

# Uncovering sources of variation in female sociality: Implications for the development of social preferences in female cowbirds (*Molothrus ater*)

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## Summary

The purpose of this study was to investigate variation in female cowbird (*Molothrus ater*) sociality. We studied four captive flocks of brown-headed cowbirds, two composed of adult females and juvenile males and two composed of juvenile females and juvenile males. From September through December, we documented sociality using affiliation measures. From the outset, we found large age differences: juvenile females interacted more with each other and with males than did adult females. We conducted perturbations of flock composition to examine the differences between juvenile and adult females. When adult females were mixed with juvenile females and juvenile males, the adult females adopted the behavior of the juvenile females, with both becoming more interactive with males. The perturbations did not appear to be a consequence of juvenile male social behavior. The results are significant as they show a special role for juvenile females to regulate male-female interactions, which in turn affect vocal and social ontogeny as well as reproductive success.

*Keywords:* female sociality, social preferences, reproductive success, brown-headed cowbird.

## Introduction

The female holds a powerful place in the social system of songbirds. She is the limiting sex, ultimately determining which genes are passed on to the

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next generation. With respect to female activity, the most studied behavior is her copulatory response to song which has been used as an assay of her song preferences (King & West, 1977; Searcy, 1992). Female songbird preferences are malleable in many species; but it was surprising to find brown-headed female cowbird song preferences were malleable at the subspecies level given their parasitic habit. When either adult or juvenile female cowbirds are housed in the rich social environment of a flock, their preferences for macrogeographic, as well as local song variants can be modified (King et al., 2003a; West et al., in press). These same preferences were not modified when the females were housed in pairs or triads where social interactions were limited to one or two individuals. Relatively little is known about how female sociality develops or about individual differences in ontogeny. Female social behavior has rarely been studied because it is subtle and hard to observe (but see Gowaty, 1997). If female song preferences are shaped by the dynamics of social groups, we need to understand the nature of those dynamics as they could be responsible for female mating and song preferences.

What we do know about female cowbird social behavior is principally limited to their reactions to male song. For example, the presence of song seems to be pivotal to age assortment, the tendency of birds to affiliate with conspecifics of the same age. It is not clear, however, what function age assortment serves. The assortment could have something to do with the shaping of preferences, perhaps permitting juvenile females to eavesdrop on adult females' reactions to song to learn which are stimulating (Gros-Louis et al., 2003). Eavesdropping is a low cost way for birds to gain information about their social companions (McGregor & Peake, 2000) and may be especially important to learn about song quality or social partners without breeding season experience, as juvenile females would need to do. We also know that juvenile females react differently to song playback depending on whether adults are present. For example, in one study, juveniles housed without adults acted as a group: they either all approached or ignored the playback speaker together but juvenile females housed with adult females did not act as a group; instead they appeared to act in a manner similar to adults who showed great individual variation to song playback (Freed-Brown, unpubl. data). Thus, in response to the playback of song, it appeared that the juvenile females copied the behaviors of the adults when they were housed with them but copied each other's behavior when they were housed without adults.

One important measure of female behavior is the frequency with which a female approaches or is in close proximity to a male. This is an especially revealing behavior if it closely follows or precedes a significant event such as the female wing stroke (a rapid wing flick given by a female during song; Gros-Louis et al., 2003) or a male song (West & King, 1988). Although male-female proximity is usually low frequency and highly variable across individual females, it is a salient event when it occurs. King et al. (2003b) found that when juvenile females who showed high rates of approaching males were moved into different aviaries, they rapidly changed the behavior of all of the other birds within the aviary. In particular, their behavior stimulated male-male singing interaction. Female vigilance and regulation of their proximity to males occurs throughout the year, allowing us to look at its influence on flock activity and potential consequences for the development of both male and female communicative behaviors. Female proximity is also known to affect individual males' song development when studied in sound-attenuating chambers: the more a female approaches and stays when a male sings, the faster he develops song and the more chances he has to stimulate the female (Freeberg et al., 1999; Smith et al., 2000).

