## Obtaining copulation solicitation displays in female canaries without estradiol implants

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Abstract. Female domesticated canaries (Serinus canaria) respond to conspecific song with copulation solicitation display (CSD) between 22 and 27 days after their first offspring has hatched. This period could be used to investigate the acoustical preferences of female canaries. This non-invasive method respects the natural reproductive cycle and could be an alternative to the invasive method of estradiol implants.

Key words. Song stimuli; sexual responses; female canaries; non-invasive method; natural reproductive cycle.

In most oscine birds the females take the more active part in selecting a mate, choosing a male partly by his song<sup>1</sup>. In order to test these song preferences, authors have usually employed the method proposed by Searcy and Marler<sup>2</sup>. The basic procedure in the laboratory experiments is to treat captive females with estradiol implants and then present them with varying numbers of song types. It has been shown that estradiol-treated females respond to conspecific songs with copulation solicitation display (CSD), a courtship behaviour that usually precedes coition<sup>2–4</sup>. The number and intensity of these displays serve as an index of the female preferences.

With this method, a large repertoire and song diversity in the male<sup>5-7</sup> have been shown to be important for females. The females are also able to discriminate between conspecific and heterospecific song<sup>3,8,9</sup>. Unfortunately, there is a drawback associated with this method, namely the introduction of hormones without any relationship to the natural hormonal cycles of the bird. In addition, according to Searcy et al.<sup>3</sup>, 50% of implanted swamp sparrows (*Melospiza georgiana*) do not show any response to the test in some experiments.

In order to alleviate these problems, we recommend a new method that can be applied to domestic birds. This method provides information as to their periods of receptivity. In our tests all females presented CSD when they heard conspecific songs.

## Materials and methods

The strain of canary used in this study, known locally as the domesticated common canary (*Serinus canaria*), is an outbred form with a heterogeneous genetic background. Eight two-year-old females and eight two-year-old males were mated at random. The pairs were placed in sound attenuation chambers (more details in Kreutzer and Vallet<sup>9</sup>). After the egg-laying period, the males were taken out of the reproduction room. All females were tested to see whether they showed CSD on

hearing songs. These experiments were carried out over 30 days, beginning 15 days after the first of the offsprings hatched.

The female canaries were tested with six song phrase stimuli: four conspecific songs (two common canary songs - CC1 and CC2 - and two wild canary songs -WC1 and WC2) and two heterospecific songs (pine siskin (Carduelis spinus) - PS1 and PS2). In order to control for variations in domesticated common canary, wild canary, and pine siskin songs, two song tapes were used in each case<sup>10</sup>. All song phrase stimuli were copies of natural songs, chosen at random and edited using software developed by J.-P. Richard<sup>11</sup>. Each song phrase stimulus was repeated six times in order to set up a song bout. The duration of each song stimulus is 8 s with a 10 s pause according to the canary rules of song organisation<sup>12</sup>. The six different song bouts were presented in random order varying from one session to another. Two test sessions were carried out each day between 10.00 h and 18.00 h from May to June 1992. The CSD was used as an index of female response to song. We measured the frequency of displays, scoring the responses as follows: 0 for no display, 0.5 for an incomplete display, and 1 for a complete display (in detail in reference 13).

## Results

Scores in relation to the different songs were compared and tested for significance using the Friedman comparison of conditions with control (the song that elicits the most of CSD), having first confirmed overall heterogeneity with Friedman's analysis of variance (df = 5,  $\bar{X} = 26.071$ , p < 0.001). We observed that the eight females gave stronger responses to songs of their own strain than to songs of the other strain or to heterospecific songs (fig.). The song CC2 elicited an intermediate level of response, between the CC1 control and the other songs. However, the number of CSD to the domesticated common canary songs CC1 and CC2 are not

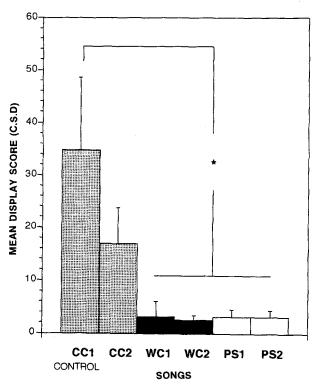


Figure. The mean display score ( $\pm$ SE) measures the number of copulation solicitation displays (CSD) of the female canaries for each song phrase stimulus. Abbreviations are: domesticated common canary 1 and 2 (CC1 – Control – and CC2), wild canary 1 and 2 (WC1 and WC2), pine siskin 1 and 2 (PSI and PS2). Significant heterogeneity was tested by using Friedman two-way analysis of variance by ranks. Label \* indicates significant differences (p < 0.01) between CC1 (Control) and WC1, WC2, PS1 and PS2 (Friedman comparison of conditions with control). All statistics were non-parametric, so mean values are for illustration only.

significantly different from each other. In contrast WC1, WC2, PS1 and PS2 stimuli showed levels of response that were significantly weaker than to the CC1 control (Friedman comparison of conditions with control; p < 0.01 for WC1, WC2, PS1 and PS2-sums of ranks: CC1 control = 8, CC2 = 18, WC1 = 35, WC2 = 34, PS1 = 37, PS2 = 36; limit value = 22.89 for p = 0.01). Kendall's coefficient of concordance showed that the females ranked the six song phrase stimuli in a similar way (w = 0.647, p < 0.01).

This set of results was obtained as a result of the discovery of a period favourable to the manifestation of CSD. This period begins 22 days after the first hatching  $(\bar{X}=22.38, SE\pm 1.34, n=8)$ . The response declines when the female again starts to lay eggs, which happens 27 days after the first hatching of young birds from the previous clutch  $(\bar{X}=26.88, SE\pm 0.73, n=8)$ .

## Discussion

The female domesticated canaries tested during these experiments responded preferentially to two songs of

domesticated canaries (CC1 and CC2). The songs of the strain of wild canaries (WC1 and WC2), as well as the heterospecific songs of the pine siskin (PS1 and PS2), gave rise to only a small number of CSD. As a whole, these results confirm those obtained with the use of estradiol implantation by Kreutzer and Vallet<sup>9</sup>.

The results obtained from these experiments on the reproductive cycle of female domesticated canaries, together with previous knowledge14,15, allow us to forecast the periods of response of the females. Thus after the first hatching, there is an interval of on average 22 days before the females restart their CSD in response to our test sessions, and on average 27 days before they lay the first egg of the next clutch. Between these two dates (22 and 27 days after the first hatching), we observed the maximum of CSD. A similar period, close to the laying of the second clutch, has been shown to be the most favourable moment for the fertilization of the eggs<sup>16</sup>. Nakamura<sup>17</sup> observes a similar period of copulation preceding the second egg-laying period for a wild species (alpine accentor Prunella collaris).

The primary interest of this technique is that it enables a non-invasive method to be substituted for an invasive one (estradiol implants). It enables females to be tested, using their natural cycle, to understand their acoustic preferences. The method could also be applied to species other than the domesticated canary.

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