

BRIEF COMMUNICATIONS

Behavioral Compatibility and Reproductive Outcomes in Two Distant Populations of Captive Brown-headed Cowbirds (*Molothrus ater*)

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Increased understanding of geographic variation in courtship behavior in animal species can provide insight into the ways behavior may influence evolutionary change. Here, the relationship of geographic variation in courtship behavior to reproductive outcomes was tested in two distant and behaviorally distinct populations of brown-headed cowbirds, *Molothrus ater*, by comparing rates of copulation and egg production. Females from an Indiana (IN) population and a South Dakota (SD) population were housed in aviaries with males from either their own population (INsame and SDsame) or the other population (INmix and SDmix). Over the breeding season, INsame females exhibited higher rates of copulation and egg production compared with INmix females, but no differences were detected between SDsame and SDmix females. The data suggest that behavioral differences between IN and SD cowbirds are the likely cause of lower egg production in the INmix females.

Keywords: courtship, cowbirds, eggs, *Molothrus ater*, population differences

Choosing behaviorally compatible individuals as mates may be adaptive. Behavioral compatibility often correlates with the population's genetic background, resulting in physiological or genetic compatibility in reproduction (Lott, 1991; Verrell, 1999; Wilczynski & Ryan, 1999). Behavioral incompatibility may stem from courtship behavior differences between populations, and if such differences reduce gene flow between those populations, they may impact population divergence (Butlin & Ritchie, 1994; Verrell, 1999). Here we asked whether behavioral differences between two populations of brown-headed cowbirds, *Molothrus ater*, might affect reproductive outcomes. Captive populations of cowbirds have been shown to court and mate assortatively by population or "cultural" background (Eastzer, King, & West, 1985; Freeberg, 2004), but the reproductive significance of these different patterns of courtship remains largely unknown.

Despite much information on cowbird reproduction and courtship (Friedmann, 1929; Holford & Roby, 1993; Lowther, 1993; Rothstein, Yokel, & Fleischer, 1986; Yokel, 1989) and the fact that

there is geographic variation in courtship behavior (King & West, 1990; Rothstein et al., 1986), little is known about the extent of reproductive compatibility between different populations. Here we report data on egg production and copulation rates for female cowbirds from a South Dakota (SD) population, *M. a. artemisiae*, and an Indiana (IN) population, *M. a. ater*, housed in aviaries with males of either their own population or the other population. SD cowbirds differ from IN cowbirds in key aspects of their courtship systems. For example, the clusters of notes in SD male songs differ from those in IN male songs, and these differences predict male courtship effectiveness with females (Freeberg, King, & West, 2001). Evidence of geographic variation in courtship does not allow us to assume that differential reproductive outcomes will necessarily result, however (Butlin & Ritchie, 1994). Thus, the present study aimed to determine whether behavioral differences might be associated with differences in reproductive outcome.

Method

Subjects

IN females. Eighteen different IN females were present in an aviary with IN males (INsame), and 14 different IN females were present in an aviary with SD males (INmix), during the 1994 breeding season. All IN females were captured as adults in Monroe County, IN, in the summer of 1993 or spring of 1994. The 18 IN females in the INsame aviary averaged 32.6 days ($SD = 10.9$; range = 15–52) and the 14 IN females in the INmix aviary averaged 50.3 days ($SD = 4.4$; range = 40–52), in their respective aviaries.

SD females. Twelve different SD females were present in an aviary with SD males (SDsame), and 12 different SD females were present in an aviary with IN males (SDmix) during the 1994

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breeding season. Twenty-one of these SD females were captured as adults or juveniles in Fall River and Custer counties, SD, in the summer of 1991 or the spring of 1992. The final three SD females resulted from matings between SD females and males in the summer of 1993. The 12 SD females in the SD_{same} aviary averaged 43.9 days ($SD = 10.7$; range = 22–51) and the 12 SD females in the SD_{mix} aviary averaged 45.2 days ($SD = 11.2$, range = 13–52) in their respective aviaries.

IN males. Ten IN males were present during the breeding season in an aviary with IN females (IN_{same}), and 12 were present in an aviary with SD females (SD_{mix}). The IN males were captured as adults in Monroe County, IN, in the summer of 1993. The 10 IN males in the IN_{same} aviary averaged 37.9 days ($SD = 16.4$; range = 12–52) and the 12 IN males in the SD_{mix} aviary averaged 34.8 days ($SD = 15.7$; range = 9–52) in their aviaries.

