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Context determines the sex appeal of male zebra finch song

OFER TCHERNICHOVSKI, HUBERT SCHWABL & FERNANDO NOTTEBOHM
The Rockefeller University Field Research Center

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Abstract. We explored the conditions under which playbacks of male zebra finch, *Taeniopygia guttata*, song induced reproduction in females. In a laboratory study, a rise in faecal oestrogen levels predicted egg laying. Song playbacks by themselves induced a decrease in oestrogen levels. There was an increase in oestrogen levels, followed by egg laying, when the song was broadcast from inside a male model positioned away from the nest. However, this effect occurred only when a second, silent male model was perched on the rim of the nest. If song was broadcast from inside the model perched on the nest, there was no increase in oestrogen levels. We conclude that tests of song efficacy in female songbirds must respect some contextual rules, which are likely to vary between species. Only then does it become possible to ascertain which sounds are most effective in inducing physiological changes leading to reproduction.

There is a rich literature on the role of avian male song in promoting a female's reproductive condition, including work on budgerigars, *Melopsittacus undulatus* (Brockway 1962), and the classic studies of Lehrman and his associates on ring doves, *Streptopelia risoria* (Lehrman 1964; Lott & Brody 1966; Lott et al. 1967; Lehrman & Friedman 1969). That work and a later study (Nottebohm & Nottebohm 1971) showed that the cooing sounds of male ring doves induce physiological and behavioural changes in females, leading to ovulation.

Studies done with songbirds have addressed similar questions as well as the slightly different question of the nature of the song stimulation that was most effective in inducing ovulation (Kroodsma 1976) or female solicitation. West et al. (1981) showed that female brown-headed cowbirds, *Molothrus bonariensis*, responded particularly strongly to the song of dominant males. Female songbirds from other species tested, however, did not respond as reliably to song playbacks, and this gave rise to the technique of pre-treating the females with oestradiol (Searcy & Marler 1981; Searcy et al. 1982). Females treated with oestradiol were more likely to give solicitation displays when presented with conspecific than heterospecific song (Searcy & Marler 1981; Clayton & Pröve 1989) and in some cases responded particularly strongly to playbacks of home dialect songs (Baker et al. 1981).

The present study evolved out of an interest in identifying features of male zebra finch song that are important for inducing receptivity and egg laying in females in the laboratory. The simplest situation, we thought, would be to take virgin, female zebra finches, *Taeniopygia guttata*, and expose them to different male songs while measuring changes in blood oestrogen levels. This approach led us, through a series of failures, to appreciate that for the song stimulus to be effective it had to occur in a narrowly defined context. The present report defines such a context. We make no claims that this context is identical to that which occurs in nature or that it is the best possible one, but hope that it identifies some traits that are germane to the breeding biology of this species. Zann (1996) extensively reviewed the breeding biology and vocal behaviour of zebra finches and a description of the vocal behaviour of this species.

We did not pre-treat our female zebra finches with oestradiol because we wanted to test whether one role of song might be to induce a rise in oestrogen levels leading to ovulation. We wanted

Correspondence: O. Tchernichovski, Rockefeller University Field Research Center, Tyrrel Rd, Millbrook, N.Y. 12545, U.S.A. H. Schwabl is now at The Department of Zoology, Washington State University, Pullman, WA 99163-4236, U.S.A.

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to monitor the oestrogen response at periodic intervals after the onset of song stimulation in a non-invasive manner. Therefore, the first step was to show that faecal oestrogen levels, presumably related to those found in the blood, were a good predictor of latency to egg laying. The second step was to show that faecal oestrogen levels, and latency to ovulation, could be modified by exposure to song. When we discovered that song by itself did not induce a rise in oestrogen levels, we examined whether the visual context in which song occurred might be important.

Figure 1. A plastic male model placed on the rear edge of a nest, in the configuration used in the experiments.

