Cultural Transmission of Vocal Traditions in Cowbirds (*Molothrus ater*) Influences Courtship Patterns and Mate Preferences

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In this study, the authors tested the cultural transmission of vocal traditions in cowbirds (*Molothrus ater*). Young cowbirds from a South Dakota (SD) population were housed over winter with adults of the SD population or with adults from an Indiana (IN) population. Song differences between the original SD and IN adult models were acquired by South Dakota culture (SDC) and Indiana culture (INC) males, respectively, and were transmitted to a 2nd cultural generation of birds. During playback tests of SDC and INC songs, SD females gave more copulatory responses to SDC songs. Finally, males with SD-like songs courted SDC females preferentially in breeding season tests, whereas males with IN-like songs courted INC females preferentially. These results indicate that the transmission of vocal traditions plays a fundamental role in the courtship patterns and mating decisions of cowbirds.

Vocal traditions in passerine birds (songbirds)—song dialects, or more generally, geographic variation in songs—have long been of interest to researchers in ethology, comparative psychology, behavioral ecology, developmental psychobiology, and evolutionary biology (Baker & Cunningham, 1985; Catchpole & Slater, 1995; Freeberg, 2000; Hauser, 1996; Kroodsma & Miller, 1996). Song in passerine species is a communicative signal that lies squarely at the interface between research inquiry into development at the level of the individual and into evolutionary processes at the level of the population. This is because, on the one hand, song represents a learned signal of communication that is often influenced largely by the social environment in which a young (often male) bird develops. On the other hand, geographic variation in song represents a possible barrier to gene flow across

populations. A male's breeding success will depend largely on the song preferences females possess in the local population; furthermore, female preferences, male courtship and mating decisions, and reproductive success in large part depend on the socially learned songs that characterize the population. To demonstrate that vocal traditions might possibly influence the patterns of courtship and mating seen in songbird populations, researchers must be able to document that birds base their courting and mating decisions at least in part on the vocal differences between populations. In other words, researchers need to demonstrate that populations exhibit mate preferences or mate assortatively (or both) on the basis of differences in their vocal traditions.

The idea that songs in songbirds may represent culturally transmitted social traditions extends back at least 4 decades to the experiments of Marler and Tamura (1964). Since then, numerous studies have addressed questions of song learning, geographic variation in songs, and vocal dialects in birds (Krebs & Kroodsma, 1980; Kroodsma & Miller, 1982, 1996; Slater, 1989). Distinct dialects in different conspecific populations of birds are often assumed to be of biological significance to the mating decisions and patterns of the birds themselves. However, without functional measures of differences in vocal traditions, this assumption is not a safe one for researchers to make (Baker & Cunningham, 1985, and commentaries therein). Indeed, Baker and Cunningham argued that in comparative studies, "we must determine the behavioral significance of song features" (p. 91)—that is, researchers need to determine the extent to which populational or group differences in vocal traditions influence mating decisions and patterns of courtship in those populations.

Results from recent studies with cowbirds from two Midwestern populations (South Dakota, *Molothrus ater artemisiae*, and Indiana, *M. a. ater*) indicate that patterns of assortative courtship and mating by population could be culturally transmitted across at least two generations of birds. In these studies, young birds captured in South Dakota (SD) were housed in large aviaries with adults from the same SD population or with adults from the Indiana (IN) population. During the breeding season, SD females housed in the South Dakota culture (SDC) paired and mated more often with

SDC males, and SD females housed in the Indiana culture (INC) paired and mated more often with INC males (Freeberg, 1996). These SDC and INC birds later served as adult social models for another cohort of young cowbirds captured in SD (so that both adult social models and young subjects were genetically SD birds). Using the SDC and INC birds as the adult social models tested whether these SD/SDC and IN/INC courtship differences could be culturally transmitted to a second generation of young SD birds in the absence of the original SD and IN adult social models (see Galef & Allen, 1995, for further discussion of this "laboratory microcultures" method of testing for cultural transmission). This second cultural generation of birds also paired assortatively by cultural background (Freeberg, 1998). In a parallel mate choice study controlling for male–male and female–female interactions, female cowbirds preferred to court males from the same cultural background (Freeberg, Duncan, Kast, & Enstrom, 1999).

In the studies carried out with the SD and IN populations, evidence of cultural transmission influencing courtship behavioral patterns was obtained, but the specific differences in communicative signals between SD and IN birds that influenced assortative pairing and mating were not determined. Here, we tested for vocal traditions in the SD, IN, SDC, and INC cowbirds of those earlier studies, asking whether those vocal traditions might influence courtship patterns and mate preferences. For vocal traditions to be a behavioral mechanism for the assortative patterns of mating by culture seen in earlier studies (Freeberg, 1996, 1998) would also require that the preferences for songs in female cowbirds had been modified by experience with different cultural backgrounds. Results from studies with other populations of cowbirds suggested that female preferences were much less modifiable than male songs and were perhaps a relatively "closed developmental program" (King & West, 1983c, 1987). Thus, the present study serves as a first step toward unraveling the culturally influenced mechanisms of assortative mating patterns in these populations from the standpoint of the male's songs (a future article will address this question from the standpoint of the female's preference for songs).

For songs of male cowbirds of two populations to represent vocal traditions that serve as behavioral mechanisms for differential courtship and mating patterns between those populations, we need to show that (a) there are measurable differences in the songs of the males of the two populations, (b) those song patterns are learned by young males, (c) those song patterns are fairly stable across generations, and (d) birds base their courtship and mating decisions on those song patterns. We tested all of these criteria in the present study. We addressed the fourth criterion in two ways: by testing female preferences for songs of males of the SD and IN cultures and by comparing SD-like and IN-like vocal characteristics to males' courtship and pairing patterns with females.

Method

General Methodology

The subjects and general methodology used are described in greater detail in Freeberg (1996, 1998). Here, we will outline the most important aspects of the design of those studies to the present study.

Population and Cultural Differences in Numbers of Notes: First Cultural Generation

Subjects. The adult male cowbirds (*Molothrus ater*) that served as social models for the young SD birds in the Freeberg (1996) study were captured in Fall River and Custer Counties, SD (SD males, $n = 17$), and in Monroe County, IN (IN males, $n = 14$). The young SD males ($n = 32$) were captured as juveniles while in wild flocks in Fall River and Custer Counties, SD, in August, 1993. They were captured along with 33 juvenile SD females and brought to Bloomington, IN.

