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**Female Cowbird Song Perception:  
Evidence for Different Developmental Programs  
within the same Subspecies**

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*With 3 figures*

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**Abstract**

Female *Molothrus ater* from Oklahoma at the subspecies border with *M.a. obscurus* were tested for their discrimination of songs from their native area and from Austin, Texas. This 400-km distance is characterized by a structural shift in the male cowbird's inclusion of the mid-song element, a structure found in the songs of Austin and the more southern *M.a. obscurus* populations but not in the songs of Oklahoma or the more northern and eastern *M.a. ater* populations. Females captured in Oklahoma showed a strong preference for their native song variants. In contrast, captive females from Oklahoma who were housed with Austin males showed no preference, responding equally to Oklahoma and Austin song. The songs of the Austin males housed with the Oklahoma females did not differ acoustically from those of wild Austin males. Thus, a different developmental pattern may operate in this region than in eastern populations of *M.a. ater*: here, the female may be more open to social influence.

**Introduction**

*Molothrus ater ater* male cowbirds from North Carolina (NC) require postnatal experience to learn to sing geographically appropriate song, with some of the experience apparently coming from the non-singing female who communicates her native preferences by social means (KING & WEST 1983a; WEST & KING 1985). NC female cowbirds, however, do not appear to require any acoustic or social experience in order to discriminate between the songs of *M.a. ater* and *M.a. obscurus*, the southwestern subspecies. Experimental attempts to modify their preferences have produced no data to suggest that NC females are vulnerable to postnatal influence (KING & WEST 1983b).

Here we studied the developmental characteristics of song discrimination in females from an *M.a. ater* population in Oklahoma which is near the morphologically described subspecies border with *M.a. obscurus* (WOLFE 1956; ROTHSTEIN 1978). The previous research on *M.a. ater* females from NC had involved the discrimination of *M.a. obscurus* songs from a population 1500 km away, a comparison not normally encountered by NC females. Intraspecific populational differences in the ontogeny of song production by males have been found in other species (e.g., KROODSMA & CANADY 1985). Relatively fewer studies exist on female song perception in general and none to our knowledge have probed for populational differences in the developmental properties of such intraspecific responsiveness.

We asked three questions. First, would *M.a. ater* females from Oklahoma prefer their own population's songs in comparison to *M.a. obscurus* songs from Austin, Texas, 400 km to the south (Study 1)? Second, would Oklahoma females retain their preference after developmental exposure to Austin males during their hatching year (Study 2)? Third, would Oklahoma females exposed to Austin males show particular preferences for their Austin companions' songs and/or would Austin males be influenced by social stimulation from Oklahoma female companions (Study 3)? Acoustic analyses of Oklahoma and Austin song accompanied all three investigations to study the auditory basis of any female preferences found and of any song modifications made by the males.

## General Methods

### Subjects

14 *M.a. ater* females served as subjects, 7 in Study 1 and 7 in Studies 2 and 3. All had been wild-caught in Marshall County at the University of Oklahoma Biological Station during the months of July and August. The Study 1 females consisted of two adults and five juveniles. They were placed in the sound attenuation chambers in mid-August in pairs or triads with other females and males from Oklahoma. The males were removed after two months. The females for Exp. 2 and 3, all juveniles, were housed immediately after capture with *M.a. obscurus* males from Austin, Texas. In the laboratory, pairs or triads of Oklahoma females were each housed with one Austin male. The four Austin male companions were adults that had been collected in July 1984 at Walter E. Long Lake Park, 8 miles east of the Brackenridge Field Station of the University of Texas at Austin. These populations were chosen because they straddled the subspecies border.

All birds were maintained on the normal photoperiod for 35° latitude throughout the winter. In Study 1, the photoperiods were accelerated to 14 h of light in February and the birds were tested from early March through April. In Studies 2 and 3, the light was increased to 14 h in May and the females were tested in May and June. See KING & WEST 1977 for details of housing and care.