The study was motivated by evidence that social influences in flocks are an important factor in shaping female preferences for males and their songs (King et al., 2003b; West et al., in press). The purpose of this study was to discover differences in sociality between juvenile and adult females and to understand the contribution that these differences might make to male-female interactivity.

The goal of this study was to determine whether or not juvenile females have competent social abilities, that is, the same social abilities as adult females or do they develop appropriate behaviors through learning or maturation? By understanding variation in female behavior that contributes to flock activity, we hoped to begin to understand both how individuals can alter the social organization of a group, as well as how the group can alter individuals by shaping their preferences and sociality. To do so, we configured four aviaries: two housing adult females with juvenile males and two housing juvenile females with juvenile males. Because we were focusing on female behaviors, we employed a skewed gender ratio with about two females to every male. This was done to increase female-female competition and behavior levels. In nature, fall and winter flocks can have skewed gender ratios, so these conditions are not improbable (Cristol et al., 1999). We chose to

use all juvenile males to assess female interactions with inexperienced males singing immature song.

## **Methods**

### *Subjects*

All birds in the study were collected in Monroe County, Indiana. We collected 24 juvenile females and 21 juvenile males in June and July 2004. The juvenile birds were estimated to be 30 to 50 days of age upon capture based on plumage (Selander & Giller, 1960) and were housed in mixed-sex outdoor aviaries measuring  $9.1 \times 20.75 \times 3.4$  m until the experiment. We also used 44 adult females between 516 and 2713 days of age. Prior to the experiment, the adult females lived in mixed sex flocks housed in outdoor aviaries measuring  $9.1 \times 20.75 \times 3.4$  m. Seven of these females were used in a playback experiment during which time they were housed in outdoor aviaries measuring  $2.4 \times 6.1 \times 2.3$  m without males from March 8 to the April 30, 2004 and then placed in sound-attenuating chambers from April 30 until July 4, 2004 (West et al., in press; for details on sound-attenuating chambers, see Freeberg et al., 1995). All birds were fed a modified version of the Bronx Zoo diet for omnivorous birds that was supplemented with Avia (Nutri-Vet Research Corporation; Poughkeepsie, NY); red millet, white millet, canary seed and vitamin treated water. All birds wore unique colored leg bands to permit individual identification.

### *Data collection*

Data were collected by two observers using voice recognition technology (White et al., 2002a). To gather data, we spoke the bird's color identification, behavioral codes and comments into a wireless microphone (Telex WT 150 & Telex FMR 150; Telex Communications Ltd, Burnsville, MN). These recordings were transcribed to text using IBM ViaVoice voice recognition software (Millennium Pro Edition) in Microsoft Word 2000 running on a PC compatible computer. The data were then imported into a database (4<sup>th</sup> Dimension v. 6.5.1; ACI Inc., San Jose, CA).

Two observers collected affiliation patterns using two protocols: nearest neighbor (NN) and approach (APP). A NN point was scored between two

birds when they were within 15 cm of each other because it seemed to be a natural spacing pattern (see Smith et al., 2002). A point was rescored between two birds if they were within 15 cm next to each other for more than a minute. A point was also rescored if the birds moved apart and then came together later. An APP was scored when a bird moved within 15 cm of another bird. NN and APP were not scored for birds on the ground, by the food, or water dishes to avoid incidental points due to foraging. NN and APP were each collected in seven-minute blocks. For each experiment, we collected 10 NN and 10 APP blocks in each flock. The two observers differed by less than 3% in total number of NN and APP points collected.

## Experiments

We conducted seven experiments over a period of four months. For most experiments, it took approximately 5 days to record the 10 NN and 10 APP in each aviary. We took an average of 3.7 blocks in each flock per day (range: 1-8). NN and APP blocks were taken alternating such that two NN blocks were rarely taken sequentially. Durations longer than 5 days reflect rain days, except in Experiment 4, which lasted 12 days, during which time we took 20 NN and 20 APP blocks. Subsequent analyses revealed no difference in affiliation patterns using NN or APP and thus the two measures were combined throughout the study and are hereafter labeled as NN points. The duration of each experiment and total number of NN points recorded appear in Table 1.

