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Cultures, genes, and neurons in the development of song and singing in brown-headed cowbirds (*Molothrus ater*)

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Abstract In brown-headed cowbirds, *Molothrus ater*, as in many songbird species, vocalizations are fundamental to reproduction. In our studies, experiments utilizing different social housing regimes and geographic comparisons have indicated the social learning of males' vocalizations and associated abilities to use vocalizations effectively during the breeding season. Here, we describe studies indicating roles of cultural and genetic background, and of social influences from females, on male vocal development. These influences can interact with neural regions, including song learning and song control nuclei, but also visual-processing nuclei, in the development of signaling. We argue that a developmental systems approach to the study of vocal behavior provides a structure to organize these different influences and how they may interact with one another over development. A systems approach requires that researchers study the social context in which signals and signalers develop – both the ontogenetic arena in which young animals learn their signals from older animals, and the functional arena in which young and older animals socially interact with one another.

Keywords Cowbirds · Development · Genetic influences · Nucleus rotundus · Social learning

Abbreviations *area X* area of the avian striatum · *DLM* medial portion of the dorsolateral thalamic nucleus · *HVc* high vocal center · *IN* Indiana · *IMAN* lateral portion of the magnocellular nucleus of the anterior neostriatum · *Rt* nucleus rotundus · *SD* South Dakota

Introduction

In this paper we present data from two experiments with brown-headed cowbirds, *Molothrus ater*, that, taken together with earlier published work, stress the need for a developmental systems approach to vocal signals. These studies indicate that the development of vocal signals and, perhaps more importantly, effectiveness at vocal signaling (vocal performance), takes place in complex and emergent social contexts. We will argue that if researchers are interested in understanding the integrative development and transmission of vocal communication systems, there is a need to focus study on understanding the dynamics within those social contexts.

In brown-headed cowbirds, as is the case with most songbird species, males must vocalize to females to succeed at courting, pairing, and reproducing (Rothstein et al. 1986). There are two major vocalizations of male cowbirds: songs and flight whistles (also known in the literature as perched songs and flight whistle songs, respectively). Males direct these vocal signals to females and to other males, primarily during the courtship and breeding seasons but also during the overwintering months (Lowther 1993; Ortega 1998; Rothstein et al. 1988). The two vocal signals are used in different contexts and there is considerable geographic variation in the use of the two signals and in the acoustical characteristics of both signals across the species range (King and West 1990; Rothstein et al. 1986; West et al. 1998). Female cowbirds base their courtship and mating

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decisions, at least in part, on the vocal signals of males (Freeberg et al. 2001; O’Loughlen and Rothstein 1995a; West et al. 1981).

Past studies of the ontogeny of cowbird vocalizations illustrate some of the ways social experience influences the development of vocal signals and vocal signaling in males. The vocal signals of young male cowbirds are affected by interactions with other males (West et al. 1983, 1997), and with females (King and West 1989; Smith et al. 2000; West and King 1988). Furthermore, male vocalizations are characterized by different dialect or vocal tradition backgrounds (Freeberg et al. 2001; O’Loughlen and Rothstein 1995a, 1995b). Vocal signaling, or more specifically, the ability to use signals effectively during the breeding season, is also affected by the different social experiential backgrounds of male cowbirds. For example, young cowbird males housed in social groups that include more experienced adult males over the winter used their signals more effectively in the following breeding season and courted and paired with female more often than did young males housed in social groups that did not include more experienced adult males (West et al. 1997).

Female cowbirds show preferences for songs of males of their own population or dialect area, as assayed by copulatory postures given in response to playbacks of male vocalizations (King and West 1977; O’Loughlen and Rothstein 1995a; West et al. 1998). Cowbirds of different populations tested in captive settings also show assortative pairing – females and males given multiple choices in courting, pairing, and mating with birds of the same or a different population preferentially pair with individuals of the same population (Eastzer et al. 1985). A series of recent studies tested whether population-typical patterns of behavior and courtship preferences could be socially transmitted across generations in cowbirds – whether a bird’s “culture”, its social traditional background, could influence its courtship and mating decisions (data summarized in Fig. 1). Young female and male cowbirds captured in South Dakota were housed over the winter in four large indoor/outdoor aviaries. Two of the aviaries contained adults from the same South Dakota (SD) population (young housed in the South Dakota “culture”, or SDC birds). The other two aviaries contained adults from a behaviorally distinct Indiana (IN) population (young housed in the Indiana “culture”, or INC birds). During the breeding season, females paired and mated more with unfamiliar males of the same cultural background than they did with unfamiliar males of the different cultural background (Freeberg 1996). Testing the courtship preferences of individual SDC and INC females, using a sequential mate choice design to control for intrasexual interactions, found that females preferred males of their own cultural background over males of the different cultural background (Freeberg et al. 1999). When this first cohort of INC and SDC birds was used as adult social models for another group of young South Dakota birds, Freeberg (1998) found that the second cultural generation also courted and paired assortatively by their social traditional background. In both cultural