### Procedures

*Recording procedures.* All playback songs were recorded during the breeding season, i.e., during the period of active courtship and egg-laying. Songs for the Test 1 playback series were obtained from 6 males recorded in Travis County, Texas in May and June 1982 and from 6 in Marshall County, Oklahoma during the month of June 1982, at the sites where the laboratory birds were caught. To obtain the recordings, males were captured in a 2 × 1.1 × 2 m decoy trap and recorded inside it with a Sennheiser electret cardioid microphone and a Uher 4200 recorder. All songs used for playback were recorded when the males were within 1 m of the microphone.

The songs of the Austin male companions were obtained in early May. The males were removed from the chambers two weeks prior to recording and placed in an aviary together for two weeks to expose them to stimulation from each other. They were then placed individually in chambers with Oklahoma females (a different group than tested here) for recording. See KING & WEST 1983 b for details of the laboratory recording procedure. The signal to noise ratios of the Austin companion songs were reduced by 10 dB by adding white noise to make them comparable to the Oklahoma field recordings.

Study 1. 16 playback songs were used: 8 from the Austin and Oklahoma sites representing a total of 12 males, one song each from four males, and two songs each from two males at each of the two sites. Playback recordings were selected on the basis of recording quality. All playback songs had a signal to noise ratio of  $40 \pm 2$  dB measured peak to peak on an oscilloscope. All songs were played back on a Uher 4000 recorder through JBL 2105 speakers located in each chamber.

Study 2. The same playback tape used in Study 1 was used but was played back using an Otari 5050 B recorder, a Urei 537 equalizer, and Crown D 60 amplifier.

Study 3. The females from Study 2 heard a new playback tape composed of the same 8 field songs from Oklahoma used in the previous test and 8 new Austin songs, two each from the Austin male companions housed with Oklahoma females.

Playback levels were measured with a Bruel and Kjaer 2209 sound pressure meter. The same sound pressure level (SPL) was used for all songs in the three studies: At .6 m from the speaker, on axis, the impulse SPL was  $82 \pm 1.5$  dB. See KING & WEST 1983 a for details.

Songs were selected for playback to sample the different structural categories represented in the two populations. In Oklahoma, we tested the only two variants found after two seasons of field recording in which 2000 songs were examined (EASTZER, unpubl. data). The two variants were songs with single (1 IPU) or double (2 IPU) inter-phrase units (Fig. 1). In Austin, we tested 2 IPU song and songs with mid-song elements (MSE), the most common variants found in 1000 field songs. Songs with MSE's do not occur in Oklahoma and songs with a single IPU and no MSE are uncommon in Austin (EASTZER, unpubl. data). Previous studies had confirmed the functional significance of the IPU for song potency in both subspecies and of the MSE for the discrimination of *M.a. ater* versus *M.a. obscurus* song (WEST et al. 1979; KING et al. 1980; KING & WEST 1983 c).

*Testing procedure.* The females were tested in their sound attenuation chambers. There were 6 playbacks each day, separated in time by approximately 90 min, over a 6-week period. On a given day, the female alternately heard three Austin and three Oklahoma songs. Only one song was presented in each playback trial. The order of songs presented each day varied but was the same for all