Social housing. The adult SD males were split into two groups, and each group was housed with adult SD females in two identical indoor–outdoor aviaries (9.1 × 21.4 × 3.4 m) in August 1993. The adult IN males were split into two groups at the same time, and each group was housed with adult IN females in separate aviaries identical in dimensions to the two SD aviaries. After the adult cowbirds were placed in these four aviaries, the young SD subjects were spread roughly equally across the four aviaries (young SDC birds in the aviaries with SD adults and young INC birds in the aviaries with IN adults). Each aviary was visually isolated from the other three aviaries. The birds were housed this way until May 1994, at which time the young SDC and INC subjects were removed to test their courtship patterns (Freeberg, 1996).

After the 1994 breeding season, in August, the birds were returned to the four social housing aviaries they had been in for the previous several months before the breeding season had begun. Six new IN males were added to the IN culture aviaries as adult social models for the INC birds. Some hand-raised birds (SD, IN, and hybrids between the two populations) were also incorporated into the SDC and INC subject pool in August 1994 (Freeberg, 1996). The SDC birds were housed with SD adults and the INC birds with IN adults in their social housing aviaries until the following May 1995, when another set of tests of courtship patterns was carried out.

Population and Cultural Differences in Numbers of Notes: Second Cultural Generation

Subjects. The SDC and INC males of the first cultural generation served as adult social models for a second cultural generation of SD cowbirds. The young males of the second cultural generation ($n = 27$) were captured as juveniles while in wild flocks in Fall River and Custer Counties, SD, in August 1995. They were captured along with 29 juvenile SD females and brought to Bloomington, IN.

Social housing. SDC males were housed with SDC females, and INC males were housed with INC females, in the same social housing aviaries in which they had been housed in the first 2 years of the study (described above). After the adult SDC and INC cowbirds were placed in these four aviaries at the end of the 1995 breeding season, birds of the second cultural generation were spread roughly equally across the four aviaries (SDC2 birds in the aviaries with SDC adults and INC2 birds in the aviaries with INC adults). The birds were housed this way until May 1996, at which time groups of the SDC2 and INC2 subjects were removed temporarily to test their courtship patterns in a separate study (Freeberg, 1998), and sets of the adult SDC and INC cowbirds were removed temporarily for a study of female mate choice (Freeberg et al., 1999).

Recording and Analyses of Songs

The vocalizations of the adult SD and IN male social models and the young SDC and INC males analyzed here were recorded in late April and early May in 1994 and 1995. The vocalizations of the adult SDC and INC male social models and the young SDC2 and INC2 males analyzed here were recorded in late April and early May in 1996. Late April and early May is the time period when mature, crystallized song has developed in males immediately prior to the beginning of the breeding season in the SD and IN populations.

Six Sennheiser RF condenser microphones were placed in locations within each aviary near perch sites frequented by the males. Vocalizations of males were recorded with a Sony TCD-D10 PRO II digital audiotape recorder. We attempted to obtain at least 50 songs from each male, as earlier studies with cowbirds indicated that this amount of documented song ensured that a male's complete repertoire had been recorded with at least 95% confidence (King & West, 1983b). In the 1st year of the study (1994), we recorded 9,319 songs: an average of 87.8 songs from each SD male (range = 15–171), 227.3 songs from each SDC male (range = 27–462), 96.8 songs from each IN male (range = 13–223), and 182.8 songs from each INC male (range = 50–354). In the 2nd year of the study (1995), we recorded 5,449 songs: an average of 111.1 songs from each SD male (range = 7–237), 80.5 songs from each SDC male (range = 17–175), 92.2 songs from each IN male (range = 16–177), and 77.3 songs from each INC male (range = 22–212). In the 3rd year of the study (1996) in which testing of the SDC and INC adult social models and the second cultural generation of birds (SDC2 and INC2) took place, we recorded 4,515 songs: an average of 76.1 songs from each SDC male (range = 18–144), 105.7 songs from each SDC2 male (range = 44–180), 52.8 songs from each INC male (range = 17–106), and 98.9 songs from each INC2 male (range = 17–199).

Songs in brown-headed cowbirds are composed of one to four clusters of low frequency notes—note clusters—followed by a high frequency whistle (so few birds in this study had a song in their repertoire with four note clusters that analyses of fourth note clusters were not conducted). Note clusters comprise largely alternating notes of lower frequency (0.2–2.0 kHz) and higher frequency (2.0–6.0 kHz) produced by the different sides of the syrinx (Allan & Suthers, 1994). Preliminary analyses of a small subset of songs of SD and IN males suggested that two major differences between populations were in numbers of note clusters and in numbers of notes within each note cluster. Songs were digitized into a zero-crossings-analyzer (ZCA) that generated a Frequency × Time display on the face of a Tektronix 5113 oscilloscope (West, King, Eastzer, & Staddon, 1979). Printouts of the ZCA plots were made of different renditions of each of the song types of each male, and the number of notes in each note cluster was counted from these printouts (see Figure 1a). Male cowbirds from SD and IN generally have from three to seven different song types in their repertoires. The number of notes in each note cluster was averaged across a given male's song types, such that each male had a single score for the average number of notes in each note cluster. For each male, we also scored the total number of songs in his song repertoire.

Female Playback Study

Results from previous work with cowbirds of different populations indicated that female cowbirds prefer the songs of males of their own population relative to the songs of males of other populations (King & West, 1990; West & King, 1986, 1996b). SD females prefer the songs of SD males over the songs of IN males, and IN females prefer the songs of IN males over the songs of SD males (West, King, & Freeberg, 1998). Females' copulatory responses also depend largely on characteristics in the note clusters in the songs (e.g., King & West, 1983a; West et al., 1979). Here, we asked whether females from the SD population could detect differences in the songs of SDC and INC males, as assayed by differential copulatory responses given to playbacks of songs of males. If the songs of SDC males were more similar to the songs of SD males than to the songs of IN males, and the songs of INC males were more similar to the songs of IN males than to the songs of SD males, as suggested by analyses of numbers of notes discussed below, we would predict that SD females would prefer (give more copulatory responses to) the songs of SDC males relative to the songs of INC males.

Playback subjects. Ten SD females served as playback subjects. All females had been collected from the same Fall River and Custer County sites as the SD birds described above. The females were between 4 and 6

years old at the time of playback testing. Each had resided in large indoor–outdoor aviaries with other SD cowbirds as parts of other experiments (Freeberg, King, & West, 1995) in the years prior to testing.

