



## A method to measure the development of song preferences in female cowbirds, *Molothrus ater*

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Male song development has been studied in detail across a range of different songbird species. Very little, however, is known about the development of preferences for songs in females. Here we describe a method to study female preferences outside the breeding season by documenting female responses to playbacks of songs of different developmental stages and of varying quality. We compared 'wingstroke' responses of juvenile and adult females in four groups across autumn, winter and spring. We found that adult, but not juvenile, females showed preferences for variable song that was developmentally advanced. We assessed female preferences for the songs in the breeding season by playing back songs in sound-attenuation chambers and recording the number of copulation solicitation displays females gave to each song. We detected a strong relationship between wingstroke patterns in spring and song preferences, but we did not detect this relationship in the autumn or winter. Female preferences, like male songs, appear to show seasonal and developmental plasticity.

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In studies of communication, receivers have been the focus of less research than producers. In songbirds, for example, there is an extensive literature on how song is learned, how it develops, and how it varies across populations (Searcy et al. 1985; Marler & Nelson 1993; Catchpole & Slater 1995; Kroodsma & Miller 1996). But production is only half of a communicative system. Far less is known about the other half of the system, female preferences. Since male song and female preferences in many species are evolutionarily linked, they may follow similar processes: being modifiable by experience, varying during development and differing across populations. Here we describe a means to study the development of female preferences in a social songbird, the brown-headed cowbird, *Molothrus ater*.

Few studies have tracked female song preferences in detail across development. One reason for this is that song preferences have typically been considered to be innate and not open to modification by experience. Since song preferences serve to assess male quality or to avoid mating with heterospecifics (Bateson 1983; Andersson 1994;

Gibson & Langen 1996; Jennions & Petrie 1997), they have been considered too important to be modifiable. Modifiable preferences could cause females to learn about wrong characteristics of males, resulting in nonadaptive mate choice decisions.

A literature has emerged recently, however, that has supported the idea that female preferences in many circumstances are indeed modifiable by experience (Riebel 2003; Coleman et al. 2004; White 2004). For example, cross-fostering experiments in zebra finches, *Taeniopygia guttata castanotis*, have shown that early social experiences can influence sexual preferences (e.g. Bischof & Clayton 1991). In addition, recent work has found that zebra finch females require exposure to male song early in life to develop normal preferences for male song (Riebel 2000, 2003; Laulay et al. 2004). Since mate preferences can play a role in assortative mating and differential reproductive success, modifiable preferences could provide the potential for genetic linkages for song and song preference to be created and broken rapidly. Sexual selection may therefore be acting on heretofore unstudied aspects of social development in addition to the immediate context of mate selection.

Most studies that have investigated the experiential effects on mate preferences have relied upon outcome measures of the developed preference. There are few

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measures that can be taken as the preference develops (but see Clayton's 1988 discrimination task). Unlike song, which can be recorded and quantified at any time during the life of a male, female preferences are difficult to measure at any time. Usually, it is only possible to study female preferences during the breeding season when copulation solicitation displays can be elicited using playbacks of male song (King & West 1977; Searcy & Marler 1981). Outside the breeding season, females rarely show overt behavioural responses to males, leaving very little available to measure when attempting to investigate the development of their preferences.

In cowbirds, females show preferences for male song and flight whistles, and these preferences can be quantified during the breeding season using the copulation solicitation display (King & West 1977; O'Loughlen & Rothstein 2003, 2004). We have recently found that social experience during the nonbreeding season affects female cowbirds' preference for male song (King et al. 2003). Females housed in large groups (12–13 females) without males during the nonbreeding season developed different preferences for male song variants than did females housed in large groups with males. Females housed singly or in small groups (two females) did not show the same malleability of preferences. Thus, social experience during the nonbreeding season apparently modified the females' innate preferences for song.