GENERAL METHODS

Animals

We used adult female zebra finches raised at the Rockefeller University Field Research Center. These birds were removed from communal aviaries 90–130 days after hatching and placed singly in 45 × 22 × 26 cm cages and held in a communal room. Their diet consisted of mixed dried seeds, seeds soaked overnight in water and ground hard-boiled eggs with shell. The photoperiod was kept constant at a 12:12 h light:dark photoperiod. Birds removed from the aviary were allowed to get used to their new cage for 5–10 days before an experiment began. Male zebra finches of comparable age were used as consorts in some of the experiments.

Male Models

We painted hollow plastic bird models slightly larger in size than male zebra finches, using Homefront decorator colours, to resemble a zebra finch male (Fig. 1). A Radio Shack NOVA 43 headphone speaker (20 Hz–20 kHz flat frequency response) was inserted into some of the models, and the sound was broadcast through a hole in the chest covered with wire-mesh. The model was placed on the rim of a white plastic nest cup full of burlap nesting material. Birds that received a nesting cup without a model on it also had this cup filled with nesting material.

Song Recording, Digitizing and Playback

We recorded three bouts of song from a 90-day-old male. Each song bout contained a few introductory notes followed by three, four and six repetitions of the same song. The initial recording was made with a cassette recorder (model PMD 221, Marantz, Chatworth, California) and digitized (16 bits, 44 100 Hz) using the Canary 1.2.1 song analysis software (Charif et al. 1995). The digitized sound was played by a Power Macintosh 7500 computer according to the following schedule: counting lights on as time zero, songs were played at 0000–0200, 0400–0600 and 0900–1200 hours (7 h daily). During playbacks, every time a song was played, one of the three song bouts was chosen at random, followed by a silent interval of 1–15 s, also chosen randomly. In this manner, there was no fixed pattern of repetition. This amount of song exposure was probably in excess of that produced by males during courtship and after a pair is formed. However, the successive song bouts of a live singing male probably include more variability than was present in our programme. Songs were played either through a Radio Shack AMX-15 amplified speaker or from inside a male model, as described; in the latter case, sound was amplified by an Optimus SA-155 amplifier. In both cases, sounds were delivered at a maximum amplitude of 85 dB (measured 12 cm in front of the sound source with a Radio Shack sound-level meter with controls set at C weighting and slow response).

Radioimmunoassay of Faecal Oestrogen Levels

The blood levels of oestrogen of female birds increase with the onset of reproduction (Wingfield & Farner 1993). In several avian species, the oestradiol concentrations in faeces are correlated

with blood levels and with reproductive state (Bishop & Hall 1991; Cockrem & Rounce 1994; Lee et al. 1995). Our protocol to measure faecal oestrogen levels was as follows. The cage bottoms were lined with paper, which was replaced 1 h after lights on and 1 h before lights off. Faeces were left to dry on the paper for 24 h at room temperature and were then separated from food scraps. Preliminary measurements showed that nocturnal faeces (collected after lights on) had a higher concentration of oestrogen and showed smoother and earlier changes associated with reproductive stages than faeces collected in the evening (before lights off). We therefore measured nocturnal faecal oestrogen levels. We weighed the dry faecal material and added 20 × the volume of phosphate buffered saline (0.1 M, 0.1% gelatin, 0.1% NaN_3 (PBSg)). Samples were vortexed for 20 min and left to equilibrate at 4°C for 24 h. After centrifugation at 2300 rpm, 75 µl of the supernatant were hydrolyzed at 38°C for 12 h by adding 10 µl of a mixture of beta-glucoronidase and sulphatase (Boehringer Mannheim Gmbh). Measurements of oestradiol concentrations therefore represent both free and conjugated oestradiol. We then transferred samples to Extralute QE columns (EM Separations Technology) and extracted them three times with 3 ml of diethyl ether/petroleum ether (1:1). Extracts were dried and re-dissolved in 250 ml of PBSg. We then performed radioimmunoassays for oestradiol on two 100 ml aliquots of each sample using tritiated oestradiol as tracer (NEN) and an oestradiol antiserum from Arnel Products (New York, cross reactivity: 14% oestrone, 5% oestriol and 0.001% all other steroids). Inter-assay variation was 9.1% ($N=5$). The standard curve ranged from 2 to 500 pg.