In September 1994, the 10 SD females were housed in an indoor–outdoor flight cage (3.2 × 3.2 × 9.1 m). In February 1995, they were moved into an indoor flight cage (1.8 × 1.8 × 2.4 m) for several weeks. In early May 1995, the 10 females were moved into six sound-attenuating chambers (1.2 m^3) for playback testing. Eight of the females were tested in pairs, and 2 were tested alone.

Selection of songs and playback procedure. To mirror the breeding season testing carried out in a different experiment (Freeberg, 1996), we conducted two separate playback experiments (Playbacks 1 and 2). Playback 1 was made of songs of SDC and INC males that had been tested together in the first half of the breeding seasons in Freeberg (1996), and Playback 2 was made of songs of SDC and INC males that had been tested together in the second half of the breeding seasons in that earlier study. Each playback set contained nine SDC and nine INC songs recorded from 5 different SDC and 5 different INC males, respectively (see exemplars in Figure 1b). Four SDC and 4 INC males each had two songs in the playback set, and the 5th SDC and INC males had one song in the playback set (thus, across Playback Sets 1 and 2, songs were tested from 10 different SDC and 10 different INC males).

Songs were recorded as described above for the analyses of numbers of notes in note clusters. To be included in Playback 1 or 2, the song had to have been recorded from a male singing within 0.3 m from, and on axis to, the microphone. Particular songs were chosen for playback on the basis of acoustic quality and as an attempt to sample the range of song types of SD/SDC and IN/INC males. For inclusion, the unweighted signal-to-noise ratio measured peak to peak had to be 53 ± 2 dB measured between 0.3 and 12.0 kHz.

In May 1995, we conducted Playback 1, and in June 1995, we conducted Playback 2. We played back six songs daily to the SD females, with one song per trial. Each trial was separated by at least 90 min. Playbacks began each morning at 0700. The females heard the songs in varying orders each day, and each song was presented an equal number of times at different times of day. We played back each song four times to each female over the course of each playback set. We scored a positive response to the song if the female adopted the copulatory posture within 1 s from the onset of the song. Songs were played back using an Otari MX5050 recorder, a Urei 537 1/3-octave equalizer, and a Crown D75 power amplifier through JBL 2105 speakers located in each chamber.

Male Mating Preference Study

Earlier work with SD and IN cowbirds indicated that male vocalizations play an important role in the courtship behavioral interactions that facilitate pairing and mating in these populations (West et al., 1998). Here, we sought to extend this research in light of note-number differences by attempting to predict a posteriori, on the basis of differences in the vocal traditions of males, their courtship interactions with females in seminatural, captive settings—namely, directed vocalizations from males to females in close proximity during the breeding season and their courtship success with females.

Subjects. The birds used in this analysis were the SDC and INC cowbirds of the first cultural generation described above, tested in their second breeding season (when we were able to collect much more data per bird than we had been able to collect in their first breeding season), and the second cultural generation of birds, who had only been tested in their first breeding season.

Social housing and breeding season testing. All birds were kept in their social housing aviaries (described above) except for the times in which they were tested in a pairing assessment aviary. In the pairing assessment aviary, an aviary identical to the social housing aviaries but novel to all the birds, we documented the courtship behavior of SDC and

(a)

(b)
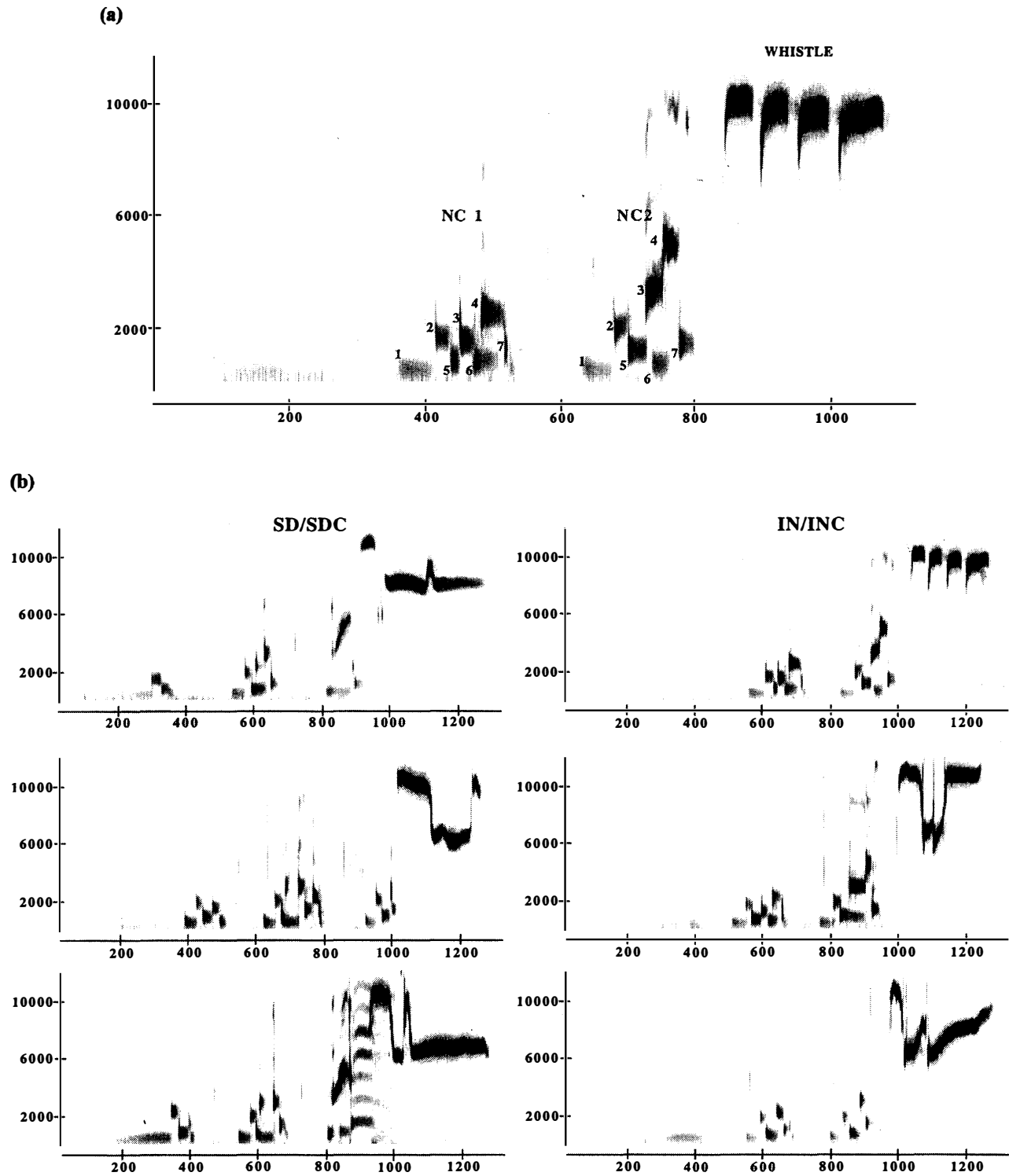
Figure 1. (a) Sonogram of an Indiana/Indiana culture (IN/INC) song exemplar illustrating note clusters (NC) and concluding whistle and the different notes within each note cluster. (b) Sonograms of exemplars of the songs of South Dakota/South Dakota culture (SD/SDC) and IN/INC male cowbirds. Left panel: sonograms of SD/SDC songs. Right panel: sonograms of IN/INC songs. The *y* axis for each sonogram measures frequency (0–12 kHz), and the *x* axis for each sonogram measures time (0–1,200 ms).