There is also evidence that female cowbirds signal their song preferences during interactions with males using extremely rapid wing movements, called 'wingstrokes' (West & King 1988). Unlike the copulation solicitation display, females give wingstrokes in response to song throughout the year. Males preferentially sing song variants that elicit wingstrokes over those that do not, and wingstroking rate is positively correlated with the rate of development of stereotyped song (King et al. 2005). West & King (1988) compared female responses to songs before and during the breeding season, and found that songs that elicited wingstrokes before the breeding season also elicited more copulatory responses from females during the breeding season. West & King suggested that the wingstroke serves as a means of nonverbal communication between females and males, allowing naïve males to develop effective, locally appropriate courtship song. The wingstroke can only function to shape song, however, if females respond differentially to songs that differ in stages of development. To date, however, little is known about how patterns of wingstroking vary with song development.

Playback of male song can elicit wingstrokes in female cowbirds; males need not be present. Females also observe and react to wingstrokes produced by other females (Gros-Louis et al. 2003). Therefore, the wingstroke can act as a signal to other females about song quality and thus be a candidate for a behavioural mechanism that affects preference development in social settings. In addition, because females give wingstrokes in response to song throughout the year, the wingstroke provides experimenters with a means to study female preferences outside the breeding season. Before we can determine whether females use wingstrokes to communicate about song with each other, we need to understand what information

is present in the wingstroke response. For example, does it reflect female preferences for song? If so, does this relationship hold throughout the year? Do juvenile females differ from experienced females in their wingstroking patterns?

Here we use a playback procedure to study wingstroking across a year in both adult and juvenile females. We played females a series of songs varying along naturally occurring dimensions; all songs were from males in the local population and varied in their level of development and quality. By studying the differences in the two age classes of females, we sought to determine whether age or experience influenced the timing and pattern of wingstroking, and by studying females across a year, we sought to determine whether preferences changed seasonally. In addition, we attempted to determine whether there were social effects on wingstroking, and finally, if there were effects of the presence or absence of males on wingstroke patterns. We housed females in four separate mixed-age groups and recorded their responses to song playbacks. We then reviewed tapes frame by frame to determine whether or not the females responded to song with wingstrokes. In the breeding season, we housed the females in sound-attenuation chambers and determined each female's preferences for a set of the playback songs by measuring each song's ability to elicit copulatory solicitation displays ('song potency'). We could then determine how wingstrokes produced before the breeding season related to the females' breeding-season song preferences.

## METHODS

### Subjects

Subjects were 12 juvenile and 12 adult female brown-headed cowbirds. Juvenile birds were wild-caught in the summer of 2002 in Monroe County, Indiana, U.S.A. The adult birds were wild-caught in the summers of 2000 and 2001 from the same location. Adults had served previously in a playback study in the 2002 breeding season. All females were housed in large, outdoor, mixed-sex aviaries from capture until the summer immediately before the experiment, then housed in single-sex groups in outdoor aviaries until the experiment began in the autumn of 2002. We provided all birds with a mix of white and red millet, canary seed, plus a modified Bronx zoo diet for omnivorous birds and vitamin-treated water, all refreshed daily. Birds wore individually distinct patterns of coloured leg bands to permit individual identification.

### Apparatus

In October 2002, we randomly assigned four groups of three juvenile and three adult females to four outdoor aviaries. Aviaries were  $2.4 \times 6.1 \times 2.3$  m, made of wood and hardware cloth, and attached to corners of a laboratory building. Each aviary was either 7.3 m away from any other and separated by two plywood walls, or separated by the building itself. We observed and recorded birds from within the building. During the winter, when

no playbacks were occurring (21 December–6 April), we combined birds into two flocks.

## Procedure

We conducted a series of rounds of playbacks during the autumn, winter and spring of 2002/2003 designed to provide females with experience hearing a variety of different types of male song (see Table 1). During each round, females heard a set of 10–18 song stimuli (see below), each repeated three to five times across 4–8 days. We placed one AIWA LCX-350 speaker (AIWA Inc., Mahwah, New Jersey, U.S.A.) in one corner of each aviary, positioned such that they broadcast away from the other aviaries. Speakers were attached to a NAD 512 compact disc player (NAD Electronics, Sharon, Massachusetts, U.S.A.) and songs were broadcast at a level approximately simulating the amplitude of a male's song sung within the aviary.

