FEMALE BUNTINGS FROM HYBRIDIZING POPULATIONS PREFER CONSPECIFIC MALES

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ABSTRACT.—I captured five female Indigo Buntings (*Passerina cyanea*) and 12 female Lazuli Buntings (*P. amoena*) from a hybridizing population in Wyoming and tested them in the laboratory for their sexual display responsiveness to male traits (live male and broadcast vocalizations) of the two species. The females responded with more copulation solicitation displays when exposed to conspecific male traits than when exposed to heterospecific male traits. Consistent preference of female buntings for conspecific male characteristics in these choice tests, together with previous results, suggests that hybrid pairs may form when females are faced with a choice of mating heterospecifically or not at all. *Received 14 Nov. 1995, accepted 20 April 1996.*

Populations of Indigo Buntings (*Passerina cyanea*) and Lazuli Buntings (*P. amoena*) overlap and hybridize in the Great Plains of North America (Rising 1983). Although hybrids are easily found in some populations, mating is non-random with positive assortment being the general rule among the pure phenotypes of the two species (Emlen et al. 1975; Baker, unpubl. data). Non-random mating in the overlap populations could have a number of proximate explanations. Mate preferences could be established in allopatric populations and emigrants from these areas into sympatric populations may retain their preferences when encountering heterospecifics, at least in initial encounters. A previous experiment gave results consistent with this hypothesis (Baker and Baker 1990). On the other hand, continuing exposure of an immigrant female to the visual and vocal courtship displays of heterospecific males could perhaps alter her preferences. An extenuating circumstance might be the availability of male mates of the alternative phenotypes.

An earlier experiment (Baker 1994) exposed females from allopatry to heterospecific males during the time they were coming into reproductive condition upon photostimulation. This exposure was restricted to 25 days and occurred in cages in the laboratory. Such exposure to displaying males had no significant effect on female preference, as indicated by an assay of copulation solicitation displays, for conspecific males and vocalizations. It is likely that females in sympatric populations experience a longer and more intense level of exposure to heterospecific courtship than was achieved in the laboratory study, and this prolonged exposure might be effective in altering female preferences. If so, such altered preferences of females could lead to the formation of observed hybrid pair-

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ings. The present experiment was conducted to address the question of whether Lazuli and Indigo Bunting females from a natural population consisting of a mix of phenotypes have significant preferences for conspecific male traits when given a choice of conspecific versus heterospecific males and vocalizations in a controlled experiment.

METHODS

Seventeen female buntings (12 Lazuli, 5 Indigo) were captured in a hybridizing population in NE Wyoming. Captures were made 18–27 July 1989 of females with fully developed brood patches, indicating that they were reproductive. All the subjects were captured during the territorial period and all had both Lazuli and Indigo males in nearby territories. Some hybrid males also defended territories in the neighborhood. None of the birds was banded, however, so the identity of the mate was not certain.

The plumage characteristics of the females were evaluated by the criteria of Emlen et al. (1975). This character index judges three areas of female plumage: throat/breast, wing bars, and back/rump. A pure Indigo would score 0 in all three regions for a total score of 0, and a pure Lazuli would score 1 in all regions for a total score of 3. Intermediates in any region were given a score of 0.5. There was some subjectivity involved in applying the scoring system, so I considered scores of 0 and 0.5 as Indigo and of 2.5 and 3 as Lazuli.

The females were housed in separate cages in a single room in the laboratory. They were fed a seed mixture of canary grass and millet, turkey starter, vitamins, grit, and water. Starting 30 July, the photoperiod was gradually reduced to 8:16 (L : D) by 9 October and retained on this cycle until 22 January 1990 when the cycle was switched overnight to 16: 8 (L : D). On 22 February, the females each received one silastic implant (10 mm long, 1.47 mm inside diameter, Dow Corning) subcutaneously in the breast region. Each implant contained 17-beta estradiol (Sigma Biochemical) and was sealed at both ends with silastic adhesive (Dow Corning). This treatment is necessary to bring females into full reproductive readiness in the laboratory.

