

The Development of Local Song Preferences in Female Cowbirds (*Molothrus ater*): Flock Living Stimulates Learning

Meredith J. West*, Andrew P. King*, David J. White†, Julie Gros-Louis* & Grace Freed-Brown*

* Department of Psychology, Indiana University, Bloomington, IN

† Department of Psychology, University of Pennsylvania, Philadelphia, PA, USA

Correspondence

Meredith J. West, Department of Psychology,
Indiana University, 1101, 10th Street,
Bloomington, IN 47405, USA.
E-mail: mewest@indiana.edu

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Abstract

We carried out two experiments across 2 yr on song perception in female cowbirds (*Molothrus ater*). In the first experiment, juvenile and adult female brown-headed cowbirds, living in same-sex flocks in outdoor aviaries, were periodically tutored with recordings of local male cowbirds' songs. In the spring, four adult male cowbirds were placed with half of the females for a 12-d period. We then tested song preferences of all females by measuring copulation solicitation displays during the breeding season. We found that the females exposed only to tape-tutor songs preferred those songs to those of the unfamiliar males used as companions and that the females allowed to interact with males preferred their songs over the familiar tape-tutor songs. These data establish the modifiability of female cowbirds' song preferences at the level of local song. In a second experiment, we studied the playback responses of juvenile females, hand-reared from the egg, who were tape-tutored only in the spring in the presence or absence of adult females. There were no differences between the responses of juveniles housed with or without adult females and the hand-reared juveniles were significantly less responsive to song than adult females. Adult females responded more to familiar songs than to the unfamiliar songs: juvenile females made no such distinction. Taken as a whole, these data are the first to document that female cowbirds' song preferences for local song can be reshaped by post-natal experience. These data complement recent study in cowbirds and other species showing that socially more complex contexts reveal plasticity in female song preferences that are not apparent when learning opportunities are constrained by impoverished laboratory settings.

Introduction

Understanding the communicative capacities of female songbirds is critical to understanding the function of male song in mate choice and to exploring how vocal cultures are transmitted from one generation to the next (Searcy & Yasukawa 1996). Studies of female song preferences have increased as techniques for measuring responsiveness have been

developed (King & West 1977; Nagle & Kreutzer 1997; Searcy 1992; Riebel & Slater 1998; Depraz et al. 2000; Payne et al. 2000; Nagle et al. 2002; Lauay et al. 2004). These studies focus on different levels of responsiveness, from recognition of conspecifics to discrimination of fine acoustic structure within songs. The results indicate that female song preference, like male song production, has a learned component in many songbird species (for reviews,

see Riebel 2003a,b). Such findings are critical to begin the task of uncovering the mechanisms integrating male and female behavior within communication networks (West & King 1986; Sturdy et al. 2001; King et al. 2003a).

Much remains to be learned about the determinants of female song preferences, especially with regard to the developmental context in which song preferences are acquired and/or modified. Many studies of female song preferences, like studies of male song production, have occurred in socially impoverished contexts in which opportunities to learn have been restricted due to the absence of chances to interact with multiple conspecifics (Riebel 2003a,b). As in the study of male song production, studying females in simplified environments has been necessary to control song experience but such restrictions may affect components of song perception. Along these lines, Sturdy et al. (2001) found that reduced auditory exposure in female zebra finches (*Taeniopygia guttata*) led to deficits in frequency range discrimination. Riebel (2000) found that isolated female zebra finches showed less repeatability in their operant responses to tutor songs. Tutored females repeatedly chose the same tutor song while isolates varied in their choices from trial to trial.

Studies of naive female cowbirds housed in auditory isolation show that post-natal experience is not necessary for species recognition or discrimination of geographic song variants (King & West 1977, 1983, 1987). Studies of females housed with males for many months also reveal no evidence of male influence, except in one border population where such experience led to a broadening of the females' preference for songs on both sides of the border (King et al. 1986). Such lack of malleability would seem to be consistent with the needs of a female brood parasite, where early experience with foster species might facilitate species-atypical song preferences leading to inappropriate mate choice.

Further evidence suggesting female cowbirds' lack of sensitivity to male vocal stimulation came from examining the individual playback preferences of females housed with individual males from local or distant populations during the fall, winter, and spring. When exposed by playback to the songs of their (now absent) male companion vs. unfamiliar but identically housed males, females show no preference or aversion to the specific songs of their companion male (e.g. West & King 1980; King & West 1983, 1987; King et al. 1986; Smith et al. 2000). Thus, long-term exposure to the same male did not modify female preferences. Observations of the

females indicated, however, that the two sexes interacted, especially in the spring, leading to changes in the males' repertoires (Smith et al. 2000; King et al. 2005).

The first evidence of female plasticity in cowbirds came from studies of mate choice in captive colonies (Freeberg 1996, 1997; Freeberg et al. 2001). When juvenile females were housed in large aviaries with males and females from a local or distant population, Freeberg et al. (2001) found that females preferentially paired and mated with males from the same cultural background, even if males from their local population were available as potential mates. The enculturated females were also able to pass on their preferences to a second generation of young females. The basis for mate choice was partially explained by different preferences for male song. Given the importance of male song to mate choice in this species, these data indicated considerable plasticity in females' song preferences and suggested that the prior methods had not been sensitive to developmental parameters in females.

