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Female canaries display to songs of early isolated males

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Abstract. We tested the sexual responsiveness of female canaries, *Serinus canaria*, to two sets of different types of male conspecific songs. In each set, three of the male songs had been recorded in a young bird which was isolated at an early age from conspecifics. Each bird was recorded during three stages of the song ontogeny (early plastic songs, late plastic songs and crystallized songs). We used copulation solicitation displays (CSD) as an index of female sexual response. Playbacks were performed during a period of natural sexual responsiveness of the females to song. We demonstrated that, in both experiments, very weak sexual displays of female canaries were recorded to the two stages of 'isolate' plastic songs. Each of the two different types of 'isolate' crystallized songs elicited high levels of CSD. These levels are not significantly different from the adult domesticated control song. Taken together, these results suggest that even isolate songs may convey sexual information. We discuss prominent acoustic features that must be present to ensure female sexual responses.

Key words. Song stimuli; acoustic isolation; sexual responses; female canaries.

It has now been clearly demonstrated in many bird species that adult male songs convey sexual information that is used by females[1]. In some of these species, special attributes of the full songs are essential to elicit female sexual responses while in others they are not[2–6].

Male song learning may explain the emergence of these acoustic 'sexual releasers' within male adult songs during the course of ontogeny. We know that song tutors play an important role in males, allowing the shaping and patterning of phonological elements through the different stages of subsongs and plastic songs[7–9]. In certain species, the phonological development of these songs may be strongly influenced by social stimulation from listeners and especially from females[10]. There is also evidence for 'self-differentiation'[11], or a predisposition for acquiring specific attributes of conspecific song[12–14].

Little is known about the sexual receptivity of female oscines to the songs of isolated males[1] but some data suggest that these types of songs convey species-specific information. Isolate songs of song sparrows and swamp sparrows elicit more sexual displays in conspecific females than heterospecific normal songs[15] or songs of deafened conspecific males[16]. Nevertheless, in the swamp sparrows, song sparrows and white-crowned sparrows, the isolate songs are clearly less effective than learned songs[15,5]. Conversely, in the brown-headed cowbird (a brood parasitic species), the sexual potential of certain isolate songs is very high compared to natural songs[12,17]. This important contrast between species suggests that further investigations are needed to verify if early isolated males of other bird species may spontaneously sing 'sexy' songs.

In canaries, the ontogeny of the song features strongly suggest that the species-specific song segmentation (especially the presence of trills) and shaping of some song elements appear progressively without any influence from an adult song tutor (Nagle, unpublished data). The influence of 'self-differentiation' rules was also demonstrated by Güttinger[18,11] and experiments carried out in canaries deafened at an early age[19].

In the present study the responses of female canaries to different types of male isolate songs (recorded through the stages of plastic and crystallized songs) were compared to the responses to a natural male song. This latter song was used in preliminary experiments and elicited high levels of female sexual response. Because of 'self-differentiation' rules appearing progressively during the course of ontogeny, we hypothesized that: 1) a plastic song of an isolated male may convey little sexual information and therefore elicit low levels of sexual display in female canaries (less than the normal control song); 2) a crystallized song of an isolated male may convey sexual information and therefore elicit high levels of sexual display in female canaries (close to that elicited by normal control song).

Materials and methods

The basic procedure was to test the sexual responsiveness of female canaries to different types of natural songs. Two experiments (EA and EB) were carried out, using the same group of eight one-year-old females. For each experiment, a set of four songs was played back to