We used relative differences in number and proportion of female-male NN points as our primary measure. The females also accumulated female-female points, but they were not used for analysis in part because there were more females than males, and in part because the bias for same sex neighbors is a reliable pattern in all of our captive flocks (Smith et al., 2002; White et al., 2002a, b; West et al., 2003).

We used nonparametric tests to analyze the data due to small sample sizes. The unit of analysis we used were individuals in flocks. We used Kruskal-Wallis, Mann Whitney, Friedman and Wilcoxon signed rank tests for comparisons across and within groups (Siegel & Castellan, 1988). Where multiple tests followed a Kruskal-Wallis or Friedman ANOVA, we used a bonferroni adjusted  $p$  value of  $p < 0.017$  for three comparisons, and  $p < 0.025$  for two comparisons (Sokal & Rohlf, 1995). All tests were two tailed and all statistical outcomes are reported in Tables 2 and 3.

**Table 1.** Duration and total number of NN points collected during Experiments 1-7

Experiments	Duration of experiments	Blocks	Flocks			
			AJ1	AJ2	JJ1	JJ2
1: Age differences	9/27-10/1	20	240	221	776	559
2: Juvenile male switch	10/2-10/6	20	279	362	814	627
3.1: Titration	10/7-10/11	20	249	315	815	581
3.2: Titration	10/12-10/19	20	282	196	889	873
3.3: Titration	10/20-10/24	20	360	207	771	1055
3.4: Titration	10/25-10/29	20	396	338	571	799
4: Age mix	10/31-11/10	40	1465	1390	1456	1338
5: Age mix baseline	11/12-11/16	20	446	468	854	803
6: New adults	11/18-11/23	20	559	570	1084	1017
7: Juvenile male switch	11/29-12/5	20	353	502	581	932

Note: All experiments were run in 2004. Blocks reflect a combination of NN and AP.

**Table 2.** Statistical test on the average number of female-male NN points for Experiments 1-7

Experiments	All flocks	AJ1 vs AJ2	JJ1 vs JJ2
	Kruskal-Wallis	Mann-Whitney	Mann-Whitney
1: Age differences	.0001	.291	.024
2: Juvenile male switch	.0001	.0001	.007
3.1: Titration	.0001	.378	.001
3.2: Titration	.0001	.001	.178
3.3: Titration	.0001	.449	.004
3.4: Titration	.0001	.118	.004
4: Age mix	.0001 <sup>a</sup>	.0001 <sup>b</sup>	.0001 <sup>c</sup>
5: Age mix baseline	.0001	.833	.410
6: New adults	.0001	.843	.266
7: Juvenile male switch	.0001	.799	.028

Across condition tests (AJ1, AJ2, JJ1, JJ2) were done using a Kruskal-Wallis test. Within condition tests (AJ1 and AJ2; JJ1 and JJ2) were done using a Mann-Whitney test. All  $N$  values = 12 per flock except for  $N_2 = 11$  during Experiments 3.3-5.

<sup>a</sup> Experiment 3.4-5 were compared using a Friedmann test ( $N = 46$ ).

<sup>b</sup> Experiment 3.4 was compared with 4 using a Wilcoxon test ( $E3.4 < E4$ ,  $N = 46$ ).

<sup>c</sup> Experiment 4 was compared to 5 using a Wilcoxon test ( $E4 > E5$ ,  $N = 46$ ).