We obtained more direct evidence of female plasticity with regard to macrogeographic song preferences in a study of females housed with or without males in large outdoor aviaries (King et al. 2003a). Juvenile and adult females, housed throughout the fall, winter, and spring without male companions in all-female flocks, showed no playback preferences during the breeding season for local vs. distant song, whereas, in previous studies, both ages had displayed strong local preferences after year-long housing without males in sound-attenuating chambers. The broadening of the aviary females' preferences to include distant song might have resulted from females hearing a range of song from local and migratory males singing outside the aviaries. When local males were placed inside the aviaries, the females showed a preference for local vs. distant song. Thus, females appeared to be sensitive to post-natal stimulation when housed in social groups.

In King et al. (2003a), we demonstrated influences on song preferences between populations that do not ordinarily interact with one another during the breeding season. But mate choice involving discrimination and plasticity at the microgeographic level is essential to understanding the function of female song preferences. Prior to the breeding season, females show sensitivity to male songs and flight whistles (O'Loughlen & Rothstein 1995; West et al. 1998). But females also observe other male behaviors, such as counter-singing (White et al. 2002) that draws attention to males and their songs and affects

females' production of eggs (West et al. 2003b). Young females may also watch older females' interactions as a basis for male assessment (Gros-Louis et al. 2003). Thus, sources of influence on females' preferences for songs may be based on diverse forms of experience.

Here, we explored female plasticity by exposing females to song by tape tutoring, by live tutoring, and by manipulating adult female presence. In the first experiment, we tutored flock-housed juvenile and adult females with recordings of local adult males in three sessions during the year. We examined whether adult and juvenile females' responsiveness and preferences for the song sets were the same and whether the addition of males to a group for a short period in the spring would affect preferences for the taped songs or the songs of the male companions. In a second experiment in the following year, we compared responses of a new set of hand-reared juvenile females tutored with the same songs used in Expt 1, either in the presence or the absence of a new set of adult females. We wanted to see if juvenile females with no physical contact with adult males could acquire preferences and we wanted to see if housing with adult females had any effect on juveniles' preferences. Previous observations suggested that adults showed more concordant preferences than juveniles that might make them stable sources of information about song. Stability of preference for specific songs would seem to be critical to repeatable mate choice if male song traits are actually reliable indicators of male quality and affect parameters of heritability (Riebel 2000). In contrast to the previous year, exposure to the tape-tutor songs was limited to one 12-d session in late-Apr. to see if massed exposure would lead to learning from the tutor song sets.

In the first experiment, we chose to tutor the females in late-fall and early-spring. We chose these times because they coincided with periods in which we had seen evidence of female influence on male song structure (King & West 1988; West & King 1988). Even though they cannot sing, female cowbirds can shape male song by emitting visual displays to preferred song variants. The process appears to begin in late-Nov. and ends in Apr. This period coincides with juvenile males' proceeding from plastic to stereotyped song, the pace of which is also affected by female stimulation.

In all experiments, females could, of course, see and hear wild males and other species outside the aviary. Based on past work with flocks, we wanted to see if inanimate or live tutoring inside the aviary

provided a robust form of stimulation when pitted against the backdrop of an acoustically rich environment. We chose very experienced adult females under the assumption that the ample experience they had had with male songs would approximate species-typical contexts in the wild and that it would be a fair assumption that all adult females could already discriminate local variants and show preferences for some songs over others as we and others have found in playback and mate choice tests (West & King 1985; O'Loughlen & Rothstein 1995; Freeberg 1996). We used wild-caught juveniles to test if species-typical early experience constrained post-natal learning.

In the two experiments, we measured copulatory behavior. First, we counted the number of copulatory solicitation postures given to playback of songs during the breeding season. Secondly, we calculated concordance among the females' responses to individual songs in each playback set.

Expt 1

Methods

Subjects and housing

Juvenile females were collected from one site in Monroe County, Indiana in Jun.–Aug. 2002. They were estimated to be approximately 30–50 d of age upon capture. The wild-caught adult females, captured from the same site in previous years, were at least 2–3 yr of age and had lived in mixed-sex flocks in outdoor aviaries except for 6 wk in 2002 when all participated in playback tests of local Indiana songs. The adults were thus very experienced: they had heard thousands of songs from over 30 different males living with them while in the aviaries for almost 2 yr (with males and females being moved between aviaries to stimulate interactions); they heard songs from 35 local males during playbacks; and during the spring, summer, and fall, they heard songs from wild males just outside or on top of the aviaries.

All females lived in same-sex aviaries in the summer prior to the onset of the present experiment. All birds were fed a modified version of the Bronx Zoo diet for omnivorous birds and given vitamin-treated water. All birds wore colored leg bands to permit individual identification.