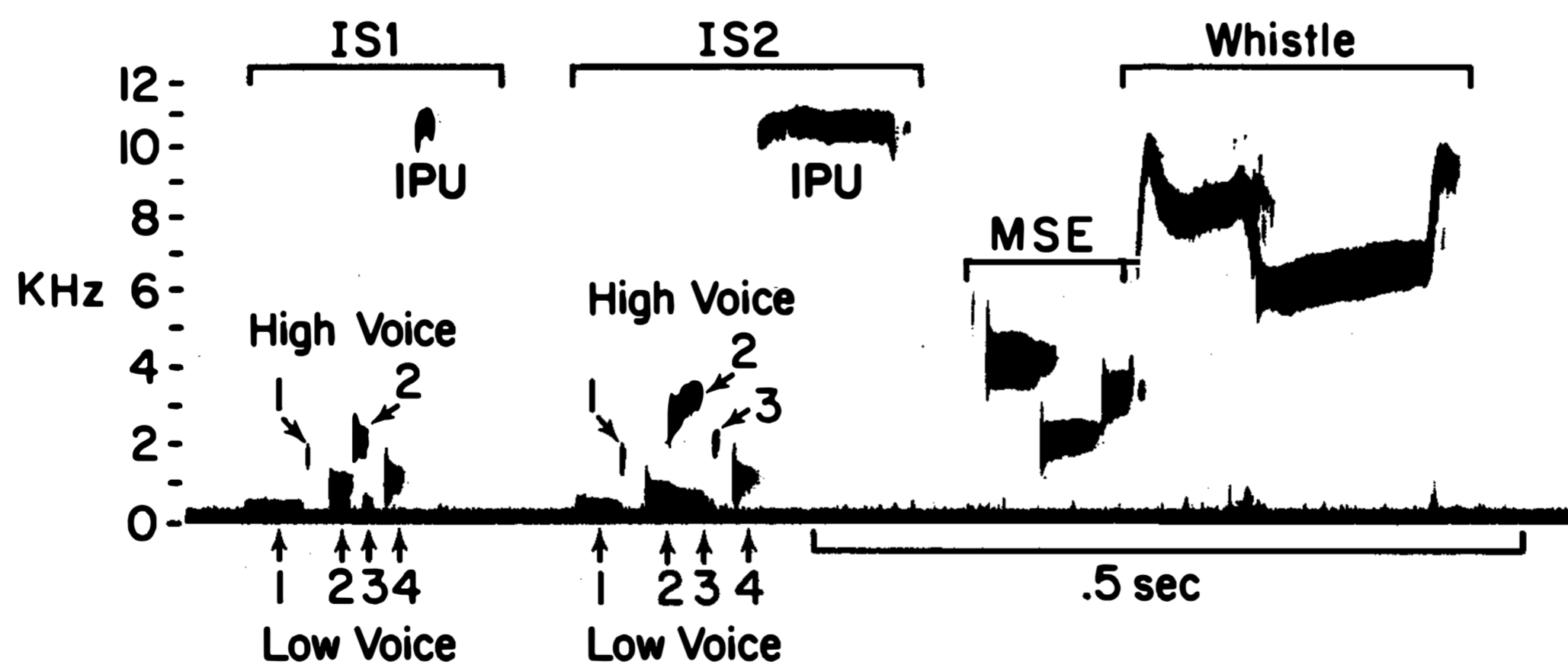


Fig. 1: Sound spectrogram of a playback song recorded from a male in Austin, Texas (see Fig. 3, AUS song #6) labelled to explain the lexical categories employed in the present study. Each introductory syllable (IS1 and IS2) consists of low voice notes (L1 to L4) ranging from 300-1700 Hz that are produced simultaneously with high voice notes (H1 to H4) ranging from 1500-5000 Hz. The inter-phrase unit (IPU) is a 8-12 kHz tone at the end of the introductory syllable. The song shown is a 2-IPU song; 1-IPU songs have an IPU only after IS2. The mid-song element (MSE) is a series of 500-5000 Hz notes found between the final IPU and the terminal whistle. MSE's are not present in Oklahoma songs. The terminal whistle is a high amplitude swept frequency tone ranging from 3-12 kHz

females. The females heard each playback song a minimum of 5 times and a maximum of 10, the average number of presentations was 7.

A positive response was scored for an individual female if she adopted a copulatory posture within 1 s of a song's onset. A copulatory posture was defined as follows: the female lowers and spreads her wings, her neck and body are arched, and the feathers around the cloacal region are separated. The onset of the posture typically occurs approximately 400 ms after the first notes of the playback song.

### Acoustic Analysis of Song Structure

Songs were measured using a Bruel and Kjaer 2033 waveform spectrum analyzer controlled by a Hewlett Packard 86 microcomputer. This system produces 25-Hz resolution in the frequency domain, the equivalent of a narrow band sonagram. Temporal measurements were made with 1 ms resolution using the waveform display, comparable to oscillogram measurements. The analysis procedure collected approximately 500 measurements on each song including frequency, amplitude, and bandwidth in the frequency domain. In the temporal domain, note onset and duration, rise/fall times of the amplitude envelopes, and time of peak amplitude of each note throughout the song were recorded.