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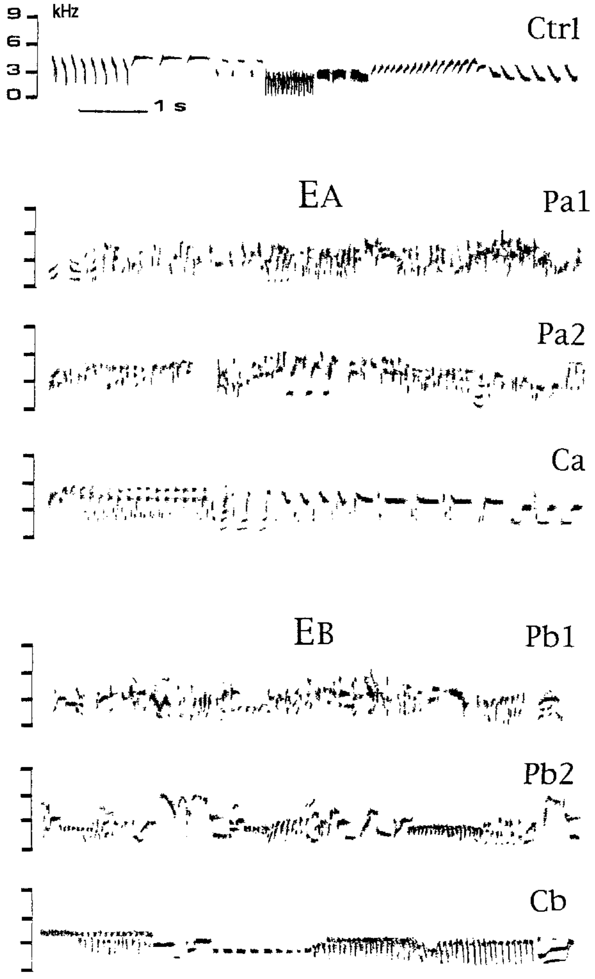

Figure 1. Domesticated canary song types used in the playback experiments EA and EB to test female sexual responsiveness. Control adult song (Ctrl) used in both experiments. Experiment EA, from top to bottom: three songs of the isolated male Ma, early plastic song (Pa1), late plastic song (Pa2) and crystallized song (Ca). Experiment EB, from top to bottom: three songs of the isolated male Mb, early plastic song (Pb1), late plastic song (Pb2) and crystallized song (Cb).

the females. Each set included a control song (Ctrl, normal adult male song) and three types of songs recorded in the same young isolated male (male «Ma» in EA and male «Mb» in EB). The females were tested during a period of natural sexual responsiveness to male song (see below). The amount of sexual response shown by the females was evaluated by measuring the number of copulation solicitation displays (CSD) to the song stimuli.

The birds were chosen from a pool of animals raised in our aviaries, all belonging to an outbred form of heterogenous genetic background locally called 'common canary'. All the females had individual contacts with some young females and males and heard singing males. All of the tested females were out of hearing of the males whose song phrases were used in the playback experiments. For the purpose of both experiments, the females were housed singly in cages within sound attenuation chambers (52 × 50 × 40 cm inside, 80 × 72 × 70 cm outside), using a 15:9 L/D photoperiod. They were provided daily with food, vitamins and nesting materials (coconut fibers, shredded linen). They were kept in the attenuation chambers during the testing period (3 weeks) and allowed to complete nesting in a bowl and to lay eggs.

In order to evaluate female sexual response, we measured the number of complete copulation solicitation displays (CSD). In a complete CSD, the responding female crouches, arches her back and simultaneously brings her tail forward and her head back. The wings are moved away from the body and vibrated. The feathers surrounding the cloacal region are separated. For each female, all of the complete CSD elicited by each type of song over the entire testing period were added together. This total was defined as the 'CSD score' and used for statistical analysis. More details may be found in Kreutzer et al.[20]

Figure 1 shows the two sets of four songs used in the playback experiments EA and EB. In each experiment, Ctrl is the copy of a natural adult song used earlier (song CC1[21]). This song fits the morphological diversity of song phrases described by Güttinger et al.[22] and it elicited strong reactions in female canaries, so we chose it as control. All the other songs were selected having the control song duration (8 s). The original songs were recorded on a Marantz CP 430 cassette recorder. All the sounds were bandpass filtered and digitized at 22 ksamples/s using an analysis and synthesis software. They were equated for loudness and edited in the time domain to eliminate discrete noises. The six songs of the young isolated males were recorded from two different males (Ma & Mb): the three songs used in experiment A (EA) from the male Ma and the three songs used in experiment B (EB) from the male Mb. Ma and Mb hatched in May 1993. They came from two different broods and had different parents. Parent males were removed from the breeding females 6 to 7 days before the first young hatched and the females raised the young birds. These birds were therefore out of hearing of any conspecific male song prior to hatching. Several data strongly suggest that, by this time, the main neural pathways allowing song learning in this species are not functional[23]. Both young birds began to sing subsongs roughly one month after hatching and, from this period on, were separated from their mother and sisters and regrouped with other isolated young males in two different sound attenuation chambers. Ma had individual contacts with three other isolated young males (same age) raised in the same conditions as him. Mb was also housed with three other isolated young males of the same age. All the isolated males were singly recorded each month from September 1993 to February 1994, using in each case a Marantz CP 430 cassette recorder.