In Oct. 2002, 12 adult and 12 juvenile females were assigned to four outdoor aviaries, each measuring $2.4 \times 6.1 \times 2.3$ m. Each aviary housed three juveniles and three adults (Table 1). In Apr. 2003,

Table 1: Dates for aviary and breeding season playbacks

<i>Expt 1</i>	
Fall and spring	Song sets in outdoor aviaries
Nov. 11–14, 2002	TT-10
Nov. 26–Dec. 4, 2002	Plastic song
Dec. 16–20, 2002	Plastic and formatted song
Apr. 8–12, 2003	TT-10
Apr. 14–28, 2003	4 adult Indiana males (MC) housed with 6 J and 6 A females and then removed
Apr. 29–May 2, 2003	TT-10
Breeding season	Playbacks in sound-attenuating chambers
May 15–25, 2003	TT-10
May 26–Jun. 8, 2003	14 local Indiana songs
Jun. 8–24, 2003	TT-8/MC-8
<i>Expt 2</i>	
Spring	Song set in outdoor aviaries
Apr. 18–30, 2004	TT-10
Breeding season	Playbacks in sound-attenuating chambers
May 16–Jun. 8, 2004	TT-10 to J females
May 31–Jun. 10, 2004	TT-10 to A females
Jun. 11–24, 2004	TT-8/MC-8 to J and A females

For aviary playbacks, the females heard 10 songs per day in different orders for 4 d; for sound-attenuating chamber playbacks, the females heard six songs per day in different orders. All fall and spring playbacks took place in each of four aviaries; male exposure took place in two combined aviaries.

the four groups were collapsed into two flocks, with equal numbers of adult and juvenile females. On Apr. 16, 2003, four local adult males from our aviaries (hereafter the MC males) were introduced to one of the two groups and remained for 12 d. The two aviaries were situated on either side of a wood building so that only the one group heard the MC males. After the males' removal, the females were reconfigured into four flocks with equal numbers of juveniles and adults. The juvenile (J) and adult (A) females exposed to the four males are hereafter referred to as the JA + MC females, as opposed to JA females who received no experience with males in aviaries. Three females (two adults and one juvenile) died during the course of the experiment and no data are reported from them.

Song/male experience between fall 2002 and spring 2003

The four groups of females were exposed to tape recordings of cowbird song beginning in the fall of 2002 and ending in the spring of 2003 (Table 1). Each session consisted of 4 d of tutoring with recordings of 10 unfamiliar, wild-caught adult Indiana (IN) males. There were 5 s of silence between the songs in the playback. After the first five playbacks each

day, 30 min elapsed before playback of the second five songs. Thus, in each 4-d session, the females heard each song four times for a total of 40 playbacks/session. The 10 songs were presented in a different order each day. The females heard the 10-song set, hereafter the TT-10 set, once in the fall and twice in the spring while housed in the four aviaries in groups of six. Among the songs on the TT-10 set, three were shared, i.e. showed the same frequency structure and timing. In that song playback to females had not been carried out at these times before, we chose the number of songs and amount of tutoring based on our best guesses about balancing exposure with habituation.

On two occasions in the fall and winter of 2002, all the females heard playbacks of plastic and formatted song recorded from unfamiliar IN males housed with non-conspecifics as part of another study (Table 1). Plastic song contains note clusters and whistles sung in varying arrangements from rendition to rendition; formatted song shows variable content but contains a stable syntactic structure. Females in the wild would hear these forms of song in the late-winter and early-spring.

The four MC males' songs were heard for 12 d on Apr. 16–30 by one group of six juveniles and six adults when the females were divided into two flocks of 12. During that time, we recorded each male. From the recorded songs, eight songs, two from each male, were combined with eight of the TT-10 songs into one test tape, hereafter the TT-8/MC-8 song set. We dropped the two songs receiving the fewest playback responses. Three of the TT-8 songs were shared and none of the MC songs was shared.

Recording and playback procedures

We recorded TT-10 male song in aviaries during the breeding season of 2002 (all TT songs) and 2003 (MC males). We placed two to four Sennheiser RF condenser microphones in locations to maximize our chances of obtaining recordings from males vocalizing <0.3 m on axis with the microphones. The vocalizations were recorded with a Sony TCD-D10 PRO II DAT recorder (Sony Corporation, 550 Madison Ave., New York, USA), sampled at 48 kHz. Songs were then digitally converted to 44.1 kHz files. Songs were selected on the basis of recording quality. Using Peak LE and Bsound with IGOR PRO V.4.1 (<http://homepage.mac.com/bsnelson/Bsound.html>), we filtered songs with a Hanning window high pass (75 kHz) filter to remove low frequency noise below the range of cowbird song.

During the year, the songs were played back through AIWA LCX-350 speakers (Aiwa Corporate, NJ07430, USA), attached in one corner of each aviary, positioned so that they broadcast away from the other aviaries. Sound was played using NAD compact disc player (NAD Electronics International Pickering, Ontario, Canada) and songs were broadcast at a level approximating the amplitude of songs sung within an aviary.