For song typing and molar structural analysis, both wide band sonagrams and zero crossing plots were employed (see KING & WEST 1983 b). To compare structural patterns in different song types, zero crossing plots were stored in digital memory and recalled simultaneously in a graphics display which allows songs to be superimposed and moved in frequency or time relative to each other. This procedure is equivalent to making an acetate tracing of a song and then superimposing the tracings to determine structural similarity and differences.

### Results

*Study 1.* The females from Oklahoma responded reliably more to songs of Oklahoma males: all 7 females preferred both 1 IPU and 2 IPU variants of Oklahoma song over the Austin variants (Table 1). Differences among the variants were tested by the Friedman two-way analysis of variance and were

*Table 1:* Mean percentage and standard deviation (in parentheses) of female copulatory responses to Oklahoma (OK) and Austin (AUS) song

|   | Oklahoma   |            | Austin         |            |
|---|------------|------------|----------------|------------|
|   | 1 IPU      | 2 IPU      | 2 IPU          | MSE        |
| Study 1:<br>OK females                      | 53<br>(19) | 56<br>(12) | 24<br>(12)     | 13<br>(7)  |
| Study 2:<br>OK females<br>with AUS<br>males | 35<br>(16) | 47<br>(13) | 29<br>(10)     | 21<br>(14) |
|   |            |            | AUS Companions |            |
| Study 3:<br>OK females<br>with AUS<br>males | 36<br>(23) | 43<br>(19) | 43<br>(19)     | 28<br>(13) |

significant ( $\chi_r^2 = 17$ ,  $df = 3$ ,  $p < .001$ ). The females responded essentially equally to 1 IPU versus 2 IPU song from Oklahoma but responded significantly less to Austin MSE song compared to Austin 2 IPU song (Wilcoxon test,  $T = 1$ ,  $p < .05$ ).

*Study 2.* In contrast, the females housed with Austin companions did not respond differentially to the same Oklahoma and Austin songs ( $\chi_r^2 = 6.9$ ,  $df = 3$ , n.s.). Three of the females responded essentially equally to Oklahoma and Austin song and four responded more to Oklahoma song.

Mann Whitney tests were used to compare responding between the Study 1 and 2 females. The tests indicated that the females with Austin companions responded significantly less to 1 IPU Oklahoma song ( $U = 10$ ,  $p < .036$ ) than had the Study 1 females and that although responding to Austin song was higher relative to the Study 1 group, the increase was not significant (Table 1).

Preferences for individual songs at each site were also examined. The responses of all females to each of the 8 songs from both sites were ranked in terms of mean percentage of copulatory responses. The ranks were then compared across the two samples of females to determine if the same songs were responded to similarly by the two groups. For the Oklahoma songs, the rankings for the two groups of females were positively correlated ( $r_s = .88$ ,  $p < .01$ ). The 8 Austin songs were not significantly correlated ( $r_s = .55$ , n.s.).

*Study 3.* The females showed even less differentiation between Oklahoma and Austin song when tested with the songs sung by the Austin male companions (Table 1). Here, four of the females responded more to Oklahoma song, one female responded equally, and two responded more to the Austin songs. Thus, again, in contrast to Study 1, the Study 3 group did not prefer Oklahoma or Austin song ( $\chi_r^2 = 4.7$ ,  $df = 3$ , n.s.). In comparison to the Study 1 females, the Study 3 females responded reliably less to Oklahoma 1 IPU song ( $U = 10$ ,  $p < .036$ ) and reliably more to Austin companion 2 IPU and MSE songs ( $U = 8$ ,  $p < .019$ ;  $U = 4$ ,  $p < .003$ ).

The responses of the females to songs on the two test tapes was also compared revealing no systematic differences in the level of responding of the females when they heard the same Oklahoma songs presented on the two tapes ( $\chi_r^2 = 4$ ,  $df = 3$ , n.s.) or between the two samples of Austin song ( $\chi_r^2 = 2$ ,  $df = 3$ , n.s.).

Finally, we examined the responding of the females to the songs of the particular males they had been housed with to determine if a preference existed at the level of the individual male in Study 3. The females responded an average of 33 % (range 0 to 67) to the songs of the individuals with whom they were housed as opposed to 32 % (range 19 to 43) to the songs of the other Austin companions. Four females responded less to their own male's songs and three responded more.