INC males (1995) and SDC2 and INC2 males (1996). Birds were generally kept in the pairing assessment aviary until they had paired or copulated or both, or had spent several consecutive days without engaging in any courtship behavior, at which time they were captured and placed back into their social housing aviaries. Then, replacement birds of the same sex and from the same aviary were placed into the pairing assessment aviary, and their behavior was followed. In this way, each bird was cycled through the pairing assessment aviary at least once, and often two or three times, allowing us to capture individual behavior over fairly long periods of time across the breeding season. Pairing here was defined as persistent courting of (directed singing to) a female by a male across at least 3 consecutive days and often included at least one copulation between the male and female. For more details on housing and testing methodology and determination of pairing status during the breeding season, see Freeberg (1996, 1998).

Each morning between roughly 0600 and 1030 EST, data on each male in the pairing assessment aviary were collected in two 10-min focal samples. Males were sampled individually with methodologies that had been successful at capturing male courtship effectiveness in previous studies (Eastzer, King, & West, 1985; Freeberg et al., 1995). For the present analysis, we had documented every song the focal male directed to females. We totaled the number of songs each male directed to SDC and INC females (1995) or to SDC2 and INC2 females (1996) and divided those numbers by the number of days the male had been in the pairing assessment aviary (as this number varied across birds). Thus, each male had a daily average directed song score for females of the two cultures. We subtracted the number of songs directed to INC/INC2 females from the number directed to SDC/SDC2 females to obtain a relative directed singing score for each male. A positive relative directed singing score indicated more courtship interaction with SDC/SDC2 females, whereas a negative relative directed singing score indicated more courtship interaction with INC/INC2 females. For those males that acquired at least one pairing with a female, we also totaled the number of times each paired with INC/INC2 females subtracted from the number of times each paired with SDC/SDC2 females. The result was a relative pairing score for each male: A positive relative pairing score indicated more pairings with SDC/SDC2 females, whereas a negative relative pairing score indicated more pairings with INC/INC2 females.

Song analyses. As outlined above, and because females' song preferences are likely based on aspects of the note clusters in songs (King & West 1983a; West et al., 1979), we compared males' courtship success to their songs in terms of the numbers of notes in their note clusters. Song differences in the numbers of notes per note cluster were so large for first and third note clusters that they represented nearly discrete categories (see below) and therefore were not useful to us for ranking song categorizations for comparison to males' relative directed singing scores and relative pairing scores. Early analyses of the numbers of notes in note clusters suggested that the SDC/SDC2 males that were most successful at obtaining pairings and copulations with SDC/SDC2 females were males that had a relatively low number of notes in their first note cluster relative to the number of notes in their second note cluster. Furthermore, the INC/INC2 males found to be the most successful at courting and mating with INC/INC2 females were males that had a relatively high number of notes in their first note cluster relative to the number of notes in their second note cluster. We therefore used the ratio of notes in the first note cluster (nc) to the notes in the second note cluster—nc1/nc2—to classify males' songs.

Statistical Analyses

We used Kruskal–Wallis one-way analyses of variance (ANOVA) by ranks to test for overall differences in the average numbers of notes per note cluster and in the numbers of songs in repertoires within a year for SD, IN, SDC, INC, SDC2, and INC2 males. When overall differences were found (using a conservative alpha level of .01), we used Dunn's method of pairwise multiple comparisons to test for significant differences between each of the six possible pairings. For the playback studies, we determined the percentage of copulatory postures given by the SD females to the songs of SDC males and to the songs of INC males. As all females heard the exact same sets of SDC and INC song playbacks, and female response rates showed great individual variation, we tested for differences in females' responses to SDC and INC songs using Wilcoxon signed-ranks tests. For the data on male mating preferences during the breeding season, we used linear regression to test for the effect of nc1/nc2 on the relative directed singing scores and relative pairing scores of males.

Results

Population and Cultural Differences in Numbers of Notes in Note Clusters

Vocal content differed by population and by cultural background in all 3 years and two cultural generations of the study. In the 1st year of the study (spring–summer 1994), males differed in the number of notes in the first note cluster: Kruskal–Wallis ANOVA, $H(3) = 43.37$, $p < .001$. Dunn's method of pairwise multiple comparisons found that both IN and INC males had more notes in their first note cluster than both SD and SDC males (Figure 2a). We could not detect differences in the number of notes in the second note cluster: Kruskal–Wallis ANOVA, $H(3) = 3.84$, $p = .28$ (Figure 2b). We found differences in the number of notes in the third note cluster, with IN and INC males frequently failing to have third note clusters in their songs: Kruskal–Wallis ANOVA, $H(3) = 47.85, p < .001$. Multiple comparisons found that both SD and SDC males had more notes in their third note clusters than both IN and INC males (Figure 2c). We did not detect differences among SD, IN, SDC, and INC males in the number of different song types in their repertoires: Kruskal–Wallis ANOVA, $H(3) = 1.35, p = .72$.