Breeding season playback

On May 2, 2003, all females were placed in two indoor flight cages ($2.4 \times 6.1 \times 2.3$ m) out of contact from males. JA + MC females were housed in one cage and JA females in the other. All were moved into sound-attenuating chambers on May 13, 2003 and playbacks began on May 15. The sound-attenuating chambers are 1 m^3 with the playback speaker placed to the left of the door. Some females were housed in pairs and some alone. No effects of pair housing have been found in past work in over 20 yr of testing (King & West 1983; West & King 1985; Smith et al. 2000).

Beginning on May 15, 2003, song playbacks took place (Table 1). Between the tests of the TT-10 and TT-8/MC-8 songs, the females heard six unfamiliar local songs from a set of 14 songs across the day to reduce the chances of a recency or frequency-dependent effect favoring the TT songs during the Jun. playback of TT-8/MC-8 songs.

Measuring playback responsiveness

For both test tapes, we played six songs daily to the females, with one song per trial and each trial separated in time by 90 min, beginning at 07.00 hours. We played back each song six times to each female over the course of the experiment. The females heard the vocalizations in varying orders each day and each vocalization was presented roughly an equal number of times at different times of the day.

We scored a positive response if a female adopted a copulatory posture (if she arched her neck and back and separated the feathers surrounding the cloacal area) within 1 s from onset of the song. We played songs using a Denon DN-C680 compact disc player (Denon Electronics (USA), LLC P.O. Box 867 Pine Brook, NJ, USA), a Crown D75 power amplifier and JBL 2105 speakers located in each chamber. The SPL was 85 ± 2 dB adjusted with a B&K 2209 sound pressure meter, set to A

weighting, impulse reading, measured 75 cm from the speaker.

Data Analysis

Because of small samples sizes and heterogeneity of variance, we used non-parametric tests. We used Mann-Whitney tests for comparisons across groups, Wilcoxon signed rank tests for comparison within groups, Kendall's coefficient of concordance for measures of inter-female agreement, and Kruskal-Wallis tests for comparisons of three groups, using a Bonferroni-adjusted α of $p < 0.01$ (Siegel & Castellan 1988). All tests were two-tailed. Some females did not respond at all to song reducing the N in each group as shown in Table 2.

Results

Response to local song

Within each group, the playback responsiveness of adults and yearlings to the local song variants on the TT-10 song set did not differ (Table 2; JA + MC, $U = 5$, $n_1 = 4$, $n_2 = 4$, $p < 0.48$ and JA, $U = 10.5$, $n_1 = 4$, $n_2 = 6$, $p < 0.76$). As a result, the responses of the two ages were averaged together. Experience with the MC males did not affect the mean proportion of responses to the TT-10 song set (Table 1; $U = 26$, $n_1 = 10$, $n_2 = 8$, $p < 0.21$). The females also did not differ in their responding to shared vs. unique songs [JA + MC, mean shared song = 0.52 (0.10) vs. unique song = 0.43 (0.08), $T = 8$, $n = 8$, $p < 0.16$; JA, mean shared song = 0.24 (0.11) vs. unique songs = 0.35 (0.07), $T = 8$, $n = 10$, $p < 0.09$].

Concordance

Experience with the MC males did, however, affect the concordance of female responding to the individual songs within the TT-10 set (Table 3). JA + MC females exposed in their aviary to males showed significant concordance in their rankings of the 10 songs ($W = 0.46$, $n = 8$, $p < 0.001$), but unexposed JA females did not ($W = 0.14$, $n = 10$, $p < 0.20$).

Responses to familiar and unfamiliar song

There were no age differences in mean responsiveness to the TT-8/MC-8 set and so data were combined across age: for the JA + MC group, $U = 2$, $n_1 = 3$, $n_2 = 3$, $p < 0.40$ for TT-8 and $U = 3$, $n_1 = 3$, $n_2 = 3$, $p < 0.70$ for the MC songs; JA group, $U = 12$, $n_1 = 5$, $n_2 = 5$, $p < 0.50$ for TT-8 songs and

		2003 Playback sets: Experiment 1				
		Test one		Test two		
		TT10		TT-8 versus MC -8		
J+A Females (w/ MC Males)		0.46 (0.08) n=8	T = 0, p < 0.027 0.38 (0.08) 0.61 (0.07) n=6 n=6			
J+ A Females		0.32 (0.07) n=10	T = 1, p < 0.024 0.42 (0.04) 0.25 (0.06) n=10 n=10			
		2004 Playback sets: Experiment 2				
		Test one		Test two		
		TT-10		TT-8 versus MC-8		
J Females (w A's)	U = 1 p < 0.008	0.30 (0.08) n=4	0.19 (0.04)	0.27 (0.06)		
J Females (w/o A's)			H = 8.4 p < 0.015	0.59 (0.09)	0.31 (0.03)	
A Females (w J's)				T = 0, p < 0.02 0.67 (0.06) 0.53 (.04) n=7 n=7	0.72 (0.07) n=7	

Table 2: Mean proportion and standard error of copulatory responses in the two experiments

Numbers in bold connected by brackets indicate significant differences as tested by Wilcoxon signed rank tests (T) for within-group comparisons; Mann-Whitney tests (U) for between group comparisons; and Kruskal-Wallis (H) ANOVA for comparison of the three groups.