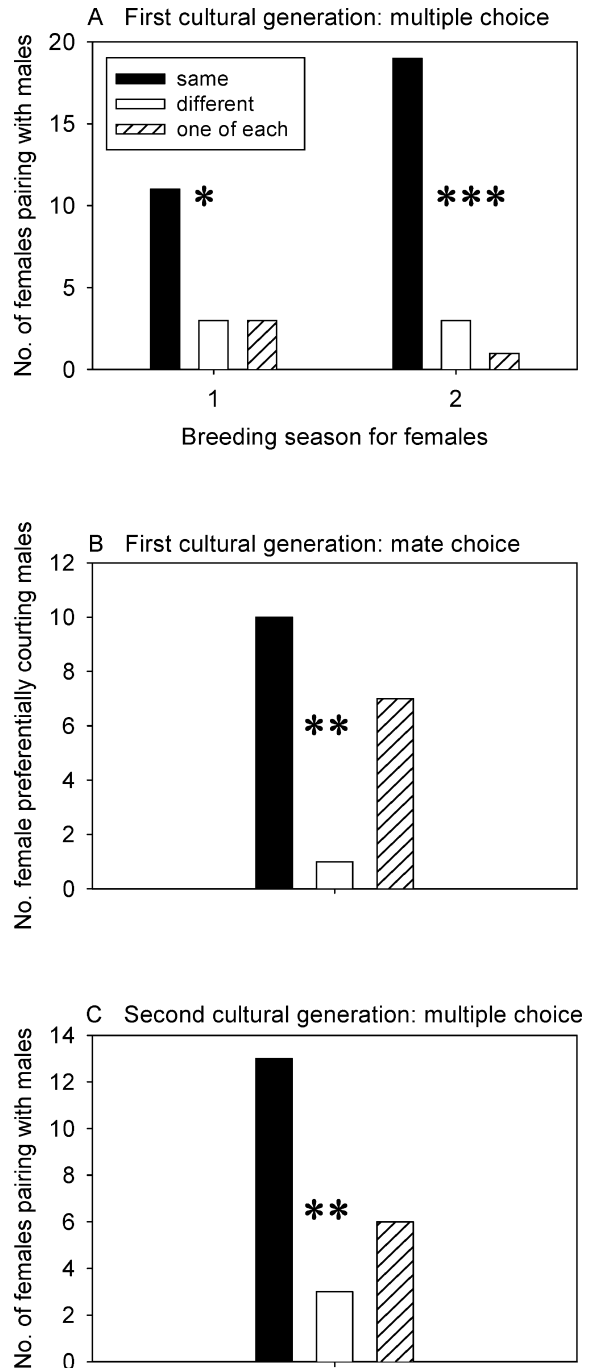


Fig. 1A–C Influences of cultural background on female cowbird courtship and mating preferences. Data indicated are female preferences for males of the same cultural background (*same*), the different cultural background (*different*), or cases where a female courted two males at different times, one male from the same and one male from the different cultural background (*one of each*). **A** South Dakota “culture” (SDC) and Indiana “culture” (INC) females of the first cultural generation, tested in a multiple-choice design over their first two breeding seasons; data redrawn from Freeberg (1996). **B** SDC and INC females of the first cultural generation, tested using a sequential mate choice design; data redrawn from Freeberg et al. (1999). **C** SDC and INC females of the second cultural generation, tested in a multiple-choice design; data redrawn from Freeberg (1998). Data analyzed using two-tailed sign tests: * $0.05 < P < 0.10$; ** $P < 0.05$; *** $P < 0.001$

generations of SDC and INC birds, furthermore, differences in males' songs – namely, the numbers of clusters of notes in the songs and numbers of notes within note clusters – were detected that differed by populational and cultural background, and that predicted male courtship interactions and success with female cowbirds (Figs. 2 and 3; Freeberg et al. 2001).

These studies indicated cultural shaping of the vocal communication system, as well as courtship patterns and mating preferences in cowbirds. The background of social traditions experienced during the fall, winter, and spring months affected male songs, male singing behavior towards females during the breeding season, female courtship interactions and pairing patterns with males, as well as female mating preferences for males. Furthermore, these patterns were socially transmitted across two cultural generations, suggesting that these social traditions related to the vocal communication system can be very stable over time.

In this report, we attempt to link the influence of cultural backgrounds to other possible influences on the development of song and singing behavior in male cowbirds. We describe new studies indicating effects of genetic background on vocal development, and possible neural correlates of vocal (and, more generally, social) development. In oscine songbird species, genetic background has been found to influence the development of the vocal communication system in a number of ways. Subspecies of marsh wrens, *Cistothorus palustris*, reared in a common environment develop repertoire sizes and singing behavior characteristic of their genetic population (Kroodsma and Canady 1985). Nelson et al. (1995) found that subspecies of white-crowned sparrows, *Zonotrichia leucophrys*, reared in a common environment developed their songs at different rates, with birds of the migratory subspecies developing their songs more rapidly (thought to be an adaptation for the shorter time period for learning as a result of migration). Munding (1995) found that in different strains of canaries, *Serinus canaria*, young males chose tutor type based upon their genetic background. In terms of neural substrates for song learning, HVC (the high vocal center), the forebrain nucleus critical to both song control and song learning,

has recently been shown to have high heritability in male zebra finches, *Taeniopygia guttata* (Airey et al. 2000). In each of these species, as is the case with oscine songbirds in general, it seems clear that young males naturally develop their songs through social interactions with, and learning from, more experienced males in their environment. These studies further indicate that genetic influences on vocal development – from ontogenetic rates to choice of a song tutor to characteristics of the songs and singing behavior that ultimately develop – occur in ways that may be adaptive for the particular population.