To reduce the possibility that females would habituate to our playback procedure, we played no more than 10 songs per day to females for no more than 4 days in a row. On each playback day, for each group, we played the first five song stimuli, three to five times each, separated by approximately 5 s, then we waited approximately 30 min before playing the second set of five. We randomized the order of songs played within each session. We recorded females' responses to songs on a Canon GL1 MiniDV camcorder (Canon Inc., New York, U.S.A.). If birds flew out of camera view during the playback, we paused the playback until the birds returned to within camera range. Two observers independently reviewed the tapes frame by frame, and for each female, scored whether the female wingstroked in response to a song. We used the published criteria for a wingstroke (West & King 1988). As such, a wing movement had to occur contingent with the song. Furthermore, a wingstroke was recorded only when there was interobserver agreement. At the end of each round for each female, we created a wingstroke score for each song stimulus based on the number of wingstrokes given to the song divided by the number of times that that song stimulus was played during which the female was in view of the camera during the playback.

Songs used for playback were all recorded using Sennheiser RF condenser microphones (Sennheiser Electronic

Corporation, Old Lyme, Connecticut, U.S.A.) less than 0.3 m away from captive vocalizing males. We recorded vocalizations with a SONY TCD-D10 PRO II DAT recorder (Sony), sampled at 48 kHz. We then digitally converted songs to 44.1-kHz files. We selected songs for playback based on recording quality and similarity in amplitude and length (the duration of each song was approximately 1.2 s). 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 Hz) filter to remove low-frequency noise below the range of cowbird song.

## Song stimuli

We designed the playback procedure to test female responses to two characteristics of male song: song development and song potency. To investigate female preferences for developing song, we played songs of different developmental stages. These songs were recorded from nine males housed in sound-attenuating chambers through their first year. The males were housed in isolation from other cowbirds, but had two canaries as companions. Although the males did copy some of the canary vocalizations, they all developed species-typical vocalizations in these conditions (King et al. 1996). We did not use any of the copied canary vocalizations for our tests. The quality of each song that we selected for playback was within the lower end of the range typical of cowbird song (King et al. 1996). From each of these nine males, we used playback songs from three phases of song development: plastic song, plastic song with note clusters and formatted song (Fig. 1). Plastic song represents some of the earliest vocalizations that males produce; these songs are extremely variable and include notes and whistles with no species-typical format. Plastic songs with note clusters are also variable songs but contain clusters of notes that are also present in mature song. For any vocalization to be regarded as a note cluster, we had to be able to detect at least two notes ranging from 300 Hz to 1400 Hz (low-frequency notes) and two notes ranging from 1400 Hz to 5000 Hz (high-frequency notes). Low- and high-frequency notes had to alternate within the note cluster and all four notes had to be represented

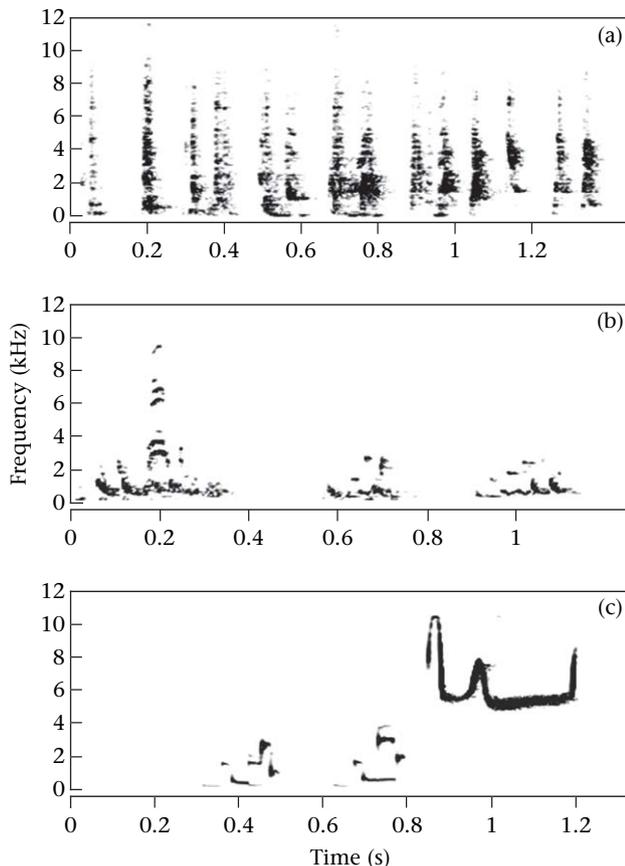
**Table 1.** Manipulations for the experiment