U = 10, n1 = 5, n2 = 5, p < 0.68 for MC songs. There were no significant differences in responding to the shared vs. unique songs in either group of females. The mean response for the JA group was 0.41 to shared songs and 0.42 to unique songs. The mean response for the JA + MC group was 0.37 to the shared songs and 0.38 to the unique songs.

The two groups of females responded significantly different to the songs presented by tape tutoring as opposed to those from the male companions (Table 2). Within the JA + MC group, all females responded more to the songs of the male companions (T = 0, n = 6, p < 0.027) while all but one of the JA females responded more to the tape-tutor songs (T = 1, n = 10, p < 0.024). Between groups,

JA + MC and JA females did not differ in their mean responsiveness to the TT-8 songs (U = 29, n1 = 6, n2 = 10, p < 0.64); however, JA + MC females responded significantly more to MC songs than did JA females (U = 5, n1 = 6, n2 = 10, p < 0.007).

Concordance

We examined concordance of individual song rankings within the two groups (Table 3). The JA + MC group showed significant concordance across the entire set of 16 songs (W = 0.35, n = 6, p < 0.01). The JA group also showed concordance (W = 0.28, n = 10, p < 0.02).

Table 3: Kendall's coefficient of concordance for juvenile and adult females for Expts 1 and 2

	Song sets	
	TT-10	TT-8–MC-8
Concordance 2003		
J + A females (w/MC males)	Yes	Yes
J + A females	No	Yes
Concordance 2004		
J females		
With adults	No	— ^a
Without adults	No	— ^a
A females	Yes	Yes

Values significant at $p < 0.05$ or lower are indicated by a 'yes' and non-significant values with 'no'.

^aConcordance not computed due to low Ns.

Discussion

The results show for the first time that female cowbirds' song preferences for local songs are affected by post-natal experience when females are tutored outdoors in groups. The different experiences afforded the two groups of females lead to a divergence in their preferences. The JA females responded more to the familiar tape-tutor songs compared with songs of unfamiliar males, while the JA + MC females preferred the male companions' songs to the tape-tutor songs. Sensitivity to song tutoring from animate or inanimate sources contrasts with all previous work studying female cowbirds housed in pairs or triads in sound-attenuating chambers: under those circumstances, naive or experienced juvenile or adult females showed absolutely no evidence of post-natal influence at microgeographic levels of song structure even after 9 mo of co-habitation with males (West & King 1980; Smith et al. 2000; King et al. 2003a). Here, we found sensitivity to acoustic and social influence with far less extended exposure. The data from the present experiment do not allow us to tell if live tutors are more effective than tape tutors and leave open many questions about the effects of timing of exposure. The females with the four MC males almost certainly heard more song in the 12-d period in late-spring compared with the 120 total exposures to the TT-10 set during the year. Further study is required to discover if one form of exposure is more effective than the other, but our goal here was to see if *any* form of post-natal experience would affect microgeographic preferences and the answer was that both sources were effective.

The tests of the MC vs. TT songs occurred later in the breeding season than the tests of the TT songs

alone, leaving open the possibility that the level of physiologic responsiveness could have changed. There was no evidence that females were more or less responsive in the Jun. test (Table 2). For the May test, the JA + MC females' mean proportion of responding was 0.46 compared with 0.49 for Jun. tests; for JA females, the mean proportion of responding was 0.32 in May and 0.33 in Jun. Two TT songs were removed to form the TT-8 song set: the deleted songs were the least potent songs from the first playback test. Could this exclusion have affected preferences when the TT and MC songs were tested together? It does not seem likely to be the case as the two groups of females did not differ in their playback responses to the TT-8 songs, responding to a mean proportion of 0.38 vs. 0.42 of the TT-8 songs (Table 2).

These data from aviary-housed birds stand in contrast to the results of previous studies indicating no effects of post-natal experience on preferences in females housed in sound-attenuating chambers who were tape-tutored or housed with individual males (West & King 1980; King & West 1983; King et al. 2003a). The preferences shown by the females in the present experiment are all the more impressive when taking into account the acoustic surroundings of the aviaries; we regularly observed singing male cowbirds outside the aviaries throughout the fall and spring. In contrast, during the same time frame, the females in the two groups heard each of the TT songs only 12 times (for a total of 120 playbacks) and half of the females experienced the MC songs from the added males for only 12 d. Thus, female cowbirds do not appear to need extensive experience with specific songs in order to develop specific preferences. Both juvenile and adult females were affected by the tutoring regimes, indicating that adult females can learn new preferences beyond the first year, as has been found in some other songbird species (Nagle et al. 2002; Riebel 2003a).