#### Acoustic Differentiation of the Playback Songs

The field recordings tested from Oklahoma and Austin were found to be extremely similar in terms of molar structure, containing no structural pattern unique to one set of songs that could explain the overall difference in the potency

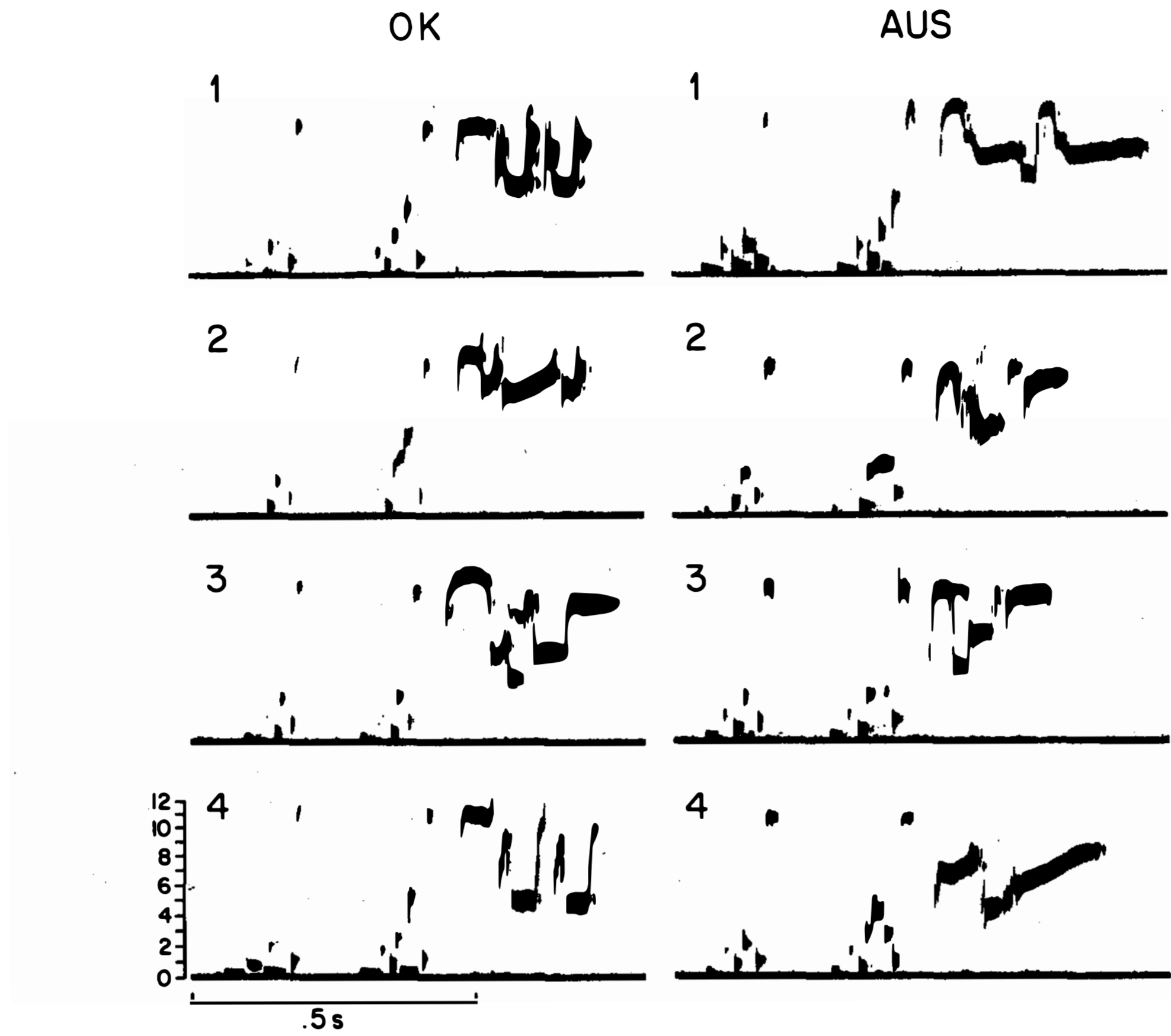


Fig. 2: Sound spectrograms of the 8 2 IPU field recordings used as playback songs, four from the Oklahoma population (OK1 — OK4) and four from the Austin population (AUS1 — AUS4)