	Date	Number of songs	Songs/day	Song stimuli
<b>Aviary playback</b>				
Round 1	11–14 Nov 2002	10	10	2002 song set
Round 2	26 Nov–4 Dec 2002	18	10	Plastic vs P+NC
Round 3	16–20 Dec 2002	18	10	P+NC vs Format
Round 4	8–12 Apr 2003	10	10	2002 song set
<b>Regrouping*</b>	14 Apr 2003			
<b>Regrouping†</b>	28 Apr 2003			
Round 5	29 Apr–2 May 2003	10	10	2002 song set
<b>Chamber playback</b>	15 May 2003	10	6	2002 song set

Plastic = Plastic song; P+NC = plastic song with note clusters; Format = formatted song.

\*Females were randomly assigned to two groups: one group with males and one group without males.

†Females were divided into four groups.



**Figure 1.** Sound spectrographs of exemplars of playback songs. (a) Plastic song without note clusters, (b) plastic song with note clusters, (c) formatted song.

within approximately 200 ms. Formatted songs have a stereotyped structure with two note clusters and a terminal whistle. We selected nine plastic, nine formatted and 18 plastic songs with note clusters from each of the nine males.

We also investigated whether females would rank formatted songs with wingstrokes during the autumn, winter and spring the way they rank the songs as measured by their copulation solicitation displays during the breeding season ('song potency'). To do so, we created a playback set of 10 songs recorded from 10 adult males wild-caught in Monroe County, Indiana. We played this set of songs (referred to as the 2002 song set) to females in the aviaries in autumn, late winter/early spring (winter), mid-spring (spring) immediately before the breeding season, and then finally in the playback chambers during the breeding season.

#### Male exposure

Past work (King et al. 2003) had shown that the song preferences of females were affected when females were housed in groups without males. We therefore gave half the females experience with males to determine whether male exposure (or lack thereof) would influence patterns of wingstroking. On 14 April 2003, we randomly assigned females from the four initial groups to two aviaries. In one

group, we housed four adult males for 2 weeks. These males were wild-caught adult birds that had been living in large, outdoor aviaries in mixed-sex groups. On 28 April we removed the males, divided the females into four groups and played back the 2002 song set again. For more details of male exposure see West et al. (in press).

#### Chamber playbacks

During the breeding season, we placed all females in sound-attenuating chambers and assessed their song preferences by playing back the 10 songs from the 2002 song set and measuring each song's effectiveness at eliciting copulation solicitation displays from females. Chambers were 1.3 m<sup>3</sup> and contained perches, food and water. We played six songs per day to females. Each song playback was separated by 90 min. We alternated the order in which we played songs with each song presented six times over the course of the experiment. We played songs on a Denon DN-C680 compact disc player (Denon Electronics, Parsippany, New Jersey, U.S.A.) and a Crown D75 power amplifier (Crown Audio Inc., Elk Hart, Indiana, U.S.A.) through JBL 2105 speakers (JBL Audio, Northridge, California, U.S.A.) placed in each sound-attenuating chamber. We scored a positive response if the female adopted a copulatory posture within 1 s from the onset of the song. To calculate song potencies, we computed mean number of responses per female for each song and averaged over all females in each condition. During the course of the experiment, two adult females and one juvenile female died. Their data were excluded from analyses. During the chamber playback, we recorded copulation solicitation displays from seven of the juvenile females and six of the adult females.

We used nonparametric tests to compare patterns of wingstroking within and between females because of heterogeneity of variance in females' responses. When results were nonsignificant, we conducted power analyses using G\*power software (<http://www.psychology.uni-duesseldorf.de/aap/projects/gpower/>). Following Nelson & Soha (2004), we multiplied obtained power levels by 0.95 to account for the lower power typical of nonparametric tests (Higgins 2004).