Expt 2

Comparison of Hand-Reared Juveniles and Experienced Adult Females

We conducted a second study to see if social experience with or without adult females during the time of tape tutoring would affect juvenile females' song preferences. The question was of interest because we thought it was possible that juveniles were acquiring their preferences by observing how adult females responded to song during the tutoring. We also used

juveniles females that had been hand-reared from the egg and thus had had no exposure to host species or to cowbirds until they were 30 or more days of age. All previous study on female perception in cowbirds had been carried out with wild-caught birds, making these fully hand-reared females a new developmental end point.

We also examined whether adults and juveniles would show a preference for tutor songs when given massed playback exposure in relation to the first experiment. In this test, we restricted the period of tutoring to one interval in the spring, at the time when females in nature would be interacting with local males prior to pairing and breeding in May. The females heard the TT songs an equal number of times but over the course of 2 wk, not 6 mo.

Methods

Subjects and procedures

Twenty-two females were used. The eight adult females had been wild-caught at the same collection site as Expt 1 in 2001 and 2002. They had lived in mixed-sex aviaries until summer 2003 and then in aviaries with only juveniles until Mar. of 2004. They had not been subjects in any other playback tests. The juveniles were hatched from eggs collected from aviaries at the laboratory in 2003. Upon hatching, they were hand-fed until independence, which generally occurred between day 18 and 25. Around day 30, adult female cowbirds were added to the outdoor aviary containing all the juveniles to facilitate independent foraging in the young birds. The hand-reared juveniles (male and female) and adult females remained together until the spring. In Mar. 8, 2004, the 14 juvenile females and eight adult females were split into two groups. The first group consisted of the eight adult females and six juveniles (JA condition). The second group consisted of eight juveniles (J condition). They were placed in two of the same aviaries used in Expt 1. All other details of care follow Expt 1.

Tutoring protocol

Both groups of females were tape-tutored for 12 d beginning in Apr. 18, 2004 (Table 1). They heard the entire TT-10 song set once per day using the previously described equipment.

Playback procedures

In Apr. 30, 2004 the birds were moved into indoor flight cages with juveniles in one cage and adults in

the other. The juvenile females were placed in sound-attenuating chambers beginning in May 15, and playbacks occurred between May 16 and Jun. 8 (Table 1). With the exception of one pair, we housed juvenile females alone. From May 31 to Jun. 10, the adults were housed in chambers and played the TT-10 song set. In Jun. 11–24, the TT-8/MC-8 playback occurred for all females. All playback and statistical procedures followed those described earlier for Expt 1. One juvenile became ill and was taken out of the experiment.

Results

Responses to local song

A Kruskal–Wallis analysis of variance indicated significant differences in responsiveness among adults, juveniles with adults, and juveniles without adults for the TT-10 song set ($H = 8.4$, $n_1 = 4$, $n_2 = 6$, $n_3 = 8$, $p < 0.015$). Adults with juvenile females were reliably more responsive than juveniles alone (A vs. J with A, $U = 1$, $n_1 = 8$, $n_2 = 4$, $p < 0.018$; A vs. J only, $U = 8$, $n_1 = 8$, $n_2 = 6$, $p < 0.04$; Table 2). The adults' song preferences for the tutor songs were concordant ($W = 0.34$, $n = 8$, $p < 0.01$). The juveniles' preferences were not concordant (JA: $W = 0.19$, $n = 4$, $p < 0.60$; J: $W = 0.28$, $n = 6$, $p < 0.20$).

In the second playback, all of the adult females responded more to the familiar TT-8 songs (Table 2; $T = 0$, $n = 7$, $p < 0.018$). The juvenile females showed more variability, with the three J + A and one J – A juveniles responding more to TT-8 songs and three J only females responding more to the K1 songs. The adults also showed concordance in their responses to the entire TT-8/MC-8 song set, $W = 0.46$, $n = 7$, $p < 0.001$ (Table 3). Too few juveniles responded in the two groups to compare concordance.

Year-to-year correlations

In both 2003 and 2004, we tested cohorts of adult and juvenile female cowbirds housed together without males but tutored with the TT-10 song set (JA condition), although at different points in time. For the two independent sets of JA adult females, the year-to-year rankings of the TT-10 songs were highly correlated, $r_s = 0.79$, $p < 0.01$; their rankings of the TT-8/MC-8 song sets were also highly correlated, $r_s = 0.80$, $p < 0.001$. For JA juvenile females, the rankings were not correlated: for the TT-10 song set, $r_s = 0.24$, $p < 0.5$ and for the TT-8/MC-8 song set, $r_s = 0.51$, $p < 0.053$.