In the 2nd year of the study (spring–summer 1995), males differed in the number of notes in the first note cluster, Kruskal–Wallis ANOVA, $H(3) = 48.80$, $p < .001$. Pairwise multiple comparisons indicated that both IN and INC males had more notes in their first note cluster than both SD and SDC males (Figure 2d). In this 2nd year of the study, males differed in the number of notes in the second note cluster: Kruskal–Wallis ANOVA, $H(3) = 15.63, p = .001$. Multiple comparisons found that the SDC males (but not SD males) had significantly more notes than both the IN and INC males (Figure 2e). We also found differences in the number of notes in the third note cluster, with IN and INC males frequently failing to have third note clusters in their songs: Kruskal–Wallis ANOVA, $H(3) = 54.42, p < .001$. Multiple comparisons found that both SD and SDC males had more notes in their third note clusters than both IN and INC males (Figure 2f). We did not detect differences among SD, IN, SDC, and INC males in the number of different song types in their repertoires: Kruskal–Wallis ANOVA, $H(3) = 6.35, p = .10$.

The song patterns seen in the first cultural generation of birds were transmitted with high fidelity to the second cultural generation of birds. Males differed in the number of notes in the first note cluster: Kruskal–Wallis ANOVA, $H(3) = 38.80, p < .001$. Dunn's method of pairwise multiple comparisons found that both INC and INC2 males had more notes in their first note cluster than both SDC and SDC2 males (Figure 2g). Although SDC and SDC2 males tended to have more notes in their second note clusters, our

Study 1   ## Study 2
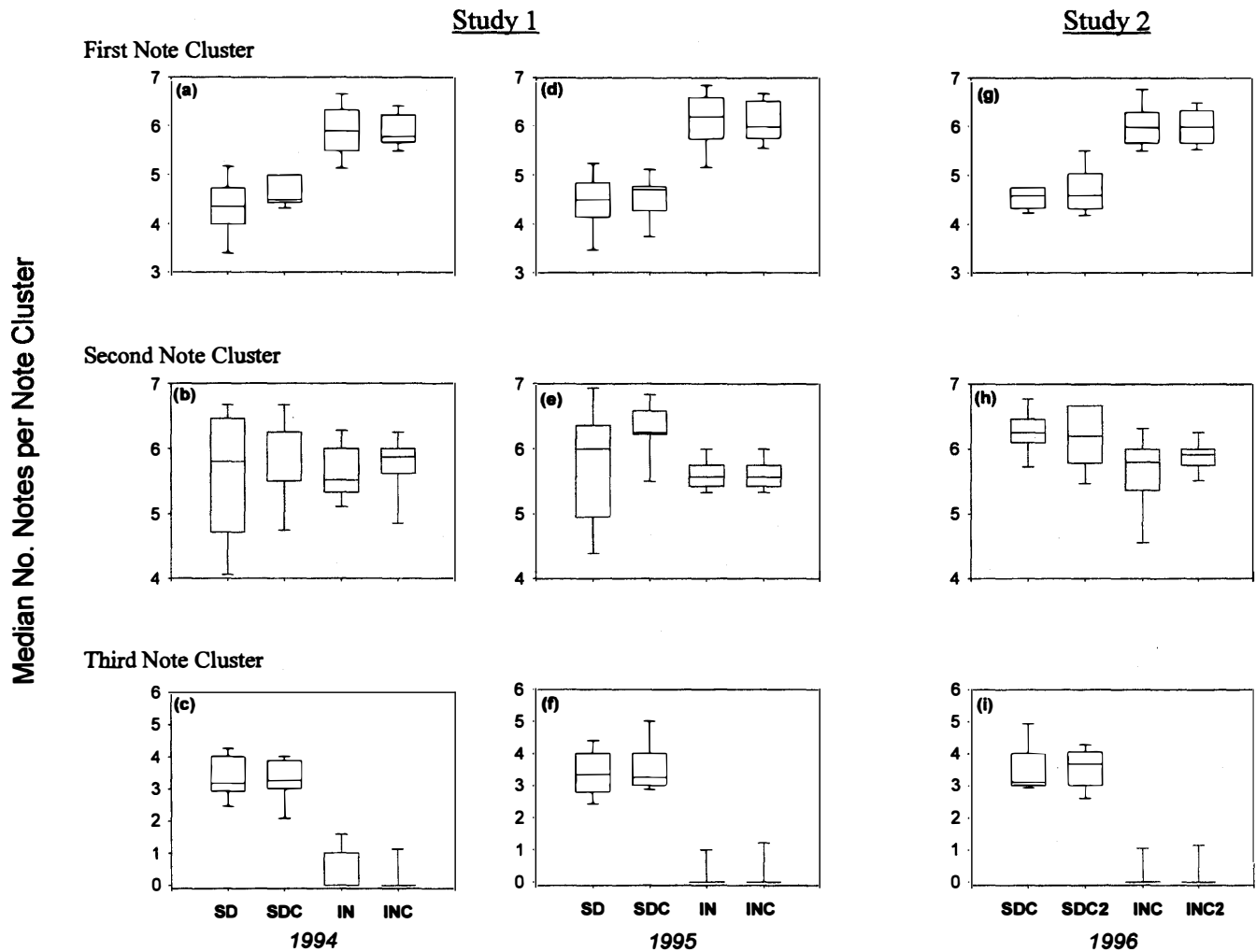
Figure 2. Median number of notes in note clusters of South Dakota (SD) and Indiana (IN) male cowbirds and the two cultural generations of SD male cowbirds. Medians are horizontal lines inside each box surrounded by 25th and 75th percentiles (lower and upper limits of each box) and 5th and 95th percentiles (lower and upper error bars). Top row: data for the first note cluster. Middle row: data for the second note cluster. Bottom row: data for the third note cluster. Left and middle panels: data from the 1st and 2nd year for birds of the first cultural generation. Right panels: data from birds of the second cultural generation. SDC and INC = first cultural generation; SDC2 and INC2 = second cultural generation.

stricter alpha level did not allow us to detect differences in the number of notes in the second note cluster: Kruskal–Wallis ANOVA, $H(3) = 10.82$, $p = .013$ (Figure 2h). We found differences in the number of notes in the third note cluster, with INC and INC2 males frequently failing to have third note clusters in their songs: Kruskal–Wallis ANOVA, $H(3) = 44.39$, $p < .001$. Multiple comparisons found that both SDC and SDC2 males had more notes in their third note clusters than both INC and INC2 males (Figure 2i). SDC, INC, SDC2, and INC2 males differed in terms of the number of different song types in their repertoires: Kruskal–Wallis ANOVA, $H(3) = 13.27$, $p = .004$, unlike the case with birds of the first cultural generation. Multiple comparisons found INC2 males (but not INC males) to have significantly larger repertoires (on average, roughly one more song in their repertoires) than both SDC and SDC2 males.