Starting 5 February, each subject in her home cage was habituated to testing circumstances by being placed in an experimental chamber containing an empty cage and a loudspeaker. An observation booth one meter away was equipped with a video camera, one-way glass, and a cassette recorder for playing stimulus tapes over the loudspeaker. Each female had nine habituation sessions of 30 min at a rate of one session every other day.

In the experiment, a test session consisted of placing a caged female into the observation chamber together with a stimulus male occupying the adjacent cage and playing vocalizations, matched with his species, from the loudspeaker located on the side of the male cage away from the female. Previous studies indicated that both male plumage characteristics and vocalizations play significant roles in the expression of female preference as indicated by the copulation display assay. Four Lazuli and two Indigo males were used as stimuli. All were captured in allopatric populations and maintained together in acoustic isolation from the females. The males did not vocalize during female testing, although they hopped around in the cage. Following 3 min of initial silence, the loudspeaker broadcast a sequence of 1 min of male songs at a rate of one song each 15 sec, 1 min of "tseep" calls (a courtship call, Thompson and Rice 1970, Thompson 1976) at a rate of one tseep every 10 sec, 1 min of male songs (different from those in the first min), 1 min of "tseep" calls, 1 min of male songs (also different from the earlier songs), and a final minute of "tseep" calls. The "tseep" call, similar in the two species, is an important component of courtship vocal behavior and increases female responsiveness in the laboratory assay (Baker and Baker 1988).

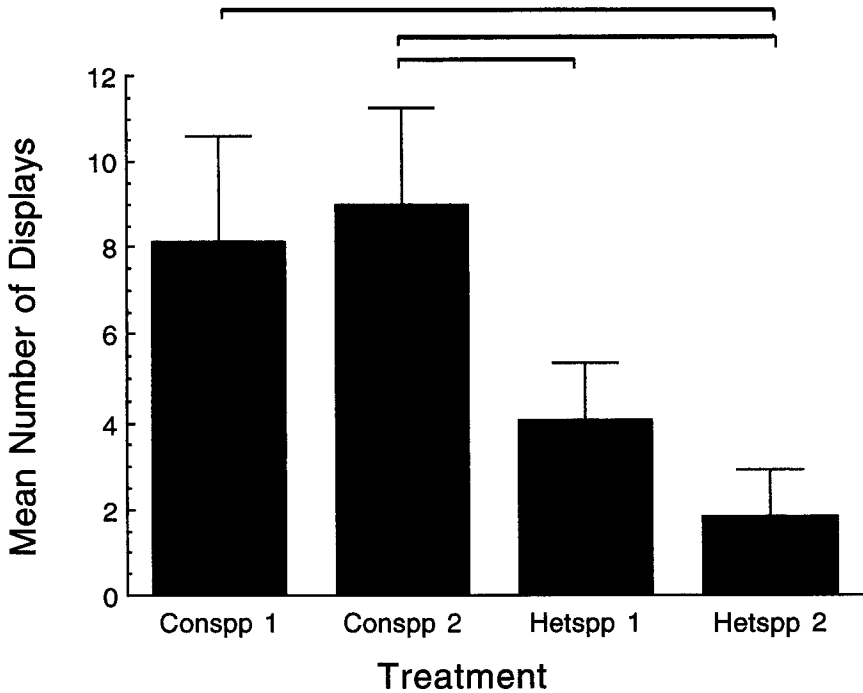From 3–12 March, half the females were tested first with Lazuli males and vocalizations

FIG. 1.   Mean number of copulation solicitation displays (+1 SEM) elicited from female buntings by conspecific versus heterospecific males and their recorded vocalizations. Horizontal lines above histogram bars connect pairs of treatments that were significantly different.