Discussion

Hand-reared juveniles were less responsive, less discriminating, and less consistent than adults when reacting to playbacks of familiar and unfamiliar song. The data suggest that the naive juveniles either needed song stimulation to occur much earlier in the year or needed direct social contact with males. It may also be that some other aspect of hand-rearing affected responsiveness. The social and acoustic experiences of the hand-reared juveniles vs. wild-caught juveniles in Expt 1 differed as the wild-caught females could have heard adult song and interacted with adult males prior to capture while the hand-reared birds could only have heard adult male song from outside the aviary after day 18. They may also have heard more immature song from their male flock mates in the fall and winter. Further investigation is required to sort through these differences but the finding that the wild-caught juveniles in Expt 1 did develop reliable preferences for TT-10 or MC-8 songs shows that new preferences can be learned even after 30–50 d of species-typical experience during the first year and after only relatively brief periods of tutoring. The lack of discrimination by hand-reared birds even with tutoring suggests that early experience with males is important to forming a preference. We found a parallel dependence in wild-caught juveniles tested with macrogeographic songs variants (King et al. 2003b). Zebra finches also appear to need experience with adult males to develop preferences for high quality songs (Lauay et al. 2004).

The Expt 2 juveniles did not show concordance in their song rankings of the TT-10 songs or did their responses correlate with those of the JA juveniles in the first experiment. The results add to the conclusion that juvenile females may require actual experience with singing males before they can form significantly concordant preferences (West et al. 1998; King et al. 2003a). The lack of correlation between the 2003 and 2004 juveniles housed with only adult females also suggest that more learning is required. By contrast, the adult females' preferences were highly correlated, suggesting song experience brings with it repeatability, even for songs heard for brief amounts of time. In zebra finches, early exposure to song leads to more repeatable preferences of songs using an operant response (Riebel 2000).

Looking collectively at the two groups across 2 yr also adds weight to the finding that exposure to

tutor songs alone affects song discrimination. Across the 2 yr, all 12 JA adults from two different cohorts responded more to the familiar TT-8 songs vs. the unfamiliar MC-8 songs and seven of eight JA juveniles responded more to TT-8 songs.

Learning by females may not be limited to the male's song. In other parts of the cowbird's range, female cowbirds display copulatory preferences for playbacks of local flight whistle dialects. These dialects are often not learned by males until their second breeding season and there is some suggestion that females may also have to learn flight whistle dialects after their first summer as they most likely learn them from males (O'Loughlen & Rothstein 1995; O'Loughlen 1995). Thus, contact with adult males, not just juvenile males, may enhance females' discrimination of song and whistle types.

Studies in white-crowned sparrows (*Zonotrichia leucophrys oriantha*) indicated that wild-caught juvenile females did not exhibit greater physiologic responses to natal vs. foreign song at the end of their first year but did respond with more copulatory responses to natal song when tested at the end of their second year (MacDougall-Shackleton et al. 2001). Exposure to foreign song at the end of their first year also affected responding to natal dialects the following spring. These data indicate the importance of testing females beyond their first breeding season and raise the possibility that song heard during the breeding season can also be considered as additional auditory experience leading to song learning. Thus, in the present study, the May playback of TT-10 songs might also be considered as further auditory learning. It was not sufficient to preclude the JA + MC females' preference for MC songs but may have consolidated the JA females' preferences. Future study is needed to look at the physiologic responses to song playback and to compare females' responses to songs in Jun. that were not preceded by the opportunity for further learning earlier in the breeding season.

As measured here, adult females did not affect juvenile females' preferences for tutored song as we had thought possible. It may be that the low response rate of the juveniles masked any effect. It may also be that hand-reared females were too naive to take advantage of observing adult females. The juveniles were housed in only two aviaries; there could have been other factors besides the presence or the absence of adult females that contributed to their post-natal experience. Thus, the data must be viewed with some caution until more groups are tested.

General Discussion

The results of the present study showing preferences for local song variants as a function of song experience complement those carried out on preferences for macrogeographic song variants (King et al. 2003a). In both studies, flock housing revealed post-natal plasticity in adult and juvenile females where chamber housing had indicated no plasticity. Concordance in song discrimination was affected by male experience and age. In both studies, adult females of 2 or more years of age with courtship experience and previously formed song preferences showed evidence of new learning, developing preferences for new songs experienced either by song or live tutoring. Thus, female cowbirds appear to show an extensive period of sensitivity to song stimulation and may, in fact, be more open-ended as learners than male cowbirds, whose ability to add song types does not appear to go past the second year (Hamilton et al. 1998).

In past studies of seven populations within and across the three subspecies, we found that acoustically isolated juvenile and adult females showed strong local preferences, even after complete deprivation from local song or many months of exposure to distant song (West et al. 1998; Smith et al. 2000; King et al. 2003a). The presence of species-typical song preferences when housing is in sound-attenuating chambers suggests that the development of song preferences proceeds through the modification of innate perceptual biases as opposed to *de novo* post-natal formation of preferences. As regards, the data gathered here on young females suggest some similarities with the male cowbird's development of song. Males with no post-natal experience produce songs that elicit copulatory responses, just as females with no post-natal experience discriminate cowbird song from heterospecific song (King & West 1977, 1980). These findings by themselves suggest that the species possesses a safety net for song recognition, a seemingly appropriate adaptation for a brood parasitic species. But such a conclusion is wrong because it is based on a performance in a physical context cowbirds and other songbirds never experience, i.e. no social contact with conspecifics. Cowbirds naturally form social groups as juveniles and that experience leads young males to sing less potent song than isolates and as the first experiment shows, appears to lead young females to override perceptual biases in favor of current exemplars of local song (West & King 1980). In sum, for both females and males, social interactions affect initial sensory states leading