SD males. Thirteen SD males were present during the breeding season in an aviary with IN females (IN_{mix}) and 14 were present in an aviary with SD females (SD_{same}). The SD males were captured as adults or juveniles in Fall River and Custer counties, SD, in the summer of 1991 or spring of 1992. The 13 SD males in the IN_{mix} aviary averaged 34.2 days ($SD = 15.5$; range = 7–52) and the 14 SD males in the SD_{same} aviary averaged 29.6 days ($SD = 15.0$, range = 4–51) in their aviaries.

All subjects caught prior to spring 1994 were housed with other cowbirds (including cowbirds from their own population) for at least the year preceding this study. All birds were fed daily with a modified Bronx Zoo diet for blackbirds and had fresh millet and canary seed ad libitum, and fresh vitamin-enriched water daily. The birds also had access to insects and other invertebrates that were naturally plentiful in the aviaries.

Breeding Season Housing

The four aviaries that housed the IN and SD females and males were each $9.1 \times 21.4 \times 3.4$ m with indoor sections. Birds in the four aviaries were visually, and largely acoustically, isolated from each other. The four aviaries were set up with their birds on May 17–18, 1994. On average during the breeding season, daily there were 11.3 IN females and 7.3 IN males in the IN_{same} aviary, 13.5 IN females and 8.6 SD males in the IN_{mix} aviary, 10.3 SD females and 8.1 SD males in the SD_{same} aviary, and 10.4 SD females and 8.0 IN males in the SD_{mix} aviary. When females and males were not in these breeding season aviaries, they were being used temporarily in other studies (from a few days to a few weeks). Males from the different aviaries were moved out of their aviary either 0 times (the male was in the aviary the entire breeding season) or 1 time, and this movement rate was similar across the four aviaries (mean number of times leaving the aviary per male: IN_{same} = 0.50, IN_{mix} = 0.79, SD_{same} = 0.88, SD_{mix} = 0.92). Females moved out of the aviaries to be used in other studies were never in contact with males from a population other than the one with which they were being housed here (e.g., an SD_{same} female may have been used in another study with SD, but not IN, males).

In the indoor section of each aviary, we packed assorted brush and branches and attached small artificial nests within the brush. Artificial nests were also placed in trees located in the outdoor aviary sections. Each aviary had 10–12 artificial nests. Artificial nests were stocked with “dummy” eggs: nonviable cowbird eggs (marked as nonviable), canary eggs, *Serinus canaria*, and zebra

finch eggs, *Taenopygia guttata*, and jelly beans. “Dummy” eggs for the artificial nests were restocked every few days.

Data Collection

Egg collection and incubation. We collected eggs each morning of the breeding season (May 18–July 8) from each of the four aviaries from 0600 to 0630. Eggs were incubated in a Petersime Model I commercial incubator at 37.5 °C. The humidity in the incubator was kept at a wet bulb reading of 28.3–28.9 °C. We monitored embryonic development by candling eggs every other day. Young cowbirds that hatched were placed into barn swallow, *Hirundo rustica*, nests that had recently hatched swallow nestlings or were judged to have eggs about to hatch. Cowbird nestlings were collected again from those nests at 5–9 days of age, and were subsequently hand-raised to independence, at which time they were released into aviaries containing other cowbirds.

Behavioral data collection. We focally sampled males (15 min/male/morning) in each aviary, using methods established in earlier studies with SD and IN birds (see West, King, & Freeberg, 1997). Data were collected between 0630 and 1030 in IN_{same}, SD_{same}, and SD_{mix} aviaries on 10 different mornings and in the IN_{mix} aviary on nine different mornings, between May 29 and June 28. One SD_{mix} female was not present in the aviaries during any of the mornings of behavioral data collection, but all the other females in the study were present on days of behavioral data collection. We scored copulations between females and males (when a female adopts a copulatory posture in response to a vocalization from a male, and the male mounts the female; West, King & Eastzer, 1981). We also scored the number of directed vocalizations from males to females, as a measure of general vocal output in the breeding season context. Directed vocalizations were those that occurred when the male was within 0.5 m of the recipient bird, and was moving, or orienting his body, toward the recipient while vocalizing (West et al., 1981).

