

ESTRADIOL DOSAGE AND THE SOLICITATION DISPLAY ASSAY IN RED-WINGED BLACKBIRDS¹

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Abstract. The majority of female Red-winged Blackbirds (*Agelaius phoeniceus*) given exogenous estradiol responded to playback of male song with copulation solicitation. Nine females given no exogenous estradiol all failed to give any displays in response to playback. We conclude that estradiol treatment is necessary to elicit response to playback in captive female Red-winged Blackbirds. Given the task of discriminating full conspecific song from a partial song, females treated with a double dose of estradiol showed a degree of discrimination nearly identical to that shown by females treated with a single dose. Variation in estradiol dosage appears not to have a strong effect on discrimination.

Key words: *song, estradiol, Red-winged Blackbirds, Agelaius phoeniceus, playback.*

A widely used method of measuring female response to male song in birds is the solicitation display assay (Searcy 1992). In this method, captive females are exposed to playback of male songs, and they respond with copulation solicitation, a courtship display given before and during copulation in many species of birds. Number, intensity, or duration of displays can be used to quantify response to different playback treatments. Because the response measured is so tightly coupled to copulation, the assay arguably gives a fairly unambiguous indication of female sexual preferences.

Following Searcy and Marler (1981), almost all applications of the solicitation display assay have primed subjects with estradiol prior to testing with playback. In the very first use of the assay, however, King and West (1977) tested untreated female Brown-headed Cowbirds (*Molothrus ater*), and in this and further studies observed strong response to song in their subjects (e.g., King et al. 1980, West et al. 1981). Recently, females have been shown to solicit in response to song without estradiol treatment in a second species, the Canary (*Serinus canaria*) (Nagle et al. 1993, Le-boucher et al. 1994). Only in Eurasian Blackbirds (*Turdus merula*) is there direct evidence that females will respond to song with solicitation display when treated with estradiol, but will not respond when untreated (Dabelsteen 1988). Taken together, these results

raise the question of whether estradiol treatment actually is necessary to obtain female response to song in most species. Here we address this question for Red-winged Blackbirds (*Agelaius phoeniceus*).

We also examine whether estradiol dosage affects discrimination in female Red-winged Blackbirds. Concern has been expressed, in peer review of past papers, that estradiol treatment may tend to distort female preferences for song. Some reviewers have worried that high levels of estradiol might cause females to show preferences that they might not otherwise exhibit, but to us the more logical argument would be that estradiol might lower discrimination by making females hyper-responsive to all songs. Previous work has shown that female Red-winged Blackbirds respond preferentially to full conspecific songs over partial ones (Searcy and Brenowitz 1988, Searcy 1990); here we present this same discrimination task to two groups of females given different dosages of estradiol.

METHODS

Subjects were 53 adult female Red-winged Blackbirds captured in the vicinity of the Pymatuning Laboratory of Ecology, Crawford County, Pennsylvania. We tested groups of 10, 10, and 7 females during May and June, 1990, and groups of 10, 10 and 6 females during May and June, 1991. Subjects were released unharmed after testing.

Estradiol treatments were in the form of crystalline 17- β -estradiol in silastic tubing of 1.96 mm outer diameter, sealed at both ends with adhesive. We used four dosage treatments: (1) one implant of 18 mm length containing 14 mm of hormone ("1-X"), (2) two implants of 18 mm length ("2-X"), (3) one implant of 12 mm length containing 7 mm of hormone ("½-X"), and (4) one implant of 18 mm length filled with glue ("sham"). The 1-X dose is the same as that used in previous experiments with female Red-winged Blackbirds (Searcy 1988, 1990). This dose is modeled on that used by Moore (1983) in White-crowned Sparrows (*Zonotrichia leucophrys*), and found to produce plasma estradiol levels in the upper part of the range shown by breeding females in that species (Wingfield and Farner 1978). The Red-winged Blackbird dose is increased over that used for White-crowned Sparrows by scaling up relative to body size raised to the 0.7 power (Searcy 1992). We placed implants under the skin of the back after application of a topical anesthetic.

We randomly assigned subjects in the first four groups to the 1-X, 2-X, and sham treatments in the approximate

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ratio of 2:2:1. We randomly assigned subjects in the final two groups to the 1-X, ½-X, and sham treatments in the same approximate ratio. After testing, implants were removed, again following application of a topical anesthetic. One subject in the 1-X group and six in the 2-X group were found to have lost one or more of their implants, and these were dropped from the analysis.