**Table 3.** Statistical tests on the proportion of female-male NN points for Experiments 1-7

Experiments	All flocks	AJ1 vs AJ2	JJ1 vs JJ2
	Kruskal-Wallis	Mann-Whitney	Mann-Whitney
1: Age differences	.002	.017	.514
2: Juvenile male switch	.0001	.0001	.052
3.1: Titration	.0001	.160	.012
3.2: Titration	.002	.128	.114
3.3: Titration	.0001	.002	.004
3.4: Titration	.0001	.044	.028
4: Age mix	.0001 <sup>a</sup>	.0001 <sup>b</sup>	.0001 <sup>c</sup>
5: Age mix baseline	.0001	.556	.101
6: New adults	.0001	.291	.008
7: Juvenile male switch	.0001	.887	.630

Across condition tests (AJ1, AJ2, JJ1, JJ2) were done using a Kruskal-Wallis test. Within condition tests (AJ1 and AJ2; JJ1 and JJ2) were done using a Mann-Whitney test. All  $N$  values = 12 per flock except for  $N_2 = 11$  during Experiments 3.3-5.

<sup>a</sup> Experiment 3.4-5 were compared using a Friedmann test ( $N = 46$ ).

<sup>b</sup> Experiment 3.4 was compared with 4 using a Wilcoxon test ( $E_{3.4} < E_4$ ,  $N = 46$ ).

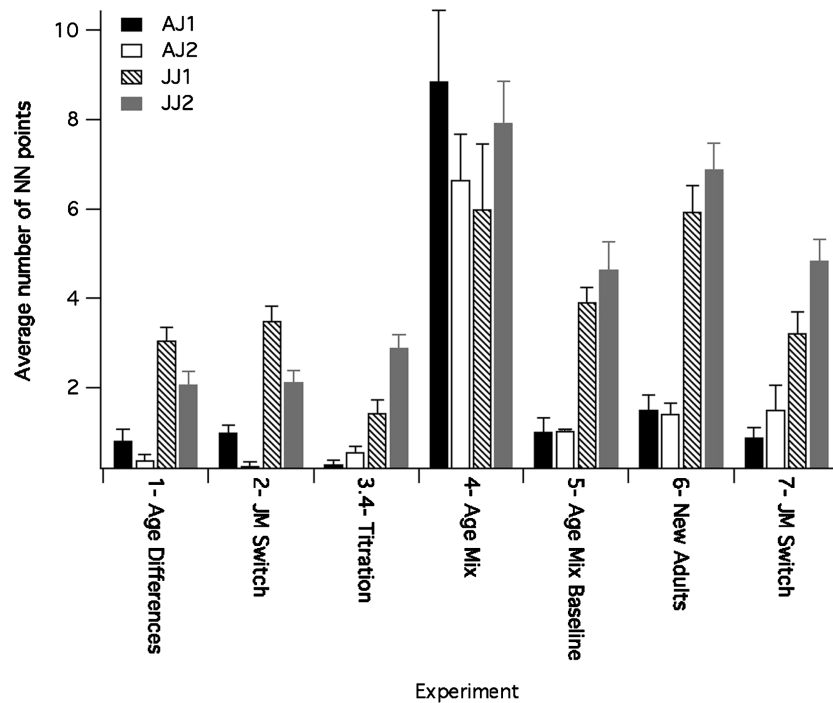
<sup>c</sup> Experiment 4 was compared to 5 using a Wilcoxon test ( $E_4 > E_5$ ,  $N = 46$ ).

### Experiment 1: Documenting differences in sociality based on age

When we housed adult and juvenile females together with juvenile males, we never found differences in female sociality based on age (e.g., King et al., 2003b). In all previous work, adult and juvenile females were housed together and thus could influence each other. Here we asked if adult and juvenile females were housed separately with juvenile males, would there be differences in sociality?

#### Method

Four flocks were composed: two of the flocks (AJ1 and AJ2) each contained 12 adult females; AJ1 had six juvenile males and AJ2 had five juvenile males. The other two flocks (JJ1 and JJ2) each contained 12 juvenile females and five juvenile males. Unless otherwise stated, each flock contained the same number of males and females throughout the seven experiments.