Statistical Analysis

Because the ages and experiential histories of the IN females differed from those of the SD females, all our statistical comparisons are within female population. We use descriptive statistics to assess numbers of eggs laid and numbers of eggs laid that ultimately hatched because, although a female cowbird can only lay one egg per day, eggs are often laid in bouts of days such that the likelihood of a female laying an egg on one day might depend in part on whether she laid an egg in the previous days (e.g., Holford & Roby, 1993; Lowther, 1993). We used two-tailed Mann–Whitney *U* tests to compare females in their rates of copulating with males and to compare males in their rates of directed vocalizations to females. Data were analyzed using SPSS (Version 15.0); *z* statistics calculated by SPSS for these tests are reported below.

Results

Egg Production

We obtained twice as many eggs, and more eggs that ultimately hatched, from females in the IN_{same} aviary compared to the IN_{mix} aviary. In total, we collected 100 eggs from the IN_{same} aviary and 47

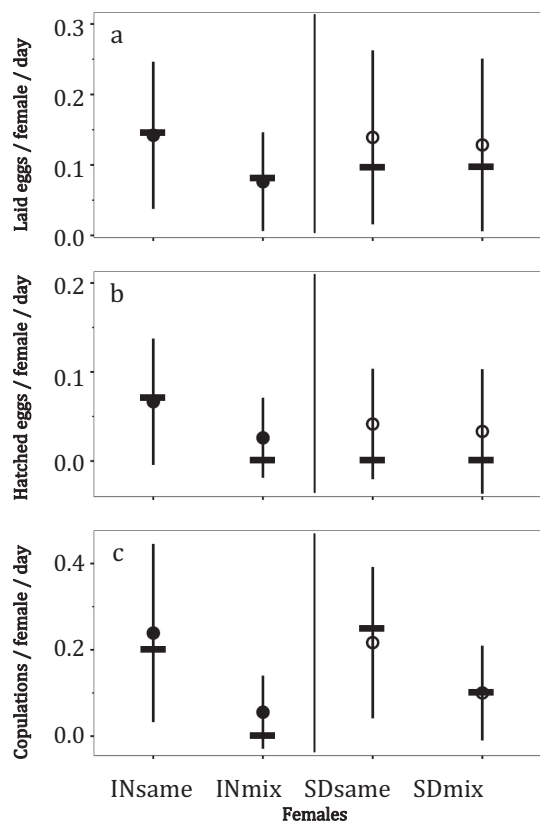


Figure 1. Mating and reproductive outcomes in the INsame (Indiana [IN] females with IN males), INmix (IN females with South Dakota [SD] males), SDsame (SD females with SD males), and SDmix (SD females with IN males) aviaries. Data represent the numbers of eggs laid (a), numbers of eggs laid that ultimately hatched (b), and numbers of copulations observed (c) per female per day in the aviaries. The data are plotted as means (circles) \pm 1 SD (whiskers), and medians (horizontal lines). Filled circles represent data for IN females, and open circles represent data for SD females.

from the INmix aviary. Laid egg rates were higher in the INsame aviary on 31 days and were higher in the INmix aviary on 16 days (Figure 1a; laid eggs/female/day were the same for the two aviaries on the remaining 5 days). Furthermore, of the eggs laid that ultimately hatched after incubation, rates were higher in the INsame aviary on 24 days and were higher in the INmix aviary on 10 days (Figure 1b; laid eggs that ultimately hatched/female/day were the same for the two aviaries on the remaining 18 days).

Unlike the case with IN females, we obtained roughly equal numbers of eggs from females in the SDsame aviary (84 eggs) compared with the SDmix aviary (72 eggs). Laid egg rates were higher in the SDsame aviary on 21 days and were higher in the SDmix aviary on 24 days (Figure 1a; laid eggs/female/day were the same for the two aviaries on the remaining 7 days). For eggs laid that ultimately hatched, rates were higher in the SDsame aviary on 15 days and were higher in the SDmix aviary on 10 days (Figure 1b; laid eggs that ultimately hatched/female/day were the same for the two aviaries on the remaining 27 days).

Copulations and Directed Vocalizations

INsame females copulated with males at higher rates than did INmix females (Figure 1c; Mann–Whitney U test, $z = 2.409$, $p = .020$). We detected no difference between SDsame and SDmix females in copulation rates (Figure 1c; Mann–Whitney U test, $z = 1.679$, $p = .104$). Across the 9 or 10 15-min morning sampling sessions, we detected no differences in directed singing between IN males ($N = 8$ observed) in the INsame aviaries and SD males ($N = 13$) in the INmix aviaries (Mann–Whitney U test, $z = 1.677$, $p = .104$), and no differences between SD males ($N = 14$) in the SDsame aviaries and IN males ($N = 9$) in the SDmix aviaries (Mann–Whitney U test, $z = 0.189$, $p = .877$).