After treatment, subjects were housed in cages within sound attenuation chambers and maintained on a 16:8 hour light:dark cycle. Songs were presented to subjects from a Tandberg TB15 tape recorder over a Realistic 40-1272 speaker (effective range 90–20,000 Hz) inside the chamber. Responses were observed using a video camera mounted on the chamber window. The response measure was the number of copulation solicitation displays performed.

Subjects were tested on day 4 after hormone administration with a tape of four song types, each recorded from local, conspecific males. Each song type was repeated eight times at the rate of one song per 10 sec, for a total of 32 songs. Subjects were tested on day 8 with a second tape, containing a different set of four conspecific song types in the same format. Between 9 and 11 days after hormone administration, subjects were tested on each of two days with 12 repetitions of a full Red-winged Blackbird song ("full song") and 12 repetitions of the same song with the introductory notes removed ("trill"). The full song and trill were presented in random order to a subject on the first day of testing and in the reversed order on the second day, with at least three hours between tests within a day. The original full song used was recorded in Ithaca, New York (Searcy and Brenowitz 1988).

RESULTS

Of the nine females given sham implants, none ever displayed during testing. Of those given the ½-X dose, 2 of 7 displayed (29%); of those given the 1-X dose, 15 of 20 displayed (75%); and of those given the 2-X dose, 5 of 10 displayed (50%). The variation in proportion displaying across dosage category was significant ($\chi^2_3 = 15.2, P = 0.002$). Lumping all the estradiol dosages together, those given exogenous estradiol were significantly more likely to display than were controls ($\chi^2_1 = 10.3, P = .001$).

Among the females that responded to song, 2-X subjects gave slightly fewer displays to both full song and trill than did 1-X subjects (Fig. 1), but in neither case was the difference between dosage groups significant ($P > 0.50$ by Mann-Whitney U -tests). To compare the degree of discrimination shown by subjects in the two treatment groups, we examined the proportion of displays given to the full song out of the total given to full song and trill. This proportion was nearly identical for the 5 2-X subjects (mean = 0.68) as for the 14 1-X subjects (mean = 0.69) that responded during the full vs. trill trials. The slight difference in discrimination was not significant (Mann-Whitney $U = 36.5, z = -0.14, P = 0.89$).

DISCUSSION

Captive female Red-winged Blackbirds in our experiments gave no copulation solicitation displays in response to song playback unless previously treated with estradiol. Dabelsteen (1988) found the same pattern in Eurasian Blackbirds. Female White-crowned Spar-

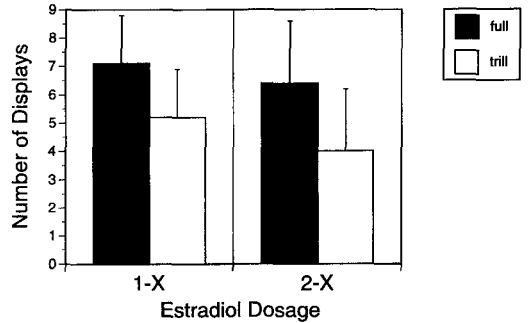


FIGURE 1. Mean (\pm se) number of copulation solicitation displays performed by female Red-winged Blackbirds in response to full song and trill. Subjects were given either one implant (1-X) or two implants (2-X) of estradiol. There is no significant difference in discrimination between the two estradiol treatment groups.

rows, observed with males but without playback, similarly give displays when treated with estradiol but give none if not treated (Moore 1983). By contrast, female Brown-headed Cowbirds (King and West 1977) and Canaries (Nagle et al. 1993, Leboucher et al. 1994) do produce displays without estradiol treatment. Canaries, which of course breed in captivity, display only during a limited time before and during egg-laying (Leboucher et al. 1994). Female Brown-headed Cowbirds copulate in captivity (Eastzer et al. 1985), and West and King (1996) imply that females only display when in breeding condition. The trend seems to be that females in species that will mate and produce eggs in captivity (Canaries, Brown-headed Cowbirds) give solicitation displays in response to male stimuli as captives without estradiol treatment, whereas females in species that will not breed readily in captivity (Red-winged Blackbirds, Eurasian Blackbirds, White-crowned Sparrows) seem to require exogenous estradiol if they are to give solicitation displays as captives.