Considerable work has been devoted to understanding the influence of neural regions on song development and learning in birds, as well as to understanding experiential influences on those neural regions (reviews in Arnold 1992; Ball 1999; Brenowitz and Kroodsma 1996; DeVogd 1994; Nottebohm 1999). The vast majority of the work on neural influences on song development has studied the anterior song learning pathway [HVC→area X (area X of the avian striatum)→DLM (medial portion of the dorsolateral thalamic nucleus)→IMAN (lateral portion of the magnocellular nucleus of the anterior neostriatum)→RA (nucleus robustus archistriatalis)] and song control/production pathway (HVC→RA) and how acoustic stimulation, either from tutor tapes, vocalizing

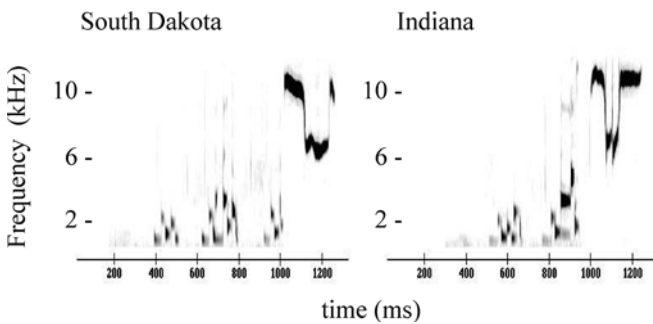


Fig. 2 Examples of sonograms of songs of South Dakota (left) and Indiana (right) male cowbirds. The y-axis for each sonogram measures frequency (1–12 kHz), and the x-axis for each sonogram measures time (0–1,200 ms)

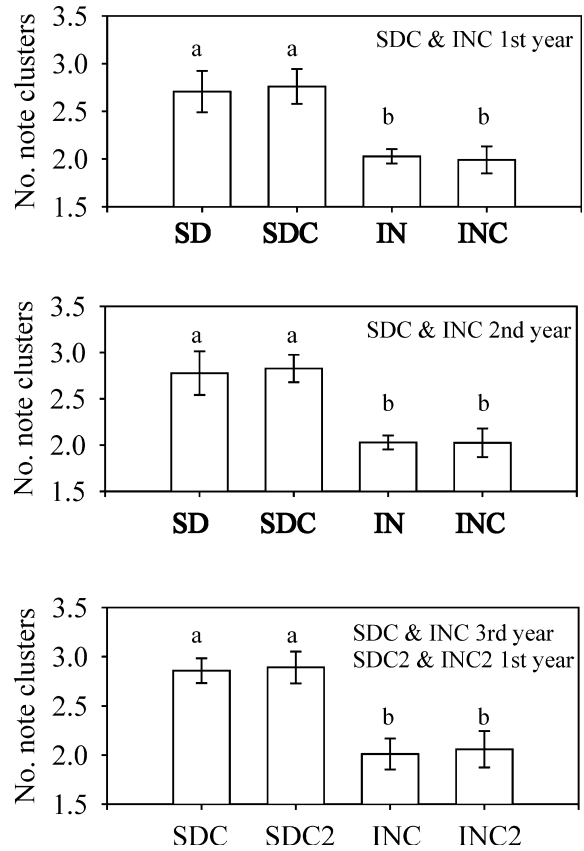


Fig. 3 The average number (\pm SE) of note clusters in the song repertoires of SD and IN males, and the SDC, INC, SDC2, and INC2 males of the two cultural generations. Data are redrawn from Freeberg et al. (2001). Different letters above bars indicate statistically significant differences between groups

social companions, or a bird's own vocalizations, may impact these regions.

Female cowbirds, even though they do not sing themselves, influence male song development (King and West 1989). Though they provide no acoustic models for males to imitate, females can influence male vocal development with a visual signal – rapid wing movements in response to male vocal behavior, called “wing strokes” (West and King 1988). Visual signals of females are also important to male courtship success with females – males pay close visual attention to female behavior during the breeding season.

Recent work with cowbirds has tested for neural correlates of song and singing behavior in male cowbirds, analyzing area X, HVc, RA, and IMAN (Hamilton et al. 1998). Because of the importance of visual stimuli to male song development and courtship effectiveness, Hamilton et al. (1998) included an analysis of a nucleus in the main visual pathway, nucleus rotundus (Rt), involved in the visual processing of texture and motion in pigeons, *Columba livia* (Shimizu and Karten 1993). Volume of Rt correlated significantly and positively with the potency of males' songs and, more importantly from a functional standpoint, with two measures of males' courtship effectiveness with females – males' rates of female-directed songs, and males' consistency at courting females across days during the breeding season (Hamilton et al. 1998). In other words, males that had better songs and that courted and paired with females more vigilantly during the breeding season had greater Rt volumes.

Together with the results of these studies, the experiments we describe below suggest there is a dynamic interplay between cultural, genetic, and social influences on the development of vocal signaling in this species. Furthermore, correlations between the volume of certain neural regions and measures of vocal development and courtship effectiveness illustrate the role neural components might play in the developmental system. We believe that by linking the salient components and processes that build song and singing behavior – in cowbirds and other songbird species – will we be able to uncover the nature of song and singing development in the social context. We conclude by briefly describing a developmental systems approach to the question of the ontogeny of vocal signals and signaling in cowbirds (e.g., Oyama 2000). Such an approach is called for when phenomena appear to be made up of interacting and interdependent components, contain much variation, and involve multiple time scales.