Discussion

Our findings suggest behavioral incompatibility and reduced reproductive outcome for INmix birds but not SDmix birds. In the INmix aviary, there were lower rates of eggs laid (and of eggs laid that hatched) and there were lower rates of copulations compared with the INsame aviary. Vocalization rates of IN and SD males did not differ appreciably across these aviaries, however. This general relationship among vocalization rates, copulation rates, and egg production is supported by a recent aviary study with IN cowbirds. Gros-Louis, White, King, and West (2006) observed lower egg production in aviaries with both adult and juvenile males compared to aviaries with only adult males—although vocalization rates did not differ among males in these aviaries, copulation rates and egg production tended to be lower in the juvenile/adult male aviaries.

In the present study, there were similar rates of eggs laid, and of eggs laid that ultimately hatched, when comparing the SDsame and SDmix aviaries. There were very low rates of eggs laid that hatched for the females in both the SDsame and SDmix aviaries (Figure 1b), perhaps because the local environmental conditions in IN were not ideal for female cowbirds from SD to produce higher rates of viable eggs.

Reasons for the asymmetric reproductive incompatibility (i.e., in the INmix aviary but not in the SDmix aviary) are not known, but the lower rate of copulations seen in the INmix aviary suggests a behavioral cause. We detected no differences across aviaries in the number of directed vocalizations by males to females. Thus, what the males were vocalizing, rather than how often they vocalized, may have caused the differential copulation rates seen (see also Freeberg et al., 2001). Earlier work suggested that IN females might be choosier and less flexible in their song and mate preferences than SD females (West et al., 1997). For example, the song preferences of female cowbirds from eastern populations (like IN females, of the *M. a. ater* subspecies) appear to be relatively narrow in breadth (King & West, 1990; but see King, West, & White, 2003), but mate preferences (likely strongly related to song preferences) of female cowbirds from the SD population appear highly modifiable by experience (reviewed in Freeberg, 2004).

A genetic incompatibility between the SD and IN populations is also a possibility, one that would seem to exist for interpopulation crosses involving IN females but not SD females. In a study with *M. a. ater* cowbirds from North Carolina (NC) and *M. a. obscurus* cowbirds from Texas (TX), King & West (1990) found that egg production in an aviary with NC females and TX males was

equivalent to egg production in an all-NC aviary. However, the number of hatched young in the interpopulation aviary was far lower than that of the all-NC aviary, and only one of the young produced from crosses between NC females and TX males survived. In the present study, we found no evidence of differential mortality among young produced from any of the four aviaries. Therefore, despite the substantial behavioral differences known to exist between SD and IN cowbirds, there appear to be no strong postmating genetic incompatibilities at work here, unless they were operating very early during embryonic development.

In cowbirds, different populations can vary enormously in their courtship behavior and mating preferences, and these can be socially transmitted (Freeberg et al., 2001; O'Loughlin & Rothstein, 1995; West et al., 1997). Our study suggests that behavioral differences between cowbird populations may lead to differences in reproductive outcome, although we must be cautious in this interpretation because of our lack of replication of treatments (though the finding that behavioral differences of the sort measured here produce different reproductive outcomes has now been replicated [e.g., White, King, & West, 2002]). One possible behavioral difference, raised above, is that SD and IN males differ in their song structures, females prefer songs of males of their own population, and IN female preferences for IN song are stronger than SD female preferences for SD song (but see West, King, & Freeberg, 1998). Another possibility may be that males in the INsame aviary may have engaged in substantial countersinging (in which males direct consecutive and often alternating songs to one another over time), whereas the males in the INmix aviary may not have engaged in such countersinging; recent work suggests that countersinging is a behavior pattern to which females closely attend (White, Gros-Louis, King, Papakhian, & West, 2007). We did not collect these data, however, and so additional research is needed to test these possible explanations for the variation in courtship behavior and egg production seen here.

To conclude, this study suggested that group-level differences in copulation rates were associated with differences in egg production, and that geographic variation in courtship behavior between SD and IN cowbirds (likely male songs and female song preferences) may have influenced these rates of copulation and egg production. Intraspecific geographic variation in courtship behavior can play a role in the evolutionary dynamics of populations (e.g., Foster & Endler, 1999). Given the influence of social transmission on courtship behavior in cowbirds, this study further suggests exciting links between social transmission of behavior and processes of population divergence.

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