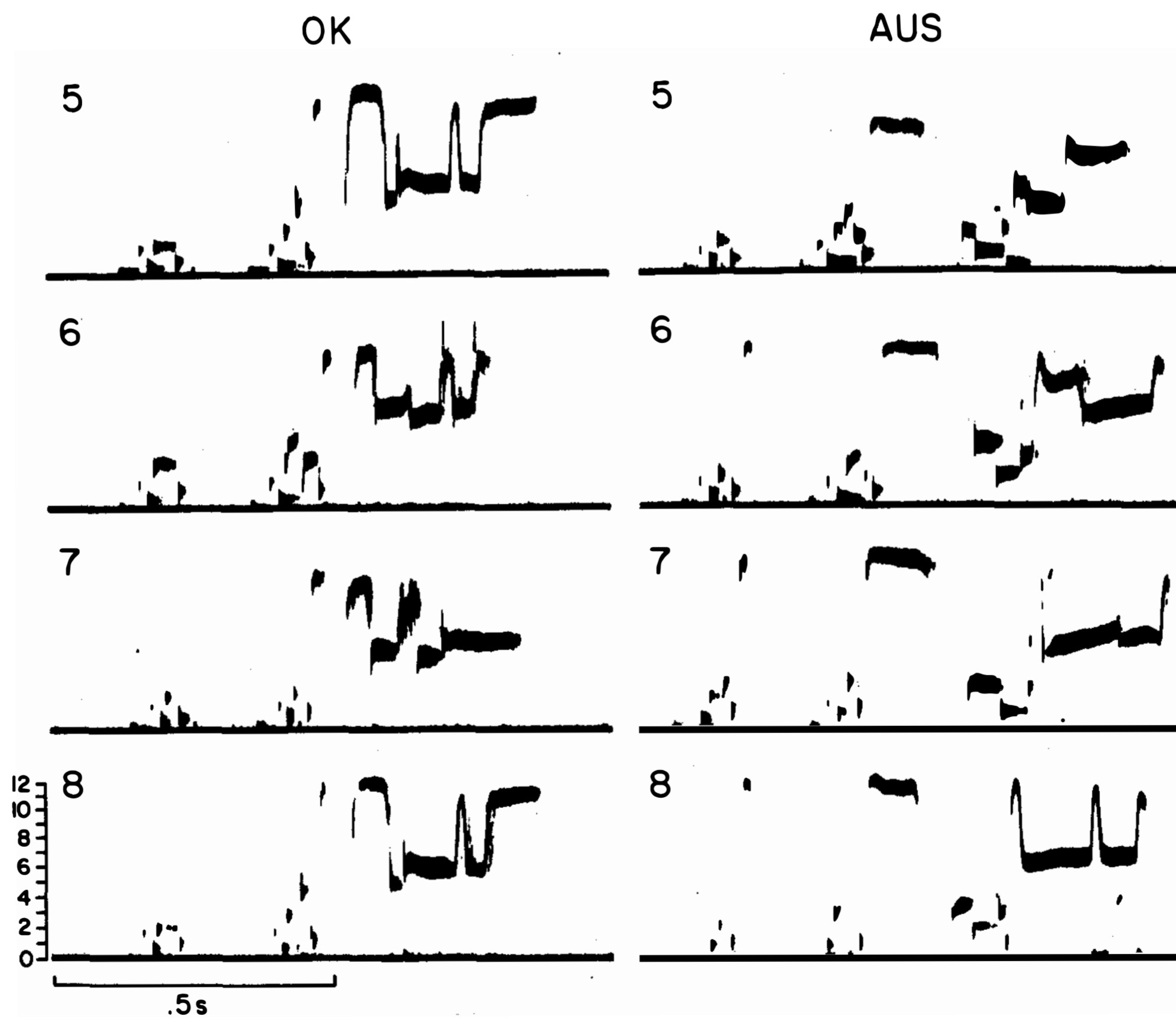


Fig. 3: Sound spectrograms of the four 1 IPU field recordings used as playback songs, four from Oklahoma (OK5 — OK8) and the four MSE field recordings from Austin (AUS5 — AUS8)