Female Playback Study

The songs of the SDC and INC males in the two playback sets represented well the differences in the numbers of notes in each note cluster for SD/SDC and IN/INC males found in Figure 1b and Figure 2. For songs of SDC males used in Playback 1, the average numbers of notes per note cluster were 4.89 (range = 3–8) for the first note cluster, 6.22 (range = 5–8) for the second note cluster, and 3.22 (range = 0–5) for the third note cluster. For songs of INC males used in Playback 1, the average numbers of notes per note cluster were 6.11 (range = 5–8) for the first note cluster, 5.67 (range = 5–7) for the second note cluster, and 0.00 (range = 0–0) for the third note cluster. In Playback 2, the average numbers of notes per note cluster for songs of SDC males were 4.78 (range = 4–5) for the first note cluster, 6.44 (range = 5–8) for the second

note cluster, and 3.78 (range = 0–5) for the third note cluster, and the average numbers of notes per note cluster for songs of INC males were 5.67 (range = 5–6) for the first note cluster, 5.78 (range = 5–7) for the second note cluster, and 0.00 (range = 0–0) for the third note cluster.

For Playback 1, all 10 SD females gave more copulatory responses to songs of SDC males than to songs of INC males: Wilcoxon signed-ranks test, p = .002 (Figure 3a). SD females responded to an average of 66% of the SDC male songs (range = 29–97) and to an average of 46% of the INC male songs (range = 19–86). The average response difference across the SD females in favor of SDC songs was 0.20 (range = 0.03–0.42). For Playback 2, one female never responded to songs of any of the males (the female had rarely responded and had the lowest response rate in Playback 1). Of the 9 remaining females, all 9 responded more to songs of SDC males than to songs of INC males: Wilcoxon signed-ranks test, p = .004 (Figure 3b). SD females responded to an average of 65% of the SDC male songs (range = 47–97) and to an average of 36% of the INC male songs (range = 17–72). The average response difference across the SD females in favor of SDC songs was 0.29 (range = 0.08–0.47).

Male Mating Preference Study

For males of both the first cultural generation and the second cultural generation, the ratio of the number of notes in the first note cluster relative to the number of notes in the second cluster predicted breeding season courtship behavior. In general, the more SD-like a male's ncl/nc2 ratio was (roughly < 1.0), the stronger his preference for pairing with and singing to SDC/SDC2 females, and the more IN-like a male's ncl/nc2 ratio was (roughly ≥ 1.0), the stronger his preference for INC/INC2 females. For birds of the first cultural generation, a male's ncl/nc2 ratio significantly predicted his relative directed singing score: R = .56, p = .002 (Figure 4a), as well as his relative pairing scores: R = .62, p = .011 (Figure 4b). For birds of the second cultural generation, a male's ncl/nc2 ratio did not significantly predict his relative directed singing score: R = .28, p = .166 (Figure 4c), but it did significantly predict his relative pairing score: R = .64, p = .008 (Figure 4d).

Discussion

In these studies, we have demonstrated that there are large differences between the songs of male cowbirds from SD and the songs of males from IN. The SD and IN song differences are so large as to be virtually nonoverlapping—for any given SD male, the average number of notes in the first note cluster is significantly less than, and the number of notes in the third note cluster is significantly more than, the number of notes in the first and third note clusters of any given IN male. These song differences may therefore represent population markers that the birds themselves might easily use to distinguish members of the same population from members of different populations.

SDC and INC males of the first cultural generation maintained their song differences into their 3rd year in the absence of the original SD and IN adult social models. Furthermore, these song patterns were transmitted to the second cultural generation of young SD birds (SDC2 and INC2). It is important to reiterate here that all of the birds in the last year of the study were genetically SD—the only difference was that the adult social models had earlier developed in either an SDC or an INC environment. Thus, in the last year of the study, we found that in the absence of true (in the sense of being caught in the wild as adults) adult SD and IN social models, SD-like and IN-like song patterns were culturally transmitted to a second generation of birds.

Taken together, the results indicate that the song differences between SD and IN males represent rather stable vocal traditions. Our data further demonstrate that these vocal traditions can be a basis on which females make courtship and mating decisions during the breeding season. SD females strongly preferred the songs of the SDC males relative to the songs of the INC males. We do not yet know whether IN females would prefer the songs of INC males over the songs of SDC males. An earlier playback study indicated that IN females do prefer songs of IN males over the songs of SD males (West et al., 1998)—in that the songs of INC and INC2 males overlapped completely with the songs of IN males in terms of the numbers of notes in their note clusters, we would predict IN females would prefer the INC songs.

These vocal traditions furthermore predicted males' courtship behavior and success with females from the different cultures during the breeding season. The ratio of the number of notes in the
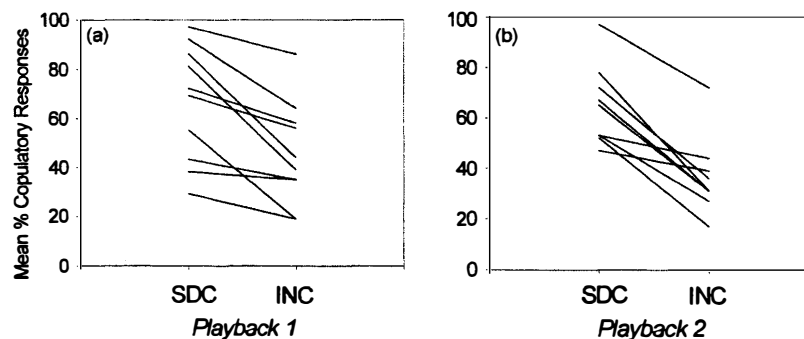