Materials and methods

Experiment 1: influences of genetic background

We tested whether genetic background (SD or IN) influenced vocal production in the spring months prior to the breeding season. In young male SD and IN cowbirds, vocal production during this time period correlates with rates of vocal development (King et al. 1996). We knew from preliminary studies that adult male cowbirds

of the IN population began to vocalize earlier in the spring months than did adult male cowbirds of the SD population. In February 1994, systematic censusing of vocalizing SD, IN, SDC, and INC males in large indoor/outdoor aviaries (for more detail see Freeberg 1996) found that IN males sang significantly more often than did SD males (see Fig. 4a).

During the breeding season of 1994 (early May through early July), we set up four large indoor/outdoor aviaries at the laboratory in Bloomington, Indiana for the purpose of collecting eggs resulting from matings between SD and IN female and male cowbirds. The four aviaries contained: (1) SD males and SD females, (2) IN males and IN females, (3) SD males and IN females, and (4) IN males and SD females. Although some SD and IN males were moved occasionally during the breeding season, on average there were 7–13 females and males, each, in each of the four aviaries throughout the breeding season.

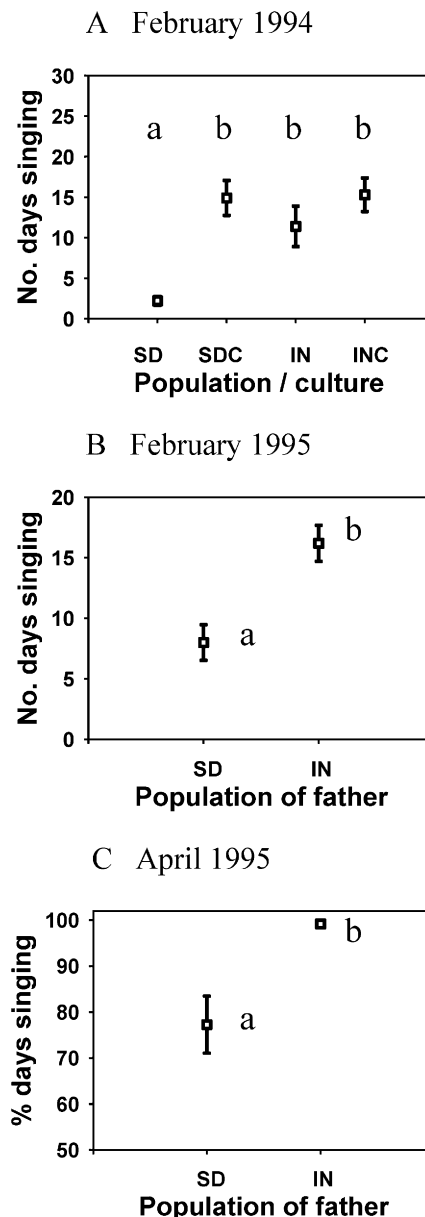


Fig. 4 Differences in the amount of singing (\pm SE) in the spring months prior to the breeding season for SD, IN, SDC, and INC birds in 1994 (A) and for the SD-fathered and IN-fathered hand-raised birds (B, C). Different letters above bars indicate statistically significant differences between groups

In the indoor section of each aviary, we placed dense configuration of assorted brush and branches, and attached small artificial nests within the brush. Artificial nests were also placed in small trees located in the outdoor sections of each of the aviaries. Throughout the breeding season, each aviary had 10–12 artificial nests. Artificial nests were stocked with “dummy” eggs: non-viable cowbird eggs (collected and marked as such from aviaries with birds of different studies), canary, and zebra finch, eggs, and jelly beans. “Dummy” eggs for the artificial nests were re-stocked every few days.

We collected cowbird eggs every morning during the breeding season (18–19 May through 8 July 1994) from each of the four aviaries from 0600 to 0630 hours. Eggs were incubated in a Petersime Model I commercial incubator at 37.5°C, with humidity at a wet bulb reading of 28.5°C. Young cowbirds that hatched were placed into barn swallow (*Hirundo rustica*) nests that had been monitored and had recently hatched swallow nestlings or were judged to have eggs on the verge of hatching. Cowbird nestlings were collected again from those barn swallow nests at 5–9 days of age, and were subsequently hand-raised to independence, at which time they were released into aviaries containing other cowbirds, beginning in mid summer. There were four hand-raised males that resulted from crosses between SD males and SD females, three that resulted from crosses between IN males and IN females, and three that resulted from crosses between IN males and SD females. In late summer, the hand-raised males were placed randomly (with respect to genetic background) into the four aviaries containing the SD, IN, SDC, and INC males, briefly described above and in more detail in Freeberg (1996). Their mature, adult vocalizations were recorded with Sennheiser RF condenser microphones using a Sony TCD-D10 PRO II digital audiotape recorder in late April and early May of 1995.

In February (very early spring, when adult and young cowbirds began to sing on a more regular basis than they did during the winter) and April (just prior to the onset of the breeding season) of 1995, we collected data on vocal production in the hand-raised males and the adults with which they were housed. We used a “vocal census” approach, where once per morning we entered each aviary and, in roughly a 5-min period, observed every male in the aviary and documented whether he was vocalizing or not. We collected 20 vocal census data points per male in February, and 20 data points for all but two males in April. These two males had to be removed because of their continuous and aggressive courtship and harassment of two females in their aviaries – the males were returned to the aviary after being caged together for a brief period. As a result of the temporary removal of, and therefore missing data points for these two males, the vocal census data collected in April are analyzed as percentage of days singing, rather than number of days singing (as they were analyzed in February).