Our second conclusion is that the estradiol dose given to female Red-winged Blackbirds does not change their discrimination between two song stimuli. Subjects given the 1-X and 2-X doses showed virtually identical discrimination between the full song and trill. We cannot report plasma estradiol levels in our subjects, but in general steroids diffuse through silastic tubing at a rate proportional to the surface area of the implant (Dzwick and Cook 1966, Stratton et al. 1973), so twice as much should diffuse from two implants as from one. Moore (1983) showed that plasma estradiol levels in female White-crowned Sparrows increase in direct proportion to the number of implants they are given. The 2-X treatments in our experiments thus should have produced plasma estradiol levels roughly twice as high as for the 1-X treatments. We conclude that changes in estradiol levels of this magnitude do not affect song discrimination.

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LITERATURE CITED

- DABELSTEEN T. 1988. The meaning of the full song of the Blackbird *Turdus merula* to untreated and estradiol treated females. *Ornis Scand.* 19:7-16.
- DZWIK, P. J., AND D. COOK. 1966. Passage of steroids through silicone rubber. *Endocrinology* 78:208-211.
- EASTZER, D. H., A. P. KING, AND M. J. WEST. 1985. Patterns of courtship between cowbird subspecies: evidence for positive assortment. *Anim. Behav.* 33:30-39.
- KING, A. P., AND M. J. WEST. 1977. Species identification in the North American cowbird: appropriate responses to abnormal song. *Science* 195:1002-1004.
- KING, A. P., M. J. WEST, AND D. H. EASTZER. 1980. Song structure and song development as potential contributors to reproductive isolation in cowbirds (*Molothrus ater*). *J. Comp. Physiol. Psychol.* 94:1028-1039.
- LEBOUCHER, G., M. KREUTZER, AND J. DITTAMI. 1994. Copulation-solicitation displays in female canaries (*Serinus canaria*): are oestradiol implants necessary? *Ethology* 97:190-197.
- MOORE, M. C. 1983. Effect of female sexual displays on the endocrine physiology and behaviour of male White-crowned Sparrows, *Zonotrichia leucophrys*. *J. Zool.* 199:137-148.
- NAGLE, L., M. L. KREUTZER, AND E. M. VALLET. 1993. Obtaining copulation solicitation displays in female canaries without estradiol implants. *Experientia* 49:1022-1023.
- SEARCY, W. A. 1988. Dual intersexual and intrasexual functions of song in Red-winged Blackbirds. *Proc. Int. Ornithol. Congr.* 19:1373-1381.
- SEARCY, W. A. 1990. Species recognition of song by female Red-winged Blackbirds. *Anim. Behav.* 40:1119-1127.
- SEARCY, W. A. 1992. Measuring responses of female birds to male song, p. 175-189. *In* P. K. McGregor [ed.], *Playback and studies of animal communication*. Plenum, New York.
- SEARCY, W. A., AND E. A. BRENOWITZ. 1988. Sexual differences in species recognition of avian song. *Nature* 332:152-154.
- SEARCY, W. A., AND P. MARLER. 1981. A test for responsiveness to song structure and programming in female sparrows. *Science* 213:926-928.
- STRATTON, L. G., L. L. EWING, AND C. DESJARDINS. 1973. Efficacy of testosterone-filled polydimethylsiloxane implants in maintaining plasma testosterone in rabbits. *J. Reprod. Fert.* 35:235-244.
- WEST, M. J., AND A. P. KING. 1996. Eco-gen-actics: a systems approach to the ontogeny of avian communication, p. 20-38. *In* D. E. Kroodsma and E. H. Miller [eds.], *Ecology and evolution of acoustic communication in birds*. Cornell Univ. Press, Ithaca, NY.
- WEST, M. J., A. P. KING, AND D. H. EASTZER. 1981. Validating the female bioassay of cowbird song: relating differences in song potency to mating success. *Anim. Behav.* 29:490-501.
- WINGFIELD, J. C., AND D. S. FARNER. 1978. The annual cycle of plasma irLH and steroid hormones in feral populations of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Biol. Reprod.* 19:1046-1056.