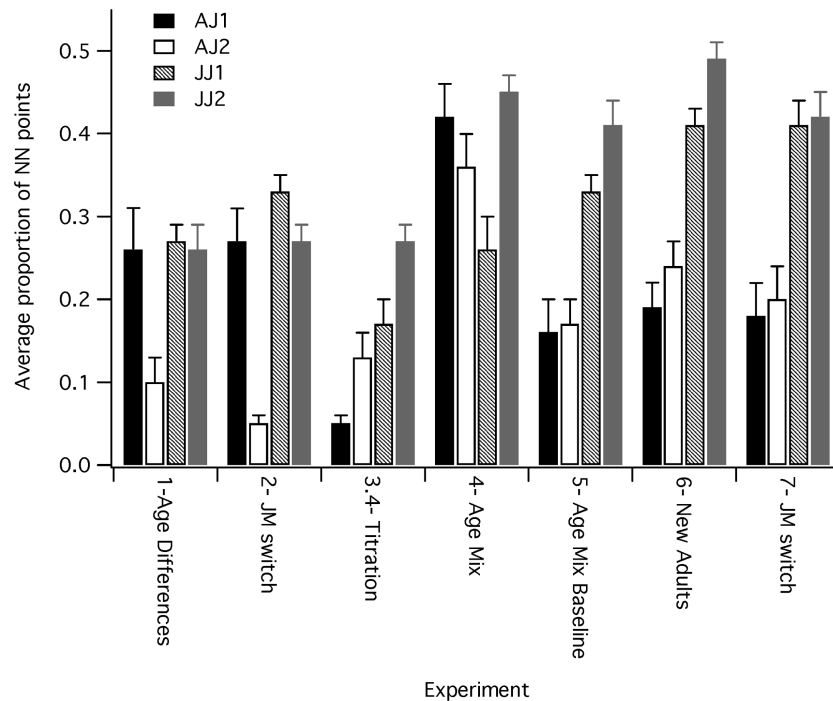


**Figure 1.** Average number of female-male NN points during Experiments 1-2, 3.4, 4-7. Data are displayed as means (SE). JM = juvenile males.

### Results and discussion

There were significant differences in the average and proportion of female-male points between juvenile and adult females (Tables 2 and 3). The juvenile females had more female-male points than the adult females (Figure 1). The juvenile females also spent a larger proportion of their time with the juvenile males while the adult females associated more with other adult females (Figure 2). There were also significant differences within the AJ and JJ flocks (Tables 2 and 3).

Experiment 1 established significant differences in adult and juvenile female social affiliations. We found the juvenile females had significantly higher numbers and proportions of NN points with juvenile males than did the adult females. We were surprised at the disparity in overall activity between adult and juvenile females. Some of it was surely due to the more playful and curious nature of the young birds: they would repeatedly chase one another, pull at feathers, and stereotypically manipulate or drop insects,



**Figure 2.** Average proportion of female-male NN points during Experiments 1-2, 3.4, 4-7. Data are displayed as means (SE). JM = juvenile males.

feathers and other objects, as has been described in young animals in general (West, 1977) and in other birds (Ficken, 1977). West & King (1987) have argued that such play is a way of perturbing behavioral states as a means of learning the dynamic properties of conspecifics or resources. For example, for a young male to pull the tail of a young female may elicit a very different reaction from pulling that of an adult, giving the young male behavioral feedback with which to form social categories. While there were gross differences in the social behavior of the adult and juvenile females within each condition, there were also differences between the flocks. Before proceeding to investigate the adult-juvenile differences, we wanted to be certain that we understood the basis of within group variation. We began by testing whether the identity of the males in each group were responsible for the within group differences.

**Experiment 2: Switching juvenile males within age class**

We know females are sensitive to male presence and often react to different kinds of male stimulation (juvenile versus adult song) by modifying their patterns of social assortment (Gros-Louis et al., 2003; West et al., 2003). Thus are the patterns of assortment or activity within age condition a result of male behavior?