During a 2-week period in March 1995 we sampled the vocal behavior of these hand-raised males. Vocal behavior was recorded with Sennheiser RF condenser microphones using a Sony TCD-D10 PRO II digital audiotape recorder. We obtained an average of 56 songs from each hand-raised male ($SD = 31.8$, range = 10–106). The songs recorded in March were compared to the males’ crystallized song recorded during the breeding season (methods described in more detail in Freeberg et al. 2001; King et al. 1996). Recorded songs were digitized into a zero-crossings analyzer that generated a frequency by time display on the face of a Tektronix 5113 oscilloscope. Printouts of zero-crossings plots were made of different renditions of males’ song types. Songs and note clusters recorded in March were compared to mature, crystallized songs recorded in May during the breeding season, by overlaying printouts of songs of a given male from the two time periods on a light table. We determined the amount of total song agreement between the March songs and the May (breeding season) songs for each male (a song from March was determined to match a song from May if they had 100% overlap of note clusters and > 50% overlap of the concluding whistle). The average number of note clusters for a males’ breeding season repertoires was determined from the songs recorded in May for each male. Finally, we compared the males in terms of the number of different song types they developed in their breeding season repertoires. We analyzed the data using non-parametric tests with SYSTAT (version 10.0).

Experiment 2: neural correlates of song and singing and influence of social experience

In the second experiment, we compared the volume of Rt in 12 SD males housed in two different sound attenuation chamber conditions (one set of SD males housed with SD females, the other set of SD males housed with IN females), to test for social experiential effects on Rt. We tested for correlations in these males between Rt and two measures of vocal effectiveness: the rates at which female companions stayed to males’ directed songs, and males’ song potencies. In addition, we compared neural volumes of targeted structures in these males to a group of 6 age-matched SD males housed in large aviaries with other males and females. We compared the chamber-housed males to these aviary-housed males to see if there were differences in overall neural volume of targeted structures that might have resulted from the greater social stimulation in the aviary condition compared to the sound attenuation chamber conditions (namely, the aviary-housed males had several females and males with which to interact, whereas the chamber-housed males each had only 2 females with which to interact).

As part of other studies (Freeberg 1998; Smith et al. 2000), a cohort of young males (estimated 50–100 days of age at time of capture) was captured in southwestern South Dakota in August 1995 and returned to the laboratory in Bloomington, Indiana. The aviary-housed group consisted of six of the 27 males used as subjects of the second cultural generation in Freeberg (1998), and was housed in large indoor/outdoor aviaries with other SD cowbirds (aviary, SD-housed males, ASDH). Twelve additional males (not part of the cultural transmission studies described above) were housed individually in 1-m³ sound-attenuation chambers, each with 2 female cowbirds. Six of the males were housed individually with pairs of South Dakota females (chamber, SD-housed males, CSDH), and 6 of the males were housed individually with pairs of Indiana females (chamber, IN-housed males, CINH).

The 6 aviary-housed males and 12 chamber-housed males were housed in their respective social contexts through the following May. In the CSDH and CINH male chambers, birds were observed almost weekly from September 1995 until May 1996, with observers documenting the percentage of males’ directed songs to females, and the proportion of songs to which females stayed in close proximity. In May, the mature, crystallized songs of the CSDH and CINH males were recorded with Sennheiser RF condenser or electret microphones with Panasonic SV-3700 Professional digital audio tape recorders. A subset of the CSDH and CINH males’ songs (for each male, the two most-sung songs in his repertoire) was played back to South Dakota and Indiana females, and their frequencies of copulatory postures given to the songs were scored (for further details on housing, recording, and testing, see Smith et al. 2000).

During the breeding season, the six ASDH and the CINH and CSDH males were killed by overdosing them with chloroform and were perfused with saline, followed by buffered formalin. Their brains were frozen-sectioned at 40 μ m in the coronal plane. Every third section was collected and then stained with thionin. Volume of Rt was estimated from the sections by viewing them with a projection microscope at $\times 32$ magnification (for further detail, see Hamilton et al. 1998). As a control for possible differences in overall brain size, the volume of neostriatum in the males was measured from the first section in which both lamina hyperstriatica and lamina medullaris dorsalis were visible, to the last section in which the ectostriatum appeared. We analyzed the data using non-parametric tests with SYSTAT (version 10.0).

Results

Influences of genetic background

We knew from earlier vocal censusing periods that adult IN males sang more in the early spring months than did

adult SD males (see Fig.4a from vocal census data collected in February 1994, with the SD, IN, SDC, and INC birds described above). Here we asked whether there might be a genetic basis to these populational differences in vocal production prior to the breeding season. Hand-raised males of IN fathers (pure IN or hybrids) sang more in the two spring sampling periods than did the males of SD fathers. In February, of the 20 vocal census days, the SD-fathered males sang on fewer days than did the pure-bred and hybrid IN-fathered males (Table 1 and Fig. 4B: two-tailed Mann-Whitney *U*-test, $P=0.014$). In April, of the vocal census days, the SD-fathered males sang on a lower percentage of days than did the IN-fathered males (Table 1 and Fig. 4C, lower panel: two-tailed Mann-Whitney *U*-test, $P=0.009$).

