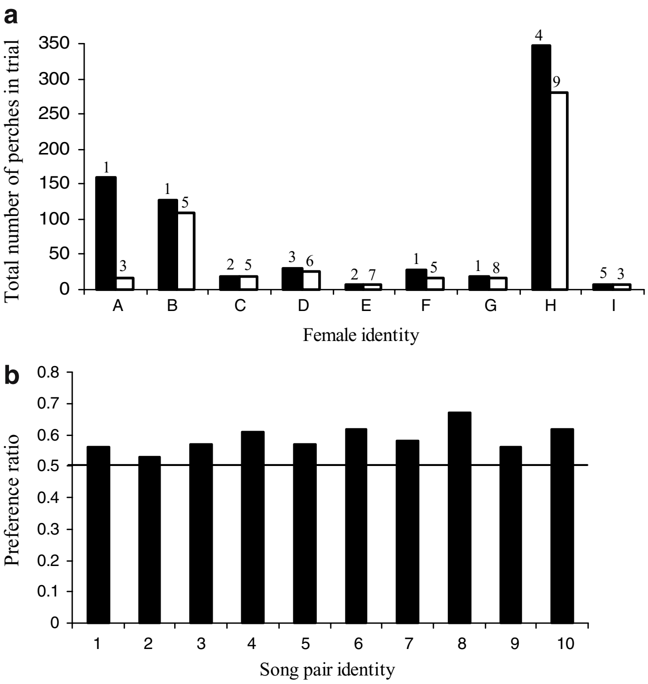

Fig. 4 **a** Individual variation in total perching activity in female zebra finches (A – I) in a trial period (50 min). First (*black bars*) and second (*white bars*) highest activity levels are shown here. The number above each bar represents the song pair that elicited the response. There are no consistent preferences for a specific song pair. **b** Overall preference ratios (total no. perches for control/total no. perches for control + stressed) in favor of control songs for each stimulus song pair (mean taken across all females). Horizontal line represents the line of equal preference. Data above this line suggest a preference for control songs. In song pairs 1–5 stressed song is from corticosterone (CORT) stressed birds, 6–10 are from food (FOOD) stressed birds

to stressed songs (T=4.81, P=0.001, n=9) and again both stress treatments showed similar patterns (Fig. 3b; CORT: T=3.99, P=0.004, n=9; FOOD: T=2.44, P=0.041, n=9). One consequence of our statistical methodology (i.e. taking a mean preference across song pairs for each female) could disguise a potential confound, namely that a particularly attractive song within a pair could skew the response data. Upon inspection we found no evidence that a single song pair elicited a consistently strong response from all females in this study (Fig. 4a) and there was very little variation in the mean preference ratios for each song pair (Fig. 4b), suggesting our analysis is robust. In addition when each song pair is taken independently (mean preference across all females, using song pair as the unit of analysis rather than female) there is still a significant effect of rearing environment (paired t test, P<0.0001).

Discussion

The results of these experiments demonstrate that developmental stress can cause significant differences in adult male song characteristics, with consequences for subsequent success in attracting a potential mate. Previous experiments have shown that developmental stress can reduce song complexity (Spencer et al. 2003, 2004; Buchanan et al. 2003), but this is the first experimental evidence that these differences have biologically meaningful consequences for female choice. The discovery that females prefer control songs over those of previously stressed males provides empirical support for the developmental stress hypothesis, confirming the important role of rearing conditions in the expression and evolution of complex acoustic signals.

One of the few studies to find differential female responses to developmentally stressed male song found that male song sparrows that had failed to copy a local model song well, failed to elicit many copulation solicitation displays from conspecific females (Nowicki et al. 2002b). Nowicki et al. (2002a) also showed in an allied study that male swamp sparrows that had undergone developmental stress were less able to copy a model song, though they found no effects of their treatment on song complexity. Although Nowicki et al. (2002b) did not directly measure female preferences across the control and stressed groups, the combined results of these two studies do suggest that females could discriminate between males of differing developmental quality, using the proportion of notes copied from a familiar model song as a cue to quality.

We suggest here that female zebra finches may discriminate between stressed and non-stressed males' songs on the basis of phrase complexity, rather than song copying ability. Females from many species of songbird use song complexity, or repertoire size, as a cue for mate choice (Catchpole and Slater 1995; Searcy and Yasukawa 1996). In the zebra finch, phrase complexity has been relatively overlooked, though one study confirmed the importance of phrase duration and complexity in mating preferences (Clayton and Prove 1989). Female zebra finches presented with a choice between simple or complex versions of zebra finch song exhibited significantly more courtship displays to the complex songs (Clayton and Prove, 1989). Another study has also suggested that female zebra finches might have a preference for more complicated male songs (Collins 1999). Another song feature that might influence female choice is song rate, but this is a condition dependent trait likely to be related to current condition rather than to conditions during early development. We found no effect of developmental stress on song rate in our larger population of males (Spencer et al. 2003), nor in the sub-sample of songs used as stimuli in this study. Therefore, although song rate may be important in mate choice in this species, it is unlikely to explain the preferences seen here for control songs.

Whilst the only differences we detected between song pairs related to phrase complexity, there may also be more subtle differences between song pairs that we did not measure in this study. Riebel and Slater (1998) showed that female chaffinches (*Fringila coelebs*) responded less to male song when the characteristic 'terminal flourish' syllable was removed. In the canary, specific syllable types are known to elicit high rates of copulation solicitation displays (Vallet and Kreutzer 1995; Vallet et al. 1998). A qualitative assay of the syllable variation between our song pairs revealed no consistent differences in terms of syllable type across song pairs. Further experimental work is required to investigate how subtle differences in songs can affect female preferences and the experimental set-up used in this study provides an excellent framework from which to do this.

Our results provide clear support for the hypothesis that stress during early development can significantly affect the quality of the song signal produced by adult male birds (Nowicki et al. 1998). Our results offer novel empirical evidence that song complexity is a reliable indicator of early developmental stress, providing a basis for the evolution of female choice based on this trait. As such, this confirms a potential mechanism by which complex song repertoires have evolved in a number of songbird species as honest indicators of past developmental history (Nowicki et al. 1998).

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