Measurement of known amounts of 100, 50 and 25 pg oestradiol that were added to a faecal extract following the above procedure gave an average of 104% of the known amount, and showed a high correlation with the known amount ($r=0.99$, $N=6$, $P<0.01$). To investigate the specificity of measurements of oestradiol, we extracted 12 randomly selected hydrolyzed faecal samples as described above, and partially separated and purified the extracted steroids on celite chromatography micro-columns (Wingfield & Farner 1975). Measurements of oestradiol in the fractions eluting oestradiol and measurements of oestradiol in unpurified extracts of faeces were highly correlated ($r=0.93$, $N=12$; $P<0.01$), suggesting that oestradiol concentrations can be estimated in organic extracts of buffer without further purification. We present oestradiol concentrations in pg/mg of dry faeces and refer to them as faecal oestrogen levels throughout the text.

Statistical Analysis

We compared initial and final values of oestrogen levels using a two-tailed paired *t*-test. We transformed data logarithmically so that data sets with different variance could be parametrically compared. All measurements of the same individual were performed within one assay, such that inter-assay variation could not influence the results.

EXPERIMENT 1: STIMULATION PROVIDED BY A LIVE MALE

Methods

Twelve females were placed in 12 standard cages, located 30 cm apart, so that the females could see each other through the bars. After 5 days we introduced a male into each of these cages. Faecal oestrogen was measured before male introduction (day 0) and 1, 3, 5 and 7 days after introducing the male. For this purpose, 30 min after lights on, a wire partition was gently driven down the middle of the cage to separate the male and female into two compartments and the paper lining the bottom of the cage was replaced. Faeces produced by the female during the next 30 min were collected and the partition was removed.

Results

Some females increased their faecal oestrogen levels, and some did not (Fig. 2). The curves recording the time change in oestrogen levels for any one bird were relatively smooth.

Seven of the 12 females laid eggs between days 7 and 21, when observations were discontinued. The latency to egg laying by the seven females was 7, 8, 9, 9, 10, 14 and 21 days after pairing. Respective oestrogen faecal levels on day 7 were 400, 200, 213, 213, 110, 31 and 22 pg/mg. Oestrogen levels on that day for the five females that did

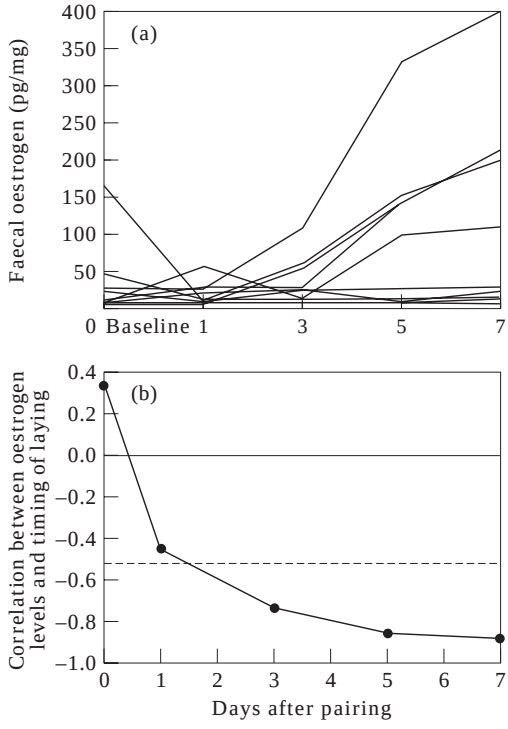

Figure 2. Experiment 1. (a) Faecal oestrogen levels immediately before a male was introduced into a female's cage and 1–7 days after. (b) Correlation between faecal oestrogen levels and the delay to egg laying during each of the first 7 days after pair formation. The five birds that had not laid eggs by day 21 after pair formation were assigned a latency to ovulation of 22 days. The correlation becomes stronger the greater the time elapsed after pairing. Data for both (a) and (b) were drawn from the same group of 12 birds. The dashed line indicates the significance threshold ($P<0.05$).