*Method*

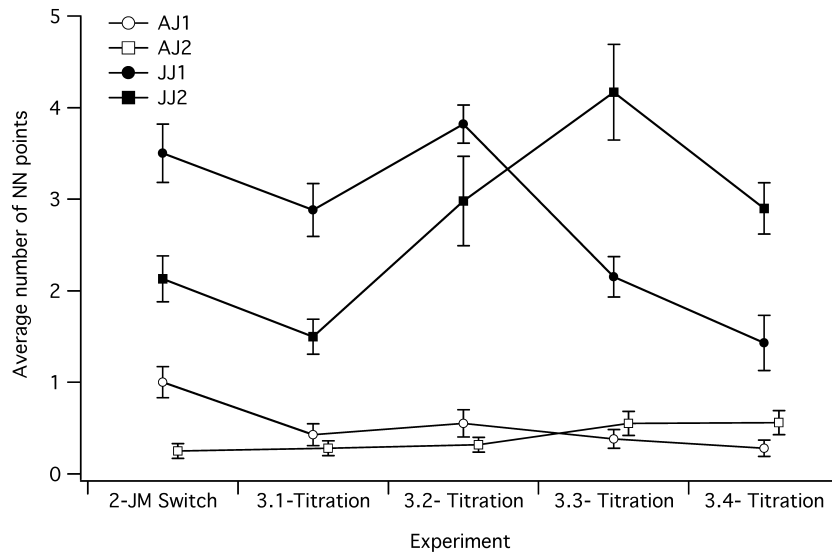
We switched all the juvenile males ( $N = 21$ ) between aviaries AJ1 and AJ2 and between aviaries JJ1 and JJ2. Each group of juvenile males was now with a different group of females from the same age class as their prior housing.

*Results and discussion*

The affiliative patterns did not change when the juvenile males were switched. The two JJ flocks still had a higher average number of female-male associations than did the two AJ flocks (Table 2; Figure 1). The two JJ flocks had reliably higher average proportion of female-male points than the two AJ flocks (Table 3; Figure 2). Experiment 2 showed that changing males within age class did not change the nature of social activity in the flocks. This replicates the King et al. (2003b) finding that the behavior of juvenile males is insufficient to cause females to alter their social patterns. Thus, individual variation in female sociality appears to account for the within group differences. Experiment 3 focuses on the contribution of individual females to account for the within group variation in flock activity.

**Experiment 3.1-3.4: Female titration**

In King et al. (2003b), the introduction of just a few females changed the social dynamics of entire flocks. Thus, we asked whether the within group differences could be attributed to individual females or was additive across individuals with no one female having any special significance. To answer this question, we gradually exchanged females within the AJ and JJ flocks and measured the resulting changes in female-male activity.



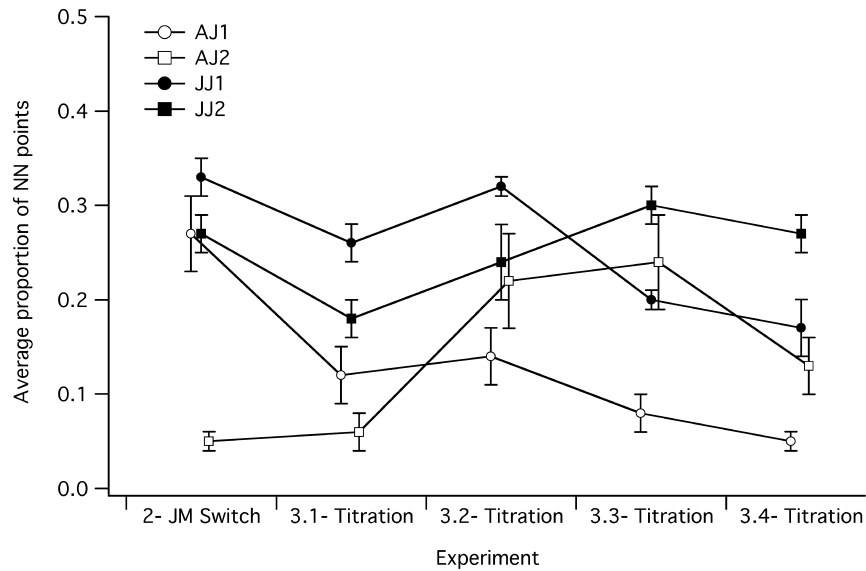
**Figure 3.** Average number of female-male NN points during Experiments 2 and 3. Data are displayed as means (SE). JM = juvenile males.