of Oklahoma and Austin songs (Figs. 2 and 3). Measurements of the frequency and duration of each note in the first two syllables of the 16 songs revealed overlapping distributions between Oklahoma and Austin on almost all measures. Of 28 Mann Whitney tests used to compare categories, only two were significant at  $p < .001$ . The frequency of the second note of the low voice (IS1 L2) in the first syllable was higher in the 8 Austin songs and the duration of first high voice note of the second syllable (IS2 H1) was longer for the Austin as opposed to the Oklahoma songs.

The songs were subjected to finer examination using the B&K/HP 86 analysis system. Two relationships were uncovered: the first was a frequency or pitch relationship between notes and the second was a difference in syllable rhythm. The pitch relationship consisted of a relative increase in frequency of the low voice when a high voice note was present. In all Austin songs, the low voice increased by more than one octave during a high voice note at least once in every song. By contrast, only two Oklahoma songs showed a low voice increase greater than an octave and one of these was the least potent of all of the Oklahoma songs. This difference is responsible for the frequency difference found in the first introductory syllable in the first set of frequency and duration comparisons.

The second relationship involved the timing of peak to peak amplitude on the low and high voice notes in the first syllable. Specifically, the duration of the high to low voice interval (H2 to L4) was found to be a multiple of the previous low to high voice interval (L2 to H2). This ratio captured the rhythm with which this part of the songs was sung: the Oklahoma songs had a mean ratio of 3.5 with a range of 1.9 to 5 and, by contrast, the ratio for the Austin songs was 1.6 with a range of .9 to 2.1. This difference in the rhythm was significant ( $U = 1, p < .001$ ).

The Study 3 songs recorded from the Austin companions were also examined for these two relationships. In all but one Austin song, the low voice increased by more than one octave when the high voice was present as was the case for the Austin field recordings. The rhythm of the first syllable also corresponded to the Austin field recordings, possessing a ratio of 1.7 with a range of .7 to 2.6, a significant difference compared to the Oklahoma song ( $U = 5, p < .001$ ).

#### **Structural Analysis of Songs of Austin Male Companions**

Prior to the removal of the Austin males from their female companions, a sample of 98 to 107 songs was recorded from each male during these final 14 days with the Oklahoma females. These songs were then analyzed to estimate the relative proportions of the major song variants used to categorize the field recordings. Songs with 2 IPU's accounted for 52 % to 63 % of the songs produced. The remainder of the songs all contained MSE's. No 1 IPU songs occurred at all. These males were again recorded approximately three weeks after their removal from the Oklahoma females. Analysis of the 272 songs from the four males revealed that three males still produced no 1 IPU songs while the fourth male sang 18 1 IPU songs out of 122 sampled.

### Discussion

The data provide the first evidence to date that postnatal stimulation can modify the female cowbird's sensitivity to song. Here, social housing with males influenced females such that they developed an essentially equal tolerance for Oklahoma and Austin song, i.e., their native preference was not reversed but substantially broadened.

The strength of the effect obtained here is best appreciated in light of our efforts to modify the song preference of NC females. Even completely naive NC females with a year's constant exposure to *M.a. obscurus* males from an area south of Austin showed as strong a preference for NC song as did wild caught NC females housed for one year with NC males (KING & WEST 1983 b). Efforts to hybridize NC *M.a. ater* also indicated the absence of modifiability: a female hybrid (NC mother  $\times$  Texas father) showed a preference for NC song as strong as non-hybridized NC females (KING & WEST unpubl. data).