## RESULTS

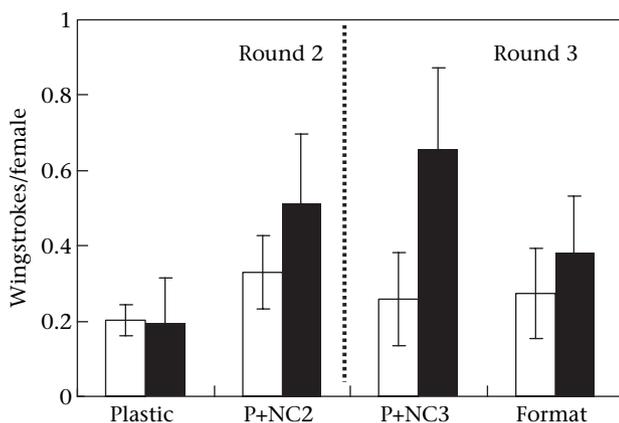
Overall, we recorded a total of 182 wingstrokes. All females produced wingstrokes during the experiment. There was large variability among females in wingstroking, ranging from one female who wingstroked to 16.14% of songs across all playbacks to one female who only produced wingstrokes to 0.36% of songs across all playbacks. There were no significant differences in the number of wingstrokes produced by adults compared to juveniles (Mann-Whitney  $U$  test:  $U = 53.5$ ,  $N_1 = 10$ ,  $N_2 = 11$ ,  $P > 0.90$ ). Rates of wingstroking did not change across the rounds of the experiment (Wilcoxon matched-pairs signed-ranks test:  $T = 72$ ,  $N = 21$ ,  $P > 0.55$ ). In round 1, females gave a mean  $\pm$  SE of  $0.27 \pm 0.11$  wingstrokes per song presentation. By round 5, females gave  $0.30 \pm 0.05$  wingstrokes per song presentation. There were no significant differences

in the overall number of wingstrokes produced across groups (Kruskal–Wallis test:  $\chi^2_3 = 3.73$ ,  $P > 0.25$ ).

We conducted a Kendall's analysis of concordance on rounds 1 and 4 (combined because there were too few responses in each round alone to allow for meaningful comparisons) and on round 5 to determine whether females in each group showed similar patterns of wingstroking to the song types within each playback round. If concordance in female wingstroking patterns was higher within groups than between groups, this would suggest that females were being influenced by the behaviour of the other females within their group. This was not the case. Concordance of relative wingstroking frequencies of females within groups was not significantly higher than that between groups (Kendall's coefficient of concordance for the four groups: rounds 1 and 4: group 1:  $W = 0.155$ ,  $\chi^2_4 = 6.18$ ,  $P > 0.19$ ; group 2:  $W = 0.09$ ,  $\chi^2_4 = 3.5$ ,  $P > 0.48$ ; group 3:  $W = 0.16$ ,  $\chi^2_4 = 6.53$ ,  $P > 0.16$ ; group 4:  $W = 0.17$ ,  $\chi^2_5 = 8.30$ ,  $P > 0.14$ ; overall concordance:  $W = 0.205$ ,  $\chi^2_{20} = 40.99$ ,  $P < 0.01$ ; round 5: group 1:  $W = 0.1$ ,  $\chi^2_4 = 4.0$ ,  $P > 0.41$ ; group 2:  $W = 0.06$ ,  $\chi^2_4 = 2.34$ ,  $P > 0.67$ ; group 3:  $W = 0.16$ ,  $\chi^2_4 = 6.22$ ,  $P > 0.18$ ; group 4:  $W = 0.16$ ,  $\chi^2_5 = 8.02$ ,  $P > 0.155$ ; overall concordance:  $W = 0.141$ ,  $\chi^2_{20} = 28.26$ ,  $P > 0.10$ ). Therefore, we combined results across groups for subsequent analyses, although doing so increased the risk of pseudoreplication.