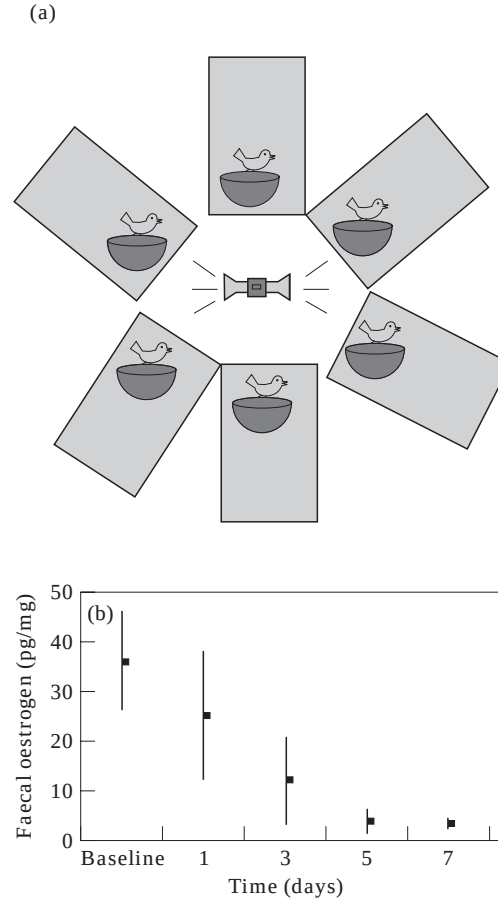

Figure 3. Experiment 2. (a) Array of six cages with a speaker in the middle; each cage contained a nest and a male model placed on the rim of the nest. (b) Faecal oestrogen levels on days 0 (baseline), 1, 3, 5 and 7. Vertical lines show standard errors ($N=12$).

not lay eggs ranged between 3 and 13 pg/mg. The correlation between level of faecal oestrogen and latency to egg laying was significant on day 3 ($N=12$; $P<0.05$) and became stronger on days 5 and 7 (Fig. 2b, c). Thus, the higher the oestrogen level, the shorter the time to egg laying. The percentage of paired birds that did not lay eggs was comparable to the percentages reported by other workers (reviewed in Zahn 1996).

We concluded that: (1) under our conditions, eggs were laid by 58% of the paired females with a latency of 7–21 days; and (2) changes in faecal oestrogen levels predicted the occurrence of and latency to egg laying.

EXPERIMENT 2: DISSOCIATION OF VISUAL AND AUDITORY STIMULI

To evaluate the stimulatory effect of the song separated from the stimulus complex of a live, live-in courting male, female zebra finches were caged singly, and their faecal oestrogen levels measured on the morning of the sixth day. Then a nest with a male model on its rim was attached to the inside wall of each cage on the side facing the middle arena. Songs were played from a speaker placed in the middle of the arena (see Methods; Fig. 3a). Faecal oestrogen levels measured during the next 10 days decreased ($P<0.01$; Fig. 3b).

EXPERIMENT 3: A SINGING MODEL AS STIMULUS

The results of experiment 2 suggested that for song playbacks to be effective in raising oestrogen levels, they should come from a correct source, that is, a male or, in its absence, a male model.

Methods

We placed three cages side by side in a stimulus array that tested for the importance of seeing a bird-like sound source. We placed a female in each cage and measured faecal oestrogen levels on the morning of the 11th day. Then each female received a nest with a male model on it. The models in cages 1 and 3 were silent; the model in cage 2 was equipped with a speaker that broadcast song (Fig. 4a). There was a cardboard separation between cages 2 and 3, but the birds in cages 1 and 2 could look into each other's cages. Song was played for 5 consecutive days, and nocturnal faeces were collected every morning. We left birds in their cages for 10 additional days to observe possible egg laying. All faecal samples were measured in one assay. We repeated this protocol six times. In each repetition, models were switched randomly between cages to ensure that results would not be attributable to a particular model. We therefore had three groups of six birds each (groups I, II and III, corresponding to cage numbers 1, 2 and 3).