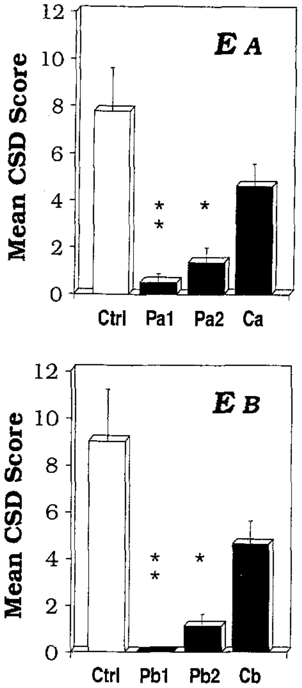

Figure 2. Mean CSD score, measuring female sexual responsiveness (vertical axis) to the four types of domesticated canary songs (horizontal axis) in each experiment (EA and EB). Mean number (+SE) of CSD elicited in female canaries by each song. Friedman two-way analysis of variance by ranks was followed by Friedman comparison of conditions with a control. Black shadings indicate isolated male songs. Labels * and ** indicate significant differences to the control (Ctrl) of $p < 0.05$ and $p < 0.01$.

Most of the songs (more than one hundred songs per male) were studied using the analysis and synthesis software developed by J. P. Richard[24]. Many of the songs recorded during each period were shared in each isolation group, as reported for other species[25]. The two sets of three songs were selected because of their frequent occurrences in most of the different utterances of each male. The plastic songs 'Pa1' of Ma and 'Pb1' of Mb were both recorded on 9 September 1993. The plastic songs 'Pa2' of Ma and 'Pb2' of Mb were recorded on 26 October 1993. The crystallized songs 'Ca' of Ma and 'Cb' of Mb were recorded on 8 December 1993. In each experiment the plastic song (Pa1 or Pb1) recorded very soon after moulting (roughly 4 months after hatching) does not have the features recorded in natural canary plastic songs as early as 60 days after hatching[23]: distinct song syllable types are very rare, the versatility is high, the frequency range is wide and the general structure is amorphous. This type of song is close to natural subsongs of this species recorded in our aviaries. Both plastic songs (Pa2 and Pb2) were recorded 45 days after Pa1 and Pb1. They include short trills and some of these are precursors of the trills developed in the crystallized songs Ca and Cb.

Each of the eight songs (duration: 8 s) is repeated six times to build a song bout. In the eight song bouts, each song is separated from the others by silence (duration: 12 s) allowing full female displaying. So, the total duration of each song bout is 108 s. During each testing session, the four song bouts were presented in random order. A delay of 3 m separated the playbacks of two successive song bouts.

A period of natural sexual receptivity in the females[21, 26] in June 1994 was used for the playback experiments. Each female was tested singly for the first time twenty days after her first young hatched (at the very beginning of a new period of sexual receptivity preceeding a second period of nesting). All the females started gathering nest material and nesting. Each experiment was carried out once a day whether in the morning or in the afternoon (alternating daily) over roughly three weeks for each female. The end of the test was linked to the cessation of sexual displays on four consecutive days. Testing was carried out each day between 10 h and 18 h.

We used non-parametric statistics[27]. Responses to the stimuli have been compared and tested for significance. We used the Friedman two-way analysis of variance by ranks for related samples, followed by Friedman comparison of conditions with a control. In all cases, low rankings indicate the serial positions that elicited the most female responses. Responses to the control songs in EA and EB have been compared and tested for significance using the Wilcoxon two-tailed signed rank test. The extent of association among the rankings was measured using the Kendall coefficient of concordance.