### Method

Three females from AJ1 were switched with three females from AJ2 and three females from JJ1 were switched with three females from JJ2. The females to be moved were chosen randomly. Every five days after 20 data blocks were collected (see Table 1), three more females were moved until all 12 females were moved into a new aviary and the original flock was reconstituted. One adult female from AJ2 died during experiment 3.3 and was removed from all subsequent analysis ( $N = 11$ ).

### Results and discussion

The sociality never changed dramatically for any flock but shifted gradually as the females were switched (Figures 3 and 4). The adult females displayed reliably lower levels of sociality than the juvenile females throughout the titration (Table 2). The adult females spent significantly less time with juvenile males than did the juvenile females based on the average proportion of NN points (Table 3). In both conditions, the flocks had defining characteristics, or flock signatures, created from their activity level and NN patterns. For example, the JJ2 generally had a lower average number of female-male points than JJ1 but as the females from these flocks were assimilated,



**Figure 4.** Average proportion of female-male NN points during Experiments 2 and 3. Data are displayed as means (SE). JM = juvenile males.

the differences dissipated but then re-emerged as the flock was reconstituted (Figures 3 and 4). Both AJ and JJ flocks returned to baseline conditions after the titration (Figures 1 and 2). The reversal of the flock activity is also shown in Figures 3 and 4 under Experiment 3.4, which is the same as Experiment 2.

Unlike the finding in King et al. (2003b), individual females did not appear to exert disproportionate influence over the flock as a whole. In King et al., adult and juvenile females were mixed. This may have created a circumstance where young females did not conform to adult female behavior norms and thus by their nonconformity produced significant flock reassortment. In this study, when the adult and juvenile females were exchanged within age class, the lack of any significant effects may be a consequence of a uniformity of social norms characteristic of each age class. In other words, mixing individuals with similar social preferences produced little change but this begs the question of what happens when individuals with different social behaviors are introduced to one another?

#### **Experiment 4: Mixing adult and juvenile females**

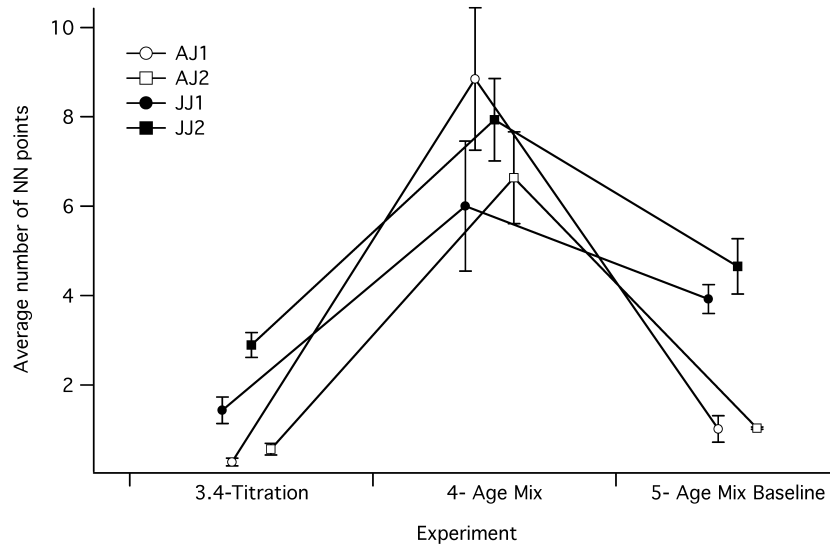
King et al. (2003b) followed four flocks of juvenile and adult females housed together with juvenile males throughout the fall. The females in that study showed strong gender assortment. Examination of the data revealed completely overlapping patterns of female-male activity for both adult and juvenile females. Miller et al. (in press) found that juvenile females, in the first 60 days of life, did not show gender assortment when they were housed without adult females. So, the data from King et al. (2003b) suggest that the juvenile females adopted the behavior of the adults and learned to avoid juvenile males. In the present study, juvenile females interacted much more with juvenile males than did the adult females. We wanted to know if the females were mixed across age class whether the juvenile females would adopt the highly discriminating behavior of the adult females and avoid the juvenile males.