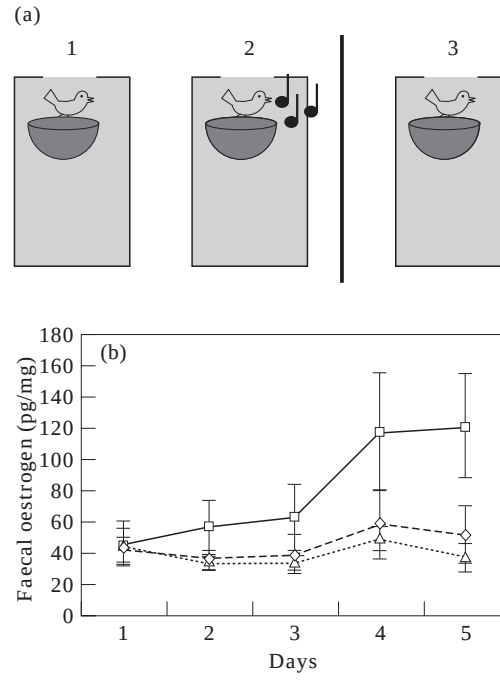

Figure 4. Experiment 3. (a) Three cages (1, 2, 3) placed side by side. Each cage contained one female (not shown). A cardboard partition blocked the view between cage 3 and the other two cages. Nest cups with a male model attached to the rim were introduced on day 11 (day 1 in the accompanying graphs). Songs were broadcast from a speaker inside the model in cage 2. (b) Average ± SE oestrogen levels in groups I (—□—), II (– –△– –) and III (– –◇– –), respectively (N=6 per group).

Results

Faecal oestrogen levels remained virtually unchanged for the females in group II, which had the singing model in their cage (P=0.36), and group III, which could not see the singing model (P=0.76). In group I females, which could see the singing model in the neighbouring cage, oestrogen levels rose significantly on day 5 (P=0.02) (Fig. 4b). During the subsequent observation period, three of the six birds of the latter group laid a clutch of three to six eggs, with latencies of 7, 9 and 14 days after the male model was introduced. The females that laid eggs started to incubate them. These three birds also showed the highest and the earliest increase of faecal oestrogen levels. None of the females in groups II and III laid eggs.

EXPERIMENT 4: THE IMPORTANCE OF MODEL PROXIMITY

Methods

Group I females in experiment 3 were exposed to a complex stimulus situation: a silent model perched on each female's nest and a singing model in an adjacent cage occupied by another female. Experiment 4 was planned to test for the relative contribution of these three variables. To this end, we repeated the paradigm used in experiment 3, but with six cages placed around a central arena, and with two variants. In one case (experiment 4a, Table I) each female received a nest with a silent male model on its rim; in the other case (experiment 4b), each female was provided with a nest but no male model. In both cases, song was played

Table I. Comparison of results

Experiment	Song stimulation from middle of arena	Stimulation from nest	Faecal oestrogen		
			Day 0	Day 5	*P*
2	Played from speaker	Silent model	36 (10)	4 (2)	<0.01
4a	Played from male model	Silent model	28 (5)	51 (14)	<0.05
4b	Played from male model	No model	39 (5)	41 (3)	NS, <0.45

Faecal oestrogen levels (pg/mg ± SE) in three experimental groups ($N = 12$ for each group) that differed in the source of the male song and in the presence of a silent model on the nest.

from a male model placed in the centre of the arena. These experiments were done twice ($N = 12$ birds).

Results

Table I compares the results of these experiments with those of experiment 2. Only experiment 4a, featuring a combination of song played from a distant male model and the presence of a nest with a silent male model inside the cage, induced a significant increase in faecal oestrogen levels. This experiment came close to reproducing the condition that had also been most effective in experiment 3. There was no increase in faecal oestrogen levels in the birds in experiment 4b. By comparing the results of experiments 2, 4a and 4b (Table I), we infer that a singing model placed away from the nest and a silent model placed on the rim of the nest helped to raise faecal oestrogen levels. These experiments did not address whether the silent model placed on the rim of the nest would have been equally effective if placed elsewhere in the cage. In experiment 3, group II, however, a singing model placed on the rim of the nest failed to induce a rise in faecal oestrogen levels.