We could detect no effect of genetic background or cultural background on vocal developmental rates, although the four males with the highest percentages of developmental-breeding season song matching (comparing March songs to May songs) were all IN-fathered males (Table 1). The average number of note clusters males developed was predicted by cultural background (Table 1; two-tailed Mann-Whitney *U*-test, $P=0.008$). IN-fathered males tended to have larger repertoires, but the differences were not statistically significant (Table 1; two-tailed Mann-Whitney *U*-test, $P=0.095$). Vocal production in February was positively correlated with percentage of developmental-breeding season song matching, but was not statistically significant (Spearman rank-order correlation coefficient = +0.421; two-tailed $P>0.2$).

Neural correlates of song and singing and influence of social experience

First, we compared neural volumes of the two chamber groups to the aviary group to see if the differences in amount of social stimulation might have a pronounced effect on the growth of nuclei. We found no differences

in volume of neostriatum when comparing the ASDH, CSDH, and CINH males (Kruskal-Wallis one-way ANOVA, $KW_2=1.825$, two-tailed $P>0.5$). We also did not detect differences in Rt volume when comparing the ASDH, CSDH, and CINH males (Fig. 5; Kruskal-Wallis one-way ANOVA, $KW_2=4.784$, two-tailed $P=0.182$).

We next compared neural volumes for the two chamber groups. We found no differences between CSDH and CINH males in volume of neostriatum (Mann-Whitney *U*-test, two-tailed $P>0.5$). The CINH males tended to have smaller Rt volumes than the CSDH males (Mann-Whitney *U*-test, two-tailed $P=0.055$).

Following the correlational findings between neural volumes and singing behavior of males in Hamilton et al. (1998), we looked at two correlates of Rt volume and differences in male vocal behavior. The larger the volume of a male's Rt, the higher his song potency tended

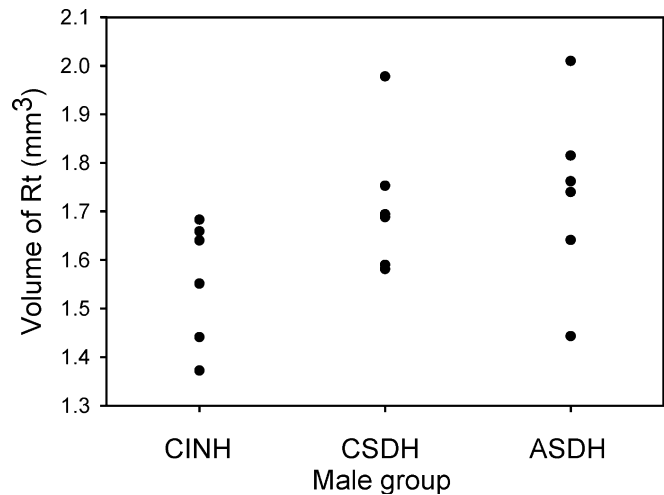


Fig. 5 Volumes of nucleus rotundus (Rt) for SD males housed in chambers with IN females (CINH), SD males housed in chambers with SD females (CSDH), and SD males housed in large aviaries containing SD females and males (ASDH)

Table 1 Genetic and cultural backgrounds of the ten hand-raised male cowbirds and their rates of vocalizing prior to the breeding season, percentages of song matching from March (developing

Male	Genetic background (father)	Cultural background	No. days singing in February	% Days singing in April	% Developmental song matching	No. songs in repertoire	Average no. note clusters in repertoire
y/bl/y	SD	SD	9	67	0.0	1	3.00
l/yr/y	SD	IN	11	95	5.9	2	2.50
o/lr/r	SD	IN	4	76	31.0	4	2.25
y/nd/r	SD	IN	8	71	19.4	2	2.00
w/dr/w	IN ^a	SD	20	100	50.0	2	3.00
d/rn/w	IN ^a	SD	16	100	35.3	3	3.00
y/nl/o	IN ^a	SD	13	100	5.6	4	2.75
n/rb/y	IN	IN	20	100	66.7	4	2.25
w/rd/r	IN	SD	17	95	13.0	4	2.75
l/ol/o	IN	IN	11	100	63.6	5	2.00

^aHand-raised hybrid males with IN fathers and SD mothers; all other birds from within-population crosses

song) to summer (crystallized song), number of songs in final repertoire, and average number of note clusters in songs of final repertoire (IN Indiana, SD South Dakota)

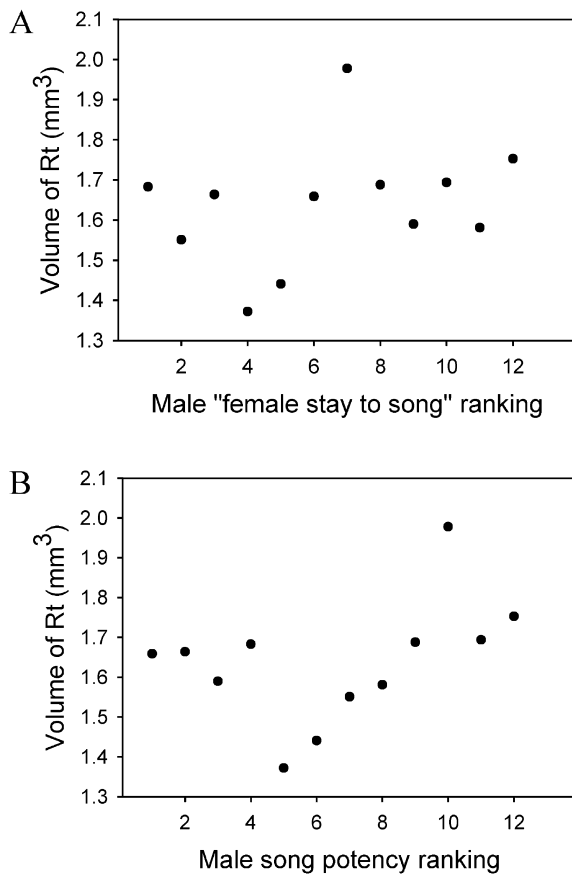


Fig. 6A,B Measures of song and singing performance compared to volume of Rt in CINH and CSDH males. Males with higher rankings on the *x*-axes were males with more effective singing behavior (A) and higher potency songs (B)