### Song development (rounds 2 and 3)

Adult and juvenile females differed in their wingstroking responses to male songs of different developmental stages (Fig. 2). In round 2, adult females produced significantly more wingstrokes to plastic song with note clusters than they did to plastic song without note clusters (Wilcoxon matched-pairs signed-ranks test:  $T = 1$ ,  $N = 10$ ,  $P < 0.02$ ). In round 3, they also responded more to plastic song with note clusters than they did to formatted song ( $T = 4$ ,  $N = 10$ ,  $P < 0.05$ ). Juveniles did not make these



**Figure 2.** Wingstrokes produced per female (corrected for the number of playbacks in which the female was viewable, see Methods) for rounds 2 and 3. □: juvenile females; ■: adult females. Plastic = playback of plastic songs; P+NC2 = plastic song with note clusters played back in round 2; P+NC3 = plastic song with note clusters played back in round 3. Format = playback of formatted song.

distinctions; they did not wingstroke to songs from one category more than they did to the other in either round 2 ( $T = 9$ ,  $N = 11$ ,  $P > 0.06$ ) or round 3 ( $T = 9$ ,  $N = 11$ ,  $P > 0.9$ ). However, owing to the large variance in female responses in round 2, there were no significant differences between juvenile and adult females in the number of wingstrokes given to plastic song with or without note clusters (Mann–Whitney  $U$  test: plastic song without note clusters:  $U = 39.5$ ,  $N_1 = 11$ ,  $N_2 = 10$ ,  $P > 0.28$ ; plastic song with note clusters:  $U = 55$ ,  $N_1 = 11$ ,  $N_2 = 10$ ,  $P > 0.97$ ). In contrast, in round 3, adult females wingstroked significantly more to plastic song with note clusters than did juveniles ( $U = 27$ ,  $N_1 = 11$ ,  $N_2 = 10$ ,  $P < 0.05$ ). There was no significant difference by age class in the number of wingstrokes given to formatted song ( $U = 48$ ,  $N_1 = 11$ ,  $N_2 = 10$ ,  $P > 0.65$ ).

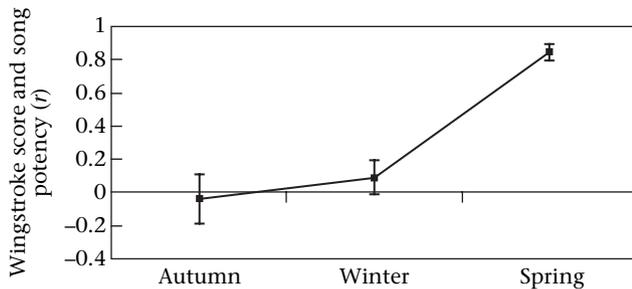
To examine whether the lack of an effect in the juvenile females resulted from a lack of statistical power, we calculated the power of the test using an estimate of effect size based on the performance of the adult females. Effect size for the adult females was 0.90 in round 2 and 0.73 in round 3. Thus, assuming similar effect sizes for juveniles, the power to detect a significant difference was 0.73 in round 2 and 0.56 in round 3.

### Song potency (rounds 1 and 4)

There was no correlation between females' wingstroking scores during the autumn and winter playbacks of the 2002 song set and their subsequent patterns of copulatory solicitation displays for the 10 song types in the song set (see Fig. 3). Only five of the females that responded during the breeding-season chamber playbacks (and thus, that provided us with a measure of their breeding-season preferences) also responded with wingstrokes during the aviary playback in the autumn, and only one of these females was an adult. Thus, we did not have a useful comparison of wingstroke responses of adult and juvenile females in autumn. In the winter, we had sufficient data for comparison of wingstroke scores and subsequent copulatory solicitation responses for each age class (three juveniles, five adults). There was, however, no correlation between wingstroke score in winter and subsequent breeding-season copulatory postures for females of either age class (Spearman rank correlation coefficient: juveniles:  $r_s = -0.05$ ,  $P > 0.46$ ; adults:  $r_s = 0.17$ ,  $P > 0.28$ ; Fig. 3).