Results

CSD scores in relation to the four different types of songs of each experiment were totaled. Figure 2 shows the mean CSD scores recorded for each song in EA and EB. We confirmed overall heterogeneity with Friedman's analysis of variance (EA: $N = 8$; $\chi^2 = 19.7$, $p = 0.0002$; EB: $N = 8$; $\chi^2 = 21.5$, $p < 0.0001$). Scores were compared and tested for significance using the Friedman comparison of conditions with a control (EA: sums of ranks: Ctrl = 9.5, Pa1 = 30, Pa2 = 24.5, Ca = 16, limit value = 14.85 for $p = 0.05$ and 18.45 for $p = 0.01$; EB: sums of ranks: Ctrl = 9.5, Pb1 = 30, Pb2 = 25.5, Cb = 15, limit value = 14.85 for $p = 0.05$ and 18.45 for $p = 0.01$). There was no significant difference in the levels of CSD elicited by the song Ctrl in EA and EB ($N = 8$, $t = -1.30$, $p = 0.23$, Wilcoxon two-tailed). We observed that, in both experiments, the control adult song elicits the highest CSD scores, while isolate plastic songs elicit very low CSD scores. Both isolate crystallized songs Ca and Cb elicit high levels of CSD. These levels do not differ significantly from levels elicited by the control. The mean CSD scores as well as the rankings of these songs are fully comparable in both experiments. Kendall's coefficient of concordance showed that the females ranked the four stimuli in a similar way (EA: $W = 0.817$, $p < 0.01$; EB: $W = 0.849$, $p < 0.01$).

Conclusion and discussion

Our results demonstrate that among male isolate songs only crystallized songs convey sexual information and that plastic songs elicit very weak sexual responses. These data may be considered with regard to our recent findings about sexual releasers of the adult male canary songs[6].

The low potential of plastic male isolate songs. The early plastic songs Pa1 and Pb1 present the features recorded in natural subsongs, probably because of a delay in song development due to isolation: in this type of song, the high versatility of elements and the absence of trills help to explain the lack of female sexual receptivity. With regard to song segmentation, we recently demonstrated that low CSD scores were recorded using common canary phonology and the versatile structure of a wild canary song[28]. With regard to phonology, we demonstrated that female canaries are clearly sensitive to special types of song trills[6]. So in early isolate plastic songs, both song structure and phonology lack main sexual features. Although trills are present in late plastic songs Pa2 and Pb2, adult species-specific clear song segmentation is still lacking and, within trills, the shape and frequency range of the elements are still highly variable, when compared to these features in crystallized isolate songs. Further investigations are needed to demonstrate how females weigh these song parameters, but as a whole, again at this stage of the song development, functional sexual releasers are not fully represented.

The high potential of crystallized male isolate songs. In this species, the levels of CSD elicited by the crystallized isolate songs are strikingly high: in fact, these levels do not differ significantly from levels elicited by the adult natural song control. It is unlikely that any bias would be due to the choice of the control: this song elicited very high levels of CSD in females in a former experiment (song 'CC1'[21]). This high sexual potential of isolate adult songs relates the canary more to the cowbird (a parasitic species) than to the swamp, song and white-crowned sparrows.

One explanation would be that in this species as in other cardueline finches, specific rules of song architecture emerge without any adult tutor influence[11,18,22,29]. We know that these rules are important to drive female responsiveness[28] and many sexual responses may be related to song segmentation. However, it is unlikely that such high levels of CSD may be recorded if crystallized songs of isolated males and normal adult canary songs had no phonological similarities. With regard to this latter point, the canary may contrast with the white-crowned sparrow studied by Spitler-Nabors and Baker[5] who demonstrated that, in this species, adult isolate songs lack particular types of syllables that may play an important role for female sexual responses. This may not be the case in canaries. Thus, further experiments are planned to compare the sexual responsiveness of female canaries to the phonologies of adult isolate trills and adult natural trills.

As a whole, the present results demonstrate that particular types of canary songs may convey sexual information that is used by females. In this species, the sexual releasers are not strictly found in adult male natural songs but also in adult isolate songs. Self-differentiation appears to be an important process leading to the shaping of certain sexual releasers at the end of male song ontogeny.

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