Figure 3. Mean percentage of South Dakota female cowbird copulatory responses given to songs of South Dakota culture (SDC) and Indiana culture (INC) male cowbirds in Playback 1 and Playback 2. Each line connects a single female's copulatory responses to SDC and to INC males.
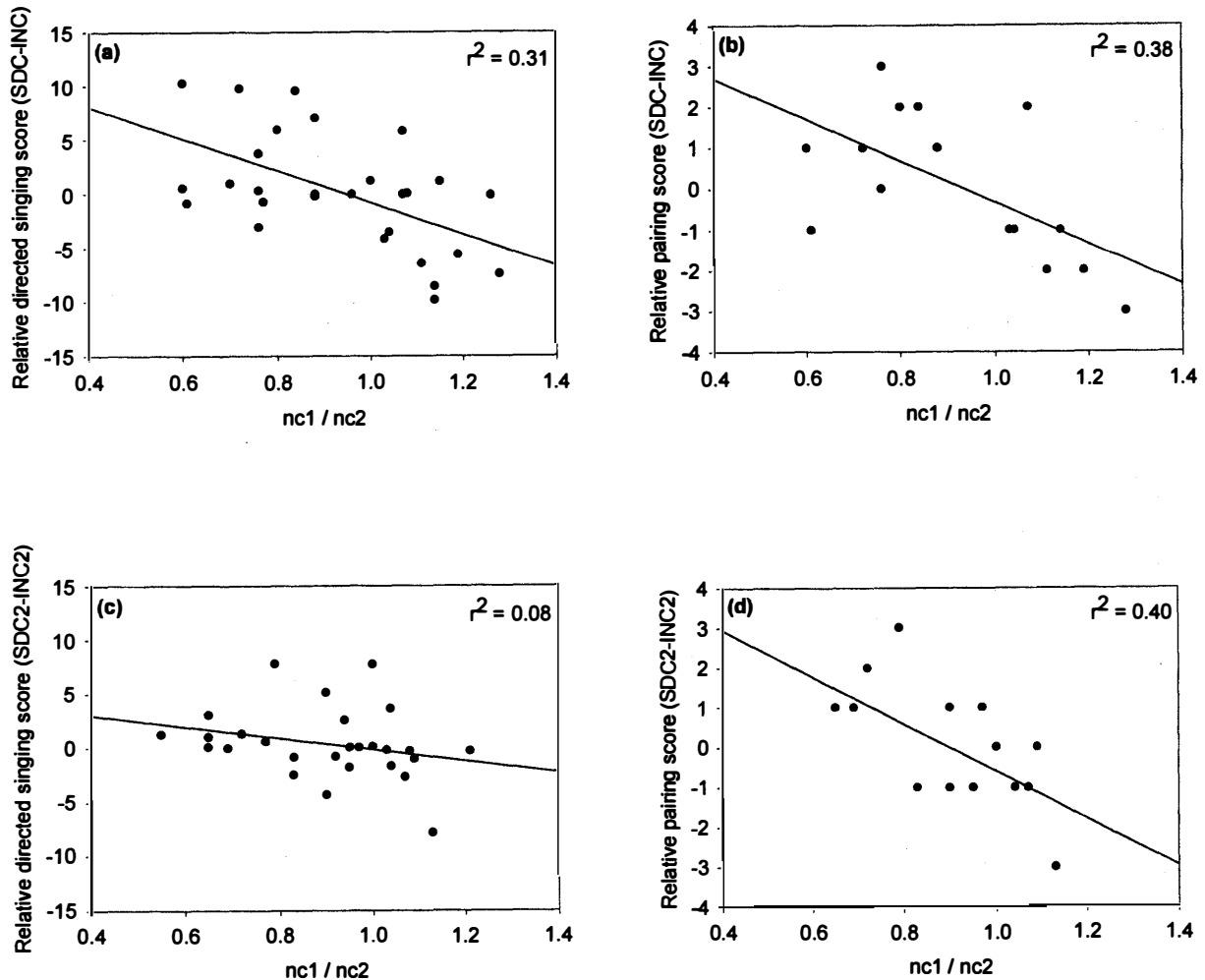
Figure 4. Courtship preferences of male cowbirds as a function of the number of notes in the first two note clusters (nc) of their songs. Top row: data from birds of the first cultural generation. Bottom row: data from birds of the second cultural generation. Left panels: data for relative directed singing scores (average daily songs directed to SDC/SDC2 females minus average daily songs directed to INC/INC2 females). Right panels: data for relative pairing scores (number of pairings with SDC/SDC2 females minus number of pairings with INC/INC2 females). The x axes for all figures represent males' ratio of the number of notes in their first note cluster relative to the number of notes in their second note cluster. SDC = South Dakota culture; INC = Indiana culture.

first note cluster relative to the number of notes in the second note cluster proved highly predictive of the direction of a male's courtship behavior toward SDC females or INC females. For the birds of the first cultural generation, the more a male's ratio of note clusters was like true SD or IN males, the more he directed songs to and was successful at pairing with SDC or INC females, respectively. The same predictive strength of the nc1/nc2 ratio held for pairings in males of the second cultural generation, but not for males' relative directed singing scores. The likely reason for this is that a high proportion of the males in the second cultural generation sang so little to females (averaging less than 1.0 directed song per morning to females of either culture; see Figure 4c) that not enough variation across the relative directed singing scores was present to detect a significant regression score. A generally low level of courtship behavior may be the norm for SD males in their first breeding season (Freeberg, 1996), as seems to be the case for

males in western populations (O'Loghlen & Rothstein, 1995b; Rothstein, Yokel, & Fleischer, 1986).

Freeberg (1996, 1998) found that the social experiential backgrounds of cowbirds predicted the courtship patterns that facilitated assortative mating in these captive populations. In the present study, we analyzed the vocal repertoires of the males of those studies and provided a behavioral mechanism for the courtship patterns demonstrated in those earlier studies—the vocal traditions of males. Song differences between SD and IN males were socially learned by SDC and INC males, respectively, and then were culturally transmitted to SDC2 and INC2 males, respectively. These song differences predicted males' courtship interactions with females during the breeding season, and SD females were able to discriminate SDC and INC songs in playback tests, preferring the SDC songs. Learned female courtship preferences together with the SD and IN vocal traditions also help explain

mechanisms for the female mate choice by cultural background that was experimentally demonstrated in cowbirds of these populations (Freeberg et al., 1999). To determine experimentally the extent to which female preferences for SD/SDC or IN/INC songs depend on the numbers of notes in the first two note clusters (as suggested by our ability to predict males' mating preferences by knowledge of their nc1/nc2 ratios), the manipulation of note numbers to generate playback sets that vary from high to low nc1/nc2 ratios would be required.