### Song potency (round 5)

Unlike in the autumn and winter, the wingstroke proved to be a strong indicator of female song preference in spring. The correlation between wingstroke score and breeding-season copulatory postures was significant for all juvenile females for which we had data (average Spearman rank correlation coefficient:  $r_s = 0.95$ ,  $N = 7$  females,  $N = 10$  songs,  $P < 0.01$ ) and for five of the six adult females (average  $r_s = 0.73$ ,  $P < 0.01$ ). Mean correlation coefficients did not differ by age class, although surprisingly, correlations for juveniles tended to be higher than those for adults (Mann–Whitney  $U$  test:  $U = 7.5$ ,  $N_1 = 7$ ,  $N_2 = 6$ ,  $P = 0.051$ ). Experience with live males did not influence the relationship between wingstroke score and song potency (females exposed to males:  $r_s = 0.83$ ;



**Figure 3.** Correlation between wingstroke score and breeding-season copulatory posture for the 10 playback songs in the 2002 song set in autumn (round 1,  $N = 5$  females), winter (round 4,  $N = 8$  females) and spring (round 5,  $N = 12$  females).

females not exposed to males:  $r_s = 0.86$ ; Mann–Whitney  $U$  test:  $U = 18$ ,  $N_1 = 7$ ,  $N_2 = 6$ ,  $P > 0.73$ ).

It is difficult to compare the correlations of wingstroke score and song potency from autumn, winter and spring because different numbers of females responded during each playback round. We therefore conducted a within-subjects analysis to compare the mean correlation between wingstroke score and song potency for each female before the spring (averaging correlations for the autumn and winter rounds if the female responded in both) with that in the spring. This produced a sample size of 10 females who responded in autumn or winter, and spring and during the breeding season. Correlations between wingstroking patterns and song potency were significantly higher in spring than in autumn or winter (Wilcoxon matched-pairs signed-ranks test:  $T = 0$ ,  $N = 10$ ,  $P < 0.005$ ).

We calculated the power to detect significant correlations between wingstroking score and song potency within each individual female for the autumn and winter samples. As an estimate for effect size, we used the average effect size found in round 5. Although the average effect size in round 5 was large ( $r_s = 0.84$ ), we think that its use is appropriate given that the wingstroke would only be an important signal if it were a strong predictor of a female's subsequent preference. Given this effect size, the power to detect a correlation between wingstroking score and song potency for a female in autumn or winter would be 0.93. That is, if a female responded to a song with wingstrokes in round 1 or 4, and subsequently responded to the same song with copulatory solicitation displays during the chamber playbacks, there was a strong likelihood that we would detect a correlation between the two measures if one existed.

## DISCUSSION

There were two main findings in this experiment. First, female wingstroking differed based on the developmental stage of played back song and the age class of females. Second, we were able to detect a strong relationship between female wingstroking patterns and their song preferences (or song potency, as measured by copulatory postures) in the spring, but not in the autumn or winter.

## Song Development

Wingstroking responses of adult females were sensitive to the developmental stage of songs. Plastic songs with note clusters elicited more wingstrokes from females than did either plastic songs without note clusters or formatted songs. The patterns of wingstroking to the songs of different developmental stages suggest that the note clusters are the critical components of song that trigger the wingstroke (King & West 1983; but see O'Loughlen & Rothstein 2004).

Evidence suggests that female cowbirds prefer stereotyped song over variable song and common song types over unique ones (O'Loughlen & Rothstein 2003). Why then did adult females in our study produce more wingstrokes in response to more variable song during the nonbreeding season? Although female preferences for variable elements in male courtship displays have been documented in many taxa (Mountjoy & Lemon 1997; Endler & Basolo 1998; Gentner & Hulse 2000; Calkins & Burley 2003), we speculate that adult females in our study may have habituated to the pattern of song signals in stereotyped songs. Plastic songs are more variable than stereotyped songs and the note clusters within them differ from those of typical formatted song. Outside the breeding season, when females are probably less attentive to song, the variability of plastic song may increase the likelihood that females notice these songs and, thus, may have reduced females' wingstroke habituation to these songs in our study.

We did not detect such a relationship between wingstroking and song development in juvenile females. Juvenile females responded with wingstrokes at similar rates to all male song types (Fig. 2). Perhaps, as Riebel (2003) suggested, experience listening to song early in development serves a perceptual fine-tuning role and thus the discriminative abilities of inexperienced juvenile females may not be as pronounced as those of adult females.