to be and the more females stayed to his directed songs in the chambers. Volume of Rt in males was positively correlated with the rates at which females stayed to CSDH and CINH males when they vocalized to the females (Fig. 6A; Spearman rank-order correlation coefficient = 0.594, two-tailed $P < 0.05$). Volume of Rt was positively, but only marginally significantly, correlated with CSDH and CINH males' song potencies (Fig. 6B; Spearman rank-order correlation coefficient = 0.510, two-tailed $0.05 < P < 0.1$).

Discussion

Influence of genetic background on song and singing

Our data indicate that the songs males developed were predicted by the social traditional background of the birds and vocal production in the spring months prior to the breeding season was predicted by genetic background. We found no effect of genetic background on vocal developmental rates, as had been suggested in earlier studies with the SD and the IN populations (King et al. 1996). In the present study we were only able to

sample male vocal development during a single, brief snapshot in time, while in the earlier study we tracked males' song development intensively and longitudinally. Results from King et al. (1996) indicated that young males that sang more in the months prior to the breeding season developed their adult, crystallized song more rapidly than males that sang less; the present study with hand-raised males differing in genetic background indicates there can be a genetic basis to rates of vocalizing prior to the breeding season. Taken together, these data suggest genetic differences in rates of vocal development in different populations of cowbirds.

Neural correlates of song and singing in male cowbirds

Volume of nucleus rotundus appeared to be influenced by the population of females with which the young SD males were housed over the winter. SD males housed in the chambers with two SD females tended to have larger Rt volumes than SD males housed in the chambers with two IN females. Further, Rt volumes in these 12 chamber-housed males correlated positively with the extent to which females stayed close to males when males directed song to them. These Rt data provide further evidence that female cowbirds can influence male vocal development through visual signals – female cowbirds offer males no acoustic stimulation on which males can base the modeling of their song development, as females do not sing. Taken together, these data are suggestive of a relationship between a male's social context and social interactions with females and neural development. Further work with SD and IN populations would prove informative to test for population differences in males' susceptibility to social influence by females – earlier studies suggested, for example, that behavioral development in IN males was less influenced by experience with heterospecifics (canaries) than was the development of behavior in SD males (West et al. 1997). A valuable follow-up to this work on Rt would be to analyze Golgi-prepared neural sections to trace dendritic growth from early in development. Does dendritic growth show similar patterns at the beginning of song ontogeny and diverge later, or is dendritic growth closely linked in time to changes in external stimulation, even at the earliest stages of song development (see DeVoogd 1994)?

Cultures, genes, and neurons in the social context of female influence on male vocal development

Earlier studies indicated social traditions in cowbird vocal behavior and courtship patterns: different cultural backgrounds influenced the vocal signals and mating preferences an individual developed. Here, we presented data from an experiment with hand-raised males of different genetic backgrounds, which pointed to

genetically-based effects on rates of vocal production during the development of males' songs. We described data from an additional study suggesting that the size of a neural region in the visual system (Rt) may correlate with male singing behavior towards females, and that different social experiential backgrounds might influence the size or growth of this nucleus. These three components of the developmental system of cowbird vocal communication may possibly intersect in important and adaptive ways in the interactions between young males and female cowbird social companions in the development of male song and singing behavior.

The aforementioned study with young SD male cowbirds housed with SD or IN female cowbirds found that females influence not just the outcome of vocal development (i.e., the songs a male develops), but they also influence developmental rates in those males. Smith et al. (2000) documented in great detail the social interactions between the young CSDH males housed in chambers with South Dakota females and between the young CINH males housed in chambers with Indiana females. The researchers found that the CSDH males developed their songs more rapidly (and developed more potent songs) than did the CINH males. CSDH males developed stereotyped song (developmentally-advanced song) more rapidly before the onset of the breeding season than did CINH males. At comparable stages of vocal development, assayed by the percentage of stereotyped song in males' repertoires, the CSDH males were as much as 2 weeks ahead of the CINH males (Smith et al. 2000).

The SD and IN females approached the CSDH and CINH males, respectively, roughly comparably across the September to following May housing period, up until the very last month. In the weeks immediately prior to the onset of the breeding season, the IN females began approaching the CINH males far more often than the SD females were approaching the CSDH males (Smith et al. 2000). The IN population comes into breeding condition two to three weeks ahead of the SD population, which is at a higher latitude. An interpretation of these data is that the IN females were coming into breeding condition during a time period when the CINH males with which they were housed were still developing their vocal signals and signaling behavior – the IN females may have been highly motivated to court males, but the particular males with which they were housed lagged behind developmentally.