and then with Indigo males and vocalizations. The other half the females were tested with the order of stimuli reversed. No subject was tested twice on the same day, and there was at least 1–2 days between successive tests on a female. With completion of these first test sessions, the stimulation of the subjects by conspecific stimuli was considered one treatment, and the stimulation by heterospecific stimuli was considered a second treatment. A second set of tests followed the first and again presented Lazuli and Indigo stimuli to each subject. In this set, the stimulus male presented to each female differed from the one she had experienced in the first set, and an entirely new group of songs was used for the vocal stimuli. The same tseep calls were used, however, because of a paucity of good quality recordings of this vocalization. With completion of this second set of tests, presentation of conspecific stimuli constituted a third treatment and presentation of heterospecific stimuli a fourth treatment. I used repeated measures ANOVA and Fisher's LSD for multiple comparisons (Winer 1971) for statistical analyses.

RESULTS

In the treatments involving exposure to conspecific males and vocalizations, the females exhibited a high level of responsiveness (Fig. 1). The

average number of copulation solicitation displays elicited by the first conspecific treatment (Conspp-1) was 8.1 and that by the second conspecific treatment (Conspp-2) was 9.0 (ns, $P > 0.05$, Fisher's LSD). The two treatments presenting heterospecific males and vocalizations elicited lower numbers of solicitation displays (Fig. 1). The average number of displays elicited by the first heterospecific treatment (hetspp-1) was 4.0 and by the second heterospecific treatment (hetspp-2) was 1.8 (ns, $P > 0.05$, Fisher's LSD).

In comparing conspecific to heterospecific treatments, the number of solicitation displays elicited by both Conspp-1 and Conspp-2 were greater than the number elicited by Hetspp-2, and the number elicited by Conspp-2 was greater than that elicited by Hetspp-1 (Fig. 1, all Ps < 0.05, Fisher's LSD). The comparison between Conspp-1 and Hetspp-1 did not give a significant difference.

Comparing responses of Indigo ($N = 5$) and Lazuli ($N = 12$) females within each treatment revealed that there were no differences in display production in three of the four treatments (Conspp-1, $P = 0.36$; Conspp-2, $P = 0.45$; Hetspp-1, $P = 0.70$; Mann-Whitney U-tests, two tailed, Siegel 1956). In the Hetspp-2 treatment, however, Indigo females averaged more displays than Lazuli females ($P = 0.01$, Mann-Whitney U-test, two tailed). In this treatment, only two of the 12 Lazuli females gave displays whereas four of the five Indigo females gave displays.

DISCUSSION

The results suggest that prolonged exposure of females to the songs and plumages of both Indigo and Lazuli bunting males in a natural breeding association does not alter preference for conspecific traits. Although the exact nature of previous experience of each female is unknown, it is possible to outline the range of possible exposures the subjects had prior to testing. These migrants arrived in the study area in late May and early June. In the immediate neighborhoods of the subjects were territorial Lazuli, Indigo, and hybrid males. Given capture in late July, it is likely that the females were exposed to males of all three types for a minimum of 5–8 weeks. Alternatively, any or all of the subjects could have hatched in a mixed population and experienced one or more breeding seasons in such a context. Thus, the experience the females had with males and songs prior to testing occurred in a natural population and was probably more intense and longer than was provided in the earlier laboratory experiment that attempted to alter adult female preferences (Baker 1994). Although the experimental females were relatively refractory to the potentially influential presence of alternative species' male phenotypes, it is possible

that more extreme amounts of exposure could affect changes in female preference.

The general inference that can be made from these results is that when given a choice between conspecific and heterospecific males and vocalizations, female buntings prefer conspecific male traits. This implies further that heterospecific pairings in natural populations may result from females making the best of a bad job, choosing to mate heterospecifically rather than not mate at all. A female with a territory retains the possible option of extra-pair fertilization (Westneat 1990) with neighboring conspecifics while having the benefit of a nest site, feeding area, and other advantages of territorial residence. A similar pattern of female behavior has been observed in Fairy-Wrens (*Malurus splendens*), (Brooker and Rowley 1995), in which social pairing by a female appears to allow the opportunity for matings with high quality neighboring males.

ACKNOWLEDGMENTS

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