Whereas this study constitutes the first positive evidence of female modifiability, it might also be interpreted as containing negative evidence regarding male cowbirds' sensitivity to stimulation from females. Previously, when naive or experienced adult NC males were housed with Texas or NC females or other species, their song development was structurally and functionally affected, whereas the females' preferences remained unchanged (KING & WEST 1983 b; WEST & KING 1985). Here, the Austin males did not appear to alter their song production. The acoustic analyses showed no differences between their songs and those of wild Austin males (the songs must, however, be played back to normally-reared females to test potency). One additional observation also suggests the Austin males' resistance to influence. All four of the Austin males were found to share more than 50 % of their song types even after almost a full year of individual housing with Oklahoma females. All had been captured from the same site, suggesting a high degree of retention despite separate housing with different females from another regional population.

At this point, the best explanation of the males' behavior is that they did not change their songs because the songs they sang suited the altered preference of their female companions. We favor this explanation for two reasons. First, observations in our laboratory show that males from other sites in Texas do alter their song production when housed with NC *M.a. ater* females whose preference remains unchanged. Second, the female companions used in the present study were juveniles. Their immaturity may have heightened their susceptibility to stimulation from their male companions and immature females may also have been less capable of influencing male song. In our work with NC populations, although we know juvenile females to be highly resistant to postnatal modification of their song preference, we do not as yet know if they are as influential as adult female *M.a. ater* in affecting male song development.

The geographic areas chosen for the present studies were selected because of the structural properties of the songs, especially the transition in use of the MSE. The data confirm the sensitivity of females to this feature: wild Oklahoma females responded less to MSE and 2 IPU Austin song and Austin-exposed Oklahoma

females showed their greatest increase in responsiveness to MSE song. The data also show that the MSE is not the only means of differentiating songs, a finding consistent with previous investigations of NC females (KING & WEST 1983 c): the properties of the syllables preceding the MSE are also involved in the female's discrimination such that the Oklahoma females responded significantly more to Oklahoma 2 IPU song than Austin 2 IPU song.

Additional evidence of the male's social influence on their female companions also emerges from an examination of the song variants sung by these males. The songs of the Austin male companions consisted of 2 IPU and MSE song, the song categories that showed the greatest increase in female responding. In contrast, the females with the Austin males were deprived of hearing 1 IPU song, a variant they normally would have heard sung by Oklahoma males. It was this lexical category (sensu MUNDINGER 1982) that showed the greatest decrease in potency when played back to the Austin-exposed females. The correspondence between exposure or deprivation of specific song variants and responsiveness to these variants also serves to validate the lexical categorizations employed.

The female's preference for Oklahoma 2 IPU songs over Austin 2 IPU songs, despite the marked structural similarity in note frequency and duration, suggests that within lexical categories, rather subtle structural differences may be of profound functional importance. The discovery of differences in relational and dynamic measures, such as octave changes of notes and the rhythm of note delivery, underscores the difficulty in using traditional descriptive categories derived from static sonographic displays to predict song function (BECKER 1982; RATCLIFFE & GRANT 1985). Not only do such data point to the need for bioassays but also for acoustic analyses sensitive to the perceptual reality of song as an event over time.

*M. a. ater* females from North Carolina prefer their own song to *M. a. obscurus* song and this preference persists even when they are postnatally exposed only to *M. a. obscurus* males (KING & WEST 1983 a). The *M. a. ater* females from Oklahoma, on the other hand, demonstrate a preference for their own population's song which can be overridden as a result of exposure to *M. a. obscurus* males. Critical species-typical behaviors such as the female cowbird's preference for male song are often assumed to be produced by the same developmental mechanisms in all populations within the species, let alone within populations of the subspecies. Methodological limitations often preclude testing many populations and can lead to premature extrapolations about the nature of the underlying developmental mechanisms (e.g., KING & WEST 1983 c).

The two consubspecific populations studied here differ in their proximity to *M. a. obscurus* populations as well as in their age and their location in the species range. Whereas Oklahoma lies in the central, ancestral part of the cowbird's range, North Carolina is on the periphery and has been colonized within the past 40 years (POTTER & WHITEHURST 1981). Investigating how the complex of lexical, geographic, and evolutionary differences between these populations has led to different developmental pathways is the next step. In taking that step we are now sensitized to the specificity of developmental programs to the particularities of populations. Developmental programs may be quite labile within species, as they

certainly are across species, but such intraspecific flexibility may have been overlooked because of the tendency to focus on macrophyletic, rather than microphyletic, behavioral comparisons.

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