Ongoing analyses of the social interactions between the CSDH males and their SD female companions and between the CINH males and their IN female companions point to large differences between the two groups. Details of the social interactions and their analyses will be described in a forthcoming paper; here we can only provide a brief overview of the SD and IN differences in these two conditions. In the chambers, social interactions recorded on videotape were analyzed according to "trigger events" that initiated sequences of interactions, and "reactions", behavioral events that occurred within

one second of a trigger event or a previous reaction. Chains of trigger events and reactions were then analyzed in terms of their conditional probabilities. In the CSDH condition, a large number of female behaviors, including gapes (beak openings), movements both away from and towards the male, neck stretching and beak wiping, and rapid wing movements, co-occur tightly in time with a male song being produced. In the CINH condition, however, only gapes by the female seemed to co-occur with high probability with a male song. Of key importance seems to be the difference between CSDH males and CINH males in their singing response to females' wing strokes. Wing strokes are rare events, but events that can have a profound impact on male vocal development (West and King 1988). For the CSDH males, nearly a third of the time a male observed a wing stroke, he followed that behavior with a song. The CINH males followed a wing stroke with a song only half as much. These differences between these two chamber-housed conditions are suggestive of the CSDH males and the SD females with which they were housed being more socially interactive with one another than were the CINH males and the IN females with which they were housed.

Towards a developmental systems view of the development of song and singing

Throughout this paper, we have tried to stress the difference between studying song as a static behavioral trait and studying singing or signaling, a dynamic measure of performance. Perhaps due to the legacy of taxonomists of looking for morphological traits that differentiate species, subspecies, or populations, we know alarmingly less about the functional value of differences in performance than we do about differences in the acoustical parameters of the song a male sings (see also Mayr 1982). When female birds assess the "trait" of birdsong in nature, however, they do so via its performance by individual males. Might it not also be the case that mate choice, and hence sexual selection, is affected as much (or more so) by vocal performance as by the static properties of a song (see Gil and Gahr 2002)? If we are right, then studies at behavioral and neural levels must include dynamic measures of song use (e.g., Jarvis et al. 1998). Such studies would necessarily lead to placing greater value on the social context in which learning occurs.

We believe studies looking for neural substrates of vocal learning should begin focusing on regions outside the traditional song control and song learning regions. Here we have addressed a possible role of nucleus rotundus, but other neural regions in the tectofugal pathway (retina→optic tectum→Rt→ectostriatum) may be important. For example, in the auditory midbrain, projections from the external nucleus of the inferior colliculus connect to the optic tectum, where maps of visual and auditory space occur (for reviews, see Carr

and Code 2000; Klump 2000). In barn owls, neural responses of the optic tectum, stimulated by both auditory and visual cues, influence head movements (Masino and Knudsen 1990). Experimental manipulations of visual stimuli (du Lac and Knudsen 1991) and of external ear morphology and therefore the acoustic stimuli reaching the inner ear (Knudsen et al. 1994) lead to changes in the optic tectum. Knudsen (1994) has proposed that integration of visual and auditory information in the optic tectum may be important for auditory learning that is visually guided to take place. Whereas this work has been carried out with barn owls, a non-songbird, and their ability to detect sounds and movements of possible prey items, it seems possible that the optic tectum may be important in both female and male songbirds for the processing of possibly subtle and rapid visual and acoustic stimuli important to the development of song, singing, and other social behaviors.

We believe researchers should also begin asking questions about social and visual effects on the song control and learning regions. For example, Bischof and Engelage (1985) found that visual stimulation influenced responses of HVC in zebra finches, and argued that visual cues from females may be important to song development and the directed singing behavior of males. Neural regions involved in filial imprinting to visual stimulation (see Bolhuis and Honey 1998) may play a role, furthermore, in the development of broader communicative interactions within the social context of many songbirds. Finally, future studies aimed at uncovering the extent to which social and perhaps non-acoustical stimuli might affect neural development now seem warranted. We hope the findings presented here offer an incentive to invite more of the natural complexity of learning into the domain of behavioral neuroscience.

To conclude, our research is beginning to uncover key components and interactions in the developmental system of cowbird vocal communication. A young male cowbird interacts with males and females during his vocal development. For the male, our data indicate that his genetic background, his cultural background, and possibly certain neural regions inside and outside the traditional song learning pathways (which conceivably may be influenced by the genetic and cultural backgrounds) will impact on the ontogeny of his vocal communication system. For a female interacting with the young male, her population (possibly genetic influences, possibly cultural influences) and neural regions in her brain (such as IMAN; Hamilton et al. 1997) can have an impact on the ontogeny of the male's vocal communication system. Importantly, these interactions between components of the developmental system likely change over ontogenetic time (e.g., Oyama 2000), just as the interactions between the young male and female may change.

We are led by our data to argue that if researchers are interested in fully understanding the acquisition and transmission of vocal communication systems, the

developmental systems view offers a stronger approach than more reductionist approaches that attempt to isolate a single component of the system. The reason is simple – if we begin by degrading the system to break it into pieces, we are often able to find some effects of those pieces on the system as a whole. But then we face the ever-growing problems of how to reconstruct what we have broken down into the natural units on which development and evolution actually work. The developmental systems view requires that we look for genetic effects on vocal development in rich social and ecological contexts; that we look for social influences on vocal development in animals differing in genetic background; that we look for hormonal, ecological, neural, and morphological influences on vocal development in animals differing in genetic and social backgrounds (see also Kroodsma 1996). Furthermore, this view requires that we document the ways developing organisms interact with their social and physical worlds, and how those interactions change over time. Attempting to take a developmental systems view is no small task, but we believe that this is the direction we must take if we truly wish to understand the nature of continuity and change of song and singing within individuals and within populations in cowbirds, and in other songbirds.

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