to different communicative outcomes. The dynamic pattern of initial bias, social perturbation, and rapid assembly of new preferences through active engagement with the environment is one that also describes developmental change in song preference learning in some other songbirds (Marler & Nelson 1993; West & King 1996; Riebel 2003a,b) and sensory-motor development in many species for many motor behaviors (Thelen & Smith 1994).

Much more is known about the contextual properties that cause transitions in male song production and use than the transitions in female perception and choice. Recent study in the laboratory has begun to outline the contextual properties important to female development. In a series of studies conducted in outdoor aviaries, we have observed the nature of female social organization and how it is affected by the presence of males, females, or the presence of songs (White et al. 2002; Gros-Louis et al. 2003; King et al. 2003b; West et al. 2003a). Briefly put, these studies show that juvenile females' social interactivity with adult females varies as a function of male presence. If males are absent, females show no particular pattern of social organization by age, but they rapidly segregate by age when adult males are introduced. Females also show immediate re-organization into age-segregated groups when male song is played into an aviary, with the age segregation disappearing when playbacks cease, and returning when playbacks resume. These changes occur as early as the fall of the female's first year. Both juvenile and adult females also use visual displays in response to song, especially rapid flicks of the wing, termed wing strokes (Gros-Louis et al. 2003). In other work with the birds studied here, D. J. White (unpubl. data) found that the adult and juvenile females wing stroked differently to primitive vs. more mature song. Females also appear to observe each other's wing strokes and appear to compete by approaching males and displacing each other (Gros-Louis et al. 2003). Females who approach males stimulate counter-singing, which, in turn, appears to affect egg production (King et al. 2003b). Ongoing work also suggests that juvenile and adult females differ in their levels of interactivity. Juvenile females are more interactive with each other and with juvenile males, compared with adult females who segregate themselves from young males. When adult females are introduced into juvenile flocks, they significantly increase their affiliations with young males, suggesting they see juvenile females as possible competitors. In the wild, flocks vary in composition with some containing

only juveniles and some containing adults as well. Young females then may learn to attend to some attributes of males by eavesdropping on adult female–juvenile male interactions.

In sum, females' reactions to song and to males affect patterns of affiliation and individual behaviors well before the breeding season. Group dynamics, such as changing patterns of flock assortment cannot occur when females are housed in sound-attenuating chambers with one or two females and one or no males. In nature, during the fall and winter, juveniles join large flocks. But within these settings, we have observed that small groups of males and females (under 20 birds) can be found in association across time (King & West 1988). Thus, opportunities for interactions like those studied here map roughly onto natural contexts. The chamber setting precludes the formation and maintenance of communication networks as described by McGregor & Peake (2000) and Sturdy et al. (2001). The rapid changes in group patterns reveal the sensitivity of network members to one another; the diversity of behavior within the network indicates the possibilities for direct and indirect learning. At present, we do not know the precise parameters of individual vs. flock housing that affect plasticity. The ambient environment in outdoor aviaries is likely to be more arousing as a result of shifts in weather, challenges from aerial predators, and changes in the acoustic environment created by resident and migrating birds. This state of arousal is likely much higher than in the stable state of chamber housing, and thus more likely to lead to behavioral transitions.

The data collected here document changes in song preferences. How these preferences affect actual mate choice is yet to be understood. Such studies are essential in that song structure represents only one means of male assessment. Studies in canaries and sparrows indicate that reproductive activity, such as nesting or egg laying may be stimulated by other cues (e.g. Depraz et al. 2000; MacDougall-Shackleton et al. 2001). In studies of flocks of cowbirds, we have found that female cowbirds' egg production and the hatching success of her eggs are best predicted by male counter-singing, not potency of particular song types. Female-directed singing by males is also an important metric (D. J. White, unpubl. data). Both of these behaviors involve song embedded in a social context. Thus, the next step is to see how plasticity in song preference relates to mate choice and reproductive success.

The data presented here show that song preferences are malleable traits, which might seem to be a

contradiction in terms as 'traits' typically refers to behavioral or morphologic properties that are relatively fixed within individuals across their lifetimes (Stamps 2003). In cowbirds, and probably other songbirds, females' preferences for songs do not appear to be stable over time and context. Perhaps, they are best not classed as traits at all, or qualified as state-dependent traits, where knowledge of context is required to answer functional and evolutionary questions. What is stable over lifetimes and individuals is the certainty that the ontogeny of preferences is situated in a social ecology. Similarities and differences in the traits of a species' ecology that must be identified to understand how selection operates on the development and evolution of communicative capacities (West et al. 2003a).

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