EXPERIMENT 5: RELATION OF SINGING MALE TO NEST

Methods

Once we became aware of the importance of the relations between sound source, model position and nest, we examined where in the cage singing occurred, and in particular the spatial relation between singing and the nest. We placed a virgin female, a male and a nest cup with nesting material in one of our standard cages and video-recorded the activity in this cage 2 hours a day (between 0900 and 1200 hours) during days 1 and 3 after the pair was constituted. This protocol was repeated six times. Then, we analysed the videotape to measure the distance from the nest at which the male sang. We divided the cage into three sections, each containing a perch but only one containing the nest (Fig. 5).

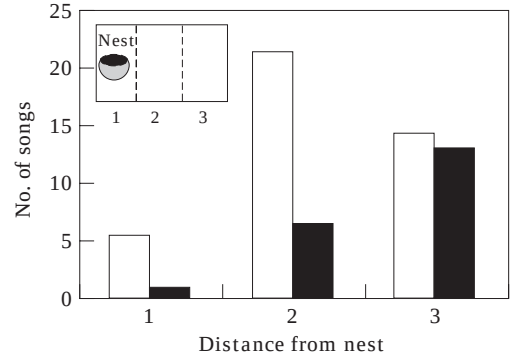

Figure 5. Spatial distribution of singing in three sections of a female's cage. Section 1 has the nest, as shown in the upper left side of the figure. A perch (not shown) ran the full length of the cage. Bars represent the frequencies of song occurrence in each of the three cage sections on days 1 (□) and 3 (■).

Results

Song could occur in any of the three areas into which we divided the cage for purposes of analysis, but tended to occur most frequently in the two areas that did not include the nest (Fig. 5). This result was significant for four of the six males on day 1 (chi-square test for each of these four birds, $P < 0.05$) and for all six males on day 3 ($P < 0.001$).

On day 3, song occurred less frequently than on day 1, and now both members of the pair spent more time at the nest.

DISCUSSION

The results of experiment 1 showed that measures of faecal oestrogen are good predictors of egg laying. Experiments 2–4 indicated that faecal oestrogen levels are also reliable indicators of the adequacy of various aspects of male stimulation. Experiments using models helped us to identify conditions that must be met for song stimulation to be effective.

There must be a confluence of visual and auditory stimulation for females to treat a model, and song, as a reproductive stimulus. This confluence was minimal: the song emanated from the model's chest. Otherwise the model was static; there was no courtship dancing, allopreening, clumping or nest site display, which in nature seem so important for bonding and reproduction (Zann 1996).

From the behaviour of the pairs in their breeding cage, we saw that even though males continued to sing after the initial acquaintance and courtship, they tended to do so away from the nest. Consistent with this result, a singing male model placed on the nest rim discouraged the change-over to a reproductive condition. Perhaps this negative effect could have been avoided by moving the singing model away from the nest after the first day of song playback. Zann (1996) described different calls that males use while leading a female to a potential nest site; apparently song seldom occurs at the nest itself.

The females seemed to benefit from stimulation from two male models, the silent model on the nest rim and the singing model away from her nest. If these two models are perceived as separate individuals, then the situation seems paradoxical because zebra finches are monogamous (Zann 1996). However, the outcome may merely be indicative of the fragmented way in which stimuli are perceived and integrated, in this case to induce ovulation.

Overall, our results show that an evaluation of song sex appeal in a highly social, sexually dimorphic species such as the zebra finch, is strongly conditioned by the visual context in which song occurs. In species such as the canary, *Serinus canaria*, in which the reproductive condition is heavily dependent on photoperiod, song broadcast from a speaker with no corresponding visual stimulation suffices to induce hormonal changes leading to ovulation (Kroodsma 1976). We have demonstrated that, even in as social a species as the zebra finch, measurements of faecal oestrogen levels and use of models, following a few contextual rules, may help to evaluate the stimulatory potency of different male songs.

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