Although the SD song patterns that were transmitted from SD males to SDC males to SDC2 males represent natural vocal traditions, it is important to emphasize that the song patterns transmitted from IN males to the INC males to the INC2 males represent neophenotypic vocal traditions. Neophenotypes are behaviors that result when an individual develops in a species- or population-atypical social context (Kuo, 1967; West, King, & Freeberg, 1994). Neophenotypic behaviors ("alien" behaviors in Lewontin, 1983) lie within the developmental norm of reaction for a species, but because the developmental context is atypical, the behaviors are rarely if ever seen outside of a laboratory preparation. Neophenotypes demonstrate the flexibility in developmental systems. In addition to indicating the breadth of developmental plasticity for a species or population, the study of neophenotypic behaviors provides the further advantage of allowing a researcher to peer into the mechanisms of what is an unambiguous learning process, one that cannot be explained solely by maturational or innate programs of development. SD cowbirds do not normally develop the songs of IN cowbirds. That the INC and INC2 males did develop these neophenotypic vocal patterns so fundamental to courtship success demonstrates the powerful role social learning plays in the development of the communicative and mate recognition systems in cowbirds.

A functional approach to the study of vocal variation (such as we have taken here) and assortative mating brings to the fore the role of females, or receivers. West and King (1986) coined the word *diacept* to describe this receptive rather than productive aspect of the courtship system on which the link between differences in vocal traditions and differences in reproductive outcomes depends. King and West used diacepts to describe populational differences in females' preferences and selectivities for males' songs in cowbirds. During 2 decades of study with this species, researchers have found that the development of male song is influenced by the social environment (King, Freeberg, & West, 1996; West & King, 1996a, 1996b; West, King, & Freeberg, 1997; West, King, & Harrocks, 1983) and that females from different populations can shape male song development on the basis of their populational preferences (King & West, 1989; Smith, King, & West, 2000; West & King, 1988).

Some evidence exists showing that females' preferences for songs can be broadened by experience, although perceptual flexibility has been found only in one population of *M. a. ater* birds to date (King & West, 1987; for suggestive evidence from wild populations of another subspecies, see O'Loghlen & Rothstein, 1995a). Female selectivity for males' songs (at the level of within-population song quality assessment and not between-population song preferences) correlates strongly with the size and density of neurons in the lateral magnocellular nucleus of the anterior neostriatum, part of the anterior forebrain pathway of the loop of the song control system that, in male cowbirds as with other male

songbirds, is devoted to song learning and acquisition (Hamilton, King, Sengelaub, & West, 1997). Results from the present work, although not designed to test for modifiability of female song preferences, together with earlier studies (Freeberg et al., 1999), suggest that female cowbirds can acquire novel song preferences and can use them as a basis for mate choice. Thus, the failure to find large amounts of plasticity in female song preferences in the earlier studies (King & West, 1983c, 1987) may have been a result of the nature of the methods used to probe modifiability (see West et al., 1997), that is, long periods of exposure to 1 or 2 males in sound-attenuating chambers. The acquisition of vocal preferences in females may require extensive social stimulation and interaction to facilitate transmission of perceptual preferences, as was found in the present study.

In the present study, we attempted a functional approach to the question of vocal traditions, linking stable differences in male vocalizations to song preferences in females and to courtship and pairing patterns among groups of cowbirds. Although much work remains (specifically testing the diacept question regarding the development of population-level preferences in songs for females), our data supply an important missing link to earlier studies of the cultural transmission of courtship patterns in this species (Freeberg, 1996, 1998). The present study provides a behavioral mechanism for the culturally transmitted patterns of assortative pairing and mating seen in those earlier studies. Taken together, these findings suggest that social learning specifies the courtship system of brown-headed cowbirds. Future work must determine the extent to which cowbirds, with their exceptional natural history as brood parasites, are exceptions in their reliance on social learning, or whether the pervasiveness of social learning in courtship and communicative systems is the rule in passerine species.

The analysis of geographic variation in behavior can provide exciting insights into evolutionary dynamics in populations, from how individuals interact adaptively with their physical and social environment, to mating patterns and preferences and rates of gene flow between populations, to speciation events (see Foster & Endler, 1999; King & West, 1990; Lott, 1991). The extent to which behaviors exhibiting intraspecific variation are ecotypes (behavioral variants due to differential selection pressures in different environments) is key to these considerations (see chapters in Foster & Endler, 1999). Song in passerines is one of the quintessential learned traits in animal behavior, yet recent research in songbirds is beginning to uncover adaptive influences on song-learning processes. For example, results from studies with marsh wrens, *Cistothorus palustris*, and two subspecies of white-crowned sparrows, *Zonotrichia leucophrys*, in which researchers used hand-raised birds to control for early environmental factors indicate that which song types are learned and when they are learned depend on whether an individual comes from a migratory or nonmigratory population (Kroodsma & Canady, 1985; Nelson, Whaling, & Marler, 1996). Both SD and IN populations of cowbirds are migratory, but the breeding season likely is shorter and begins later in SD, which is further north than IN. Thus, song learning in SD and IN cowbirds may be adapted to these seasonal differences. Data from hand-raised cowbirds, resulting from SD–SD, IN–IN, and SD–IN crosses, suggest a genetic influence on the onset of the high rates of vocalizing seen in male cowbirds in the months before the breeding season (Freeberg, West, & King, 2001). Further work addressing adaptive differences in song learning would be highly

informative to efforts aimed at understanding how vocal traditions might reduce or facilitate gene flow between populations.

To consider different ecotypes in the learning process brings one to consider the question of culture-like processes in nonhuman animals more deeply. "Cultural" traits are often described as those characters that show group- or population-level differences and that result from imitation or other social learning mechanisms (Boyd & Richerson, 1985; Freeberg, 2000; Laland, 1992). We are led by our work to argue that the focus of research attention in studies of animal culture, however, should be shifted toward a view of culture as being the process that gives rise to those different characters, rather than the characters themselves. In other words, the traits are the products of the cultural process, and cultural differences would represent differences in the social learning processes that give rise to those traits. The present developmental study was a study of ontogenetic endpoints and how those endpoints—the songs males develop—influence courtship patterns and mating preferences. In the future, researchers will need to look much more closely at the developmental processes that led to these different endpoints, and addressing possible cultural differences will require researchers to study how the social learning process differs in timing onsets, rates, flexibilities and stabilities, and behavioral outcomes in different populations under different selection pressures. Probing more deeply into the cultural process and into adaptive differences in the transmission of traits will further aid our understanding of how courtship systems in these populations evolve and, indeed, how populations may diverge and may become reproductively isolated from one another.

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