Alternatively, because of the variability in females' responses in rounds 2 and 3 of our experiment, the power to detect significant effects may have been too low. Thus, juvenile females may have wingstroked differently to songs of different developmental stages, but the effect size was too small to detect this difference. From the perspective of a juvenile male attempting to gain information about song development, wingstroking responses of juvenile females may, at best, provide little useful information.

In contrast, because adult females differed significantly in their wingstroke responses to songs of different developmental stages, their wingstrokes have the potential to serve a shaping role for male song development (sensu West & King 1988). Thus, wingstroking responses of adult females may provide more valuable information to a male during song development than those of juvenile females. These results are concordant with other evidence from aviary studies revealing that juvenile females show low levels of consistency in their final song preferences (King et al. 2003). In addition, juvenile males housed with adult females develop more effective courtship song than juvenile males housed with juvenile females (D. J. White, unpublished observations), and juvenile males that sing

more to adult females than to juvenile females are also more successful in mating (Smith et al. 2002). While the wingstroke may provide the juvenile male with information about song development, results of our experiment suggest that the wingstroke may not provide information about the male's song quality until just before the breeding season.

### Song Quality

That wingstrokes only correlated with the females' preferences in the spring and not in the autumn and winter suggests that female song preferences may vary across the year, showing stages of plasticity similar to those of male song. In the autumn and winter, females may show no distinct preferences for male song variants, but in the spring, their preferences may be reset quickly.

Alternatively, female song preferences may remain intact throughout the year, but wingstrokes do not relate to song quality at any time outside the spring. Why this relationship would change across the year is unclear. It may be that interactions with males keep female song preferences intact throughout the year and it is only when males are absent that female song preferences vary (e.g. King et al. 2003). Furthermore, females' wingstroking patterns may also differ depending on whether males are present or absent. However, in our study, wingstroking and song preferences of females formerly housed with and without males did not differ following the introduction of males in the spring.

Another possibility is that the relationship between wingstroking and song potency may exist throughout the year, but the effect size in our study was too small to detect this effect outside of the spring (because fewer females responded in the autumn and winter). Therefore, more study on preferences in autumn and winter will be needed to determine how females' preferences may change seasonally. For our purposes, we were only interested in determining whether there was a large effect between wingstroking and song potency. Again, from the point of view of a male developing his song, the information obtained from the wingstroke would only be valuable if it accurately and reliably reflects underlying female preferences.

The wingstroke is a low-frequency signal, occurring in response to only 1–3% of songs (West & King 1988; King et al. 2005). While this seems like a rare behaviour per song, given that a male can sing thousands of songs to a female in a day, a substantial number of wingstrokes may be produced in a day. Furthermore, a rare signal does not suggest an unimportant signal. A copulatory posture is also rare, but it is certainly not unimportant. In this experiment, the low frequency of wingstroking and our concern for habituation resulted in a small data set, but, owing to the strength of the effects, the experiment maintained enough power to produce at least some statistically significant results.

This work is a preliminary investigation into how female song preferences may vary and be shaped. It remains to be seen whether female wingstroking, as measured in our experiment, will be heuristic in studying the development of female preferences. Although females in our study

appeared to be less interested in the songs as playbacks continued, suggesting that habituation to playback may have occurred, there was no overall change in the rates of wingstroking across the study. In the future, we will fine-tune our playback procedure to examine the level of exposure to song stimuli that results in habituation.

We created four social conditions to examine whether social experience affects female wingstroking patterns, but we found no group-level differences. Whether female wingstrokes are truly unaffected by their social group, or our preparation was not sensitive enough to detect such influences can only be determined with more information on wingstroking patterns within females and across groups. Future studies will be designed with juveniles housed either with or without adult females to study how different learning environments might influence wingstroking patterns and development of song preference.

At this point, the experiment has revealed that the development of female song preference has the potential to be measured and studied in ways comparable to that of male song development. Given that, at least in the spring, the wingstroke serves as an honest indicator of female preference, it may also serve as a source of public information for young and/or inexperienced females to learn how to choose quality mates (Nordell & Valone 1998; Gros-Louis et al. 2003).

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