#### *Method*

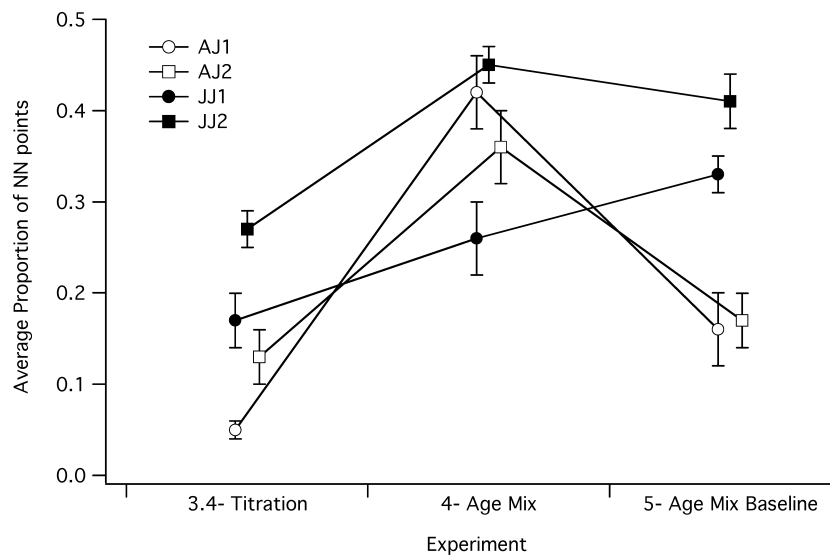
Six adult females from AJ1 were switched with six juvenile females from JJ1 and six adult females from AJ2 were switched with six juvenile females from JJ2. The birds that were switched were chosen randomly. The flocks placed in these aviaries had had no prior contact. One adult female was removed from the analysis because she was sick and no NN data were collected.

#### *Results and discussion*

All 46 females across the four aviaries increased their affiliations with males (Figures 5 and 6) as indicated by average number and proportion of NN points (Figures 1 and 2). We found consistent changes in behavior across the flocks with all groups showing the most female-male points in the mixing. For the purposes of baseline analysis, we compared a female's score for the aviary she occupied in Experiment 3.4 (before) and 5 (after). We found significant differences in female-male points between the Experiment 3.4 baseline and the Experiment 4 mix in all four aviaries and between the Experiment 4 and Experiment 5 baseline measure. We found that there was no 'home court advantage' during this experiment. The females increased their behavior regardless of the location and erased the highly significant differences in female-male affiliation observed in previous rounds. Analysis



**Figure 5.** Average number of female-male NN points during Experiment 3.4, 4, and 5. Data are displayed as means (SE).



**Figure 6.** Average proportion of female-male NN points during Experiment 3.4, 4, and 5. Data are displayed as means (SE).

of differences between adult and juvenile females in average and proportion of female-male points for all flocks revealed that the juvenile females had more NN points than the adult females in AJ1 and AJ2 but all other comparisons did not show significant differences (Tables 2 and 3). In addition, there was no age assortment between adult and juvenile females during this experiment.

We were surprised at the overall increase in the females' activity and the finding that adult females behaved more like juveniles than vice versa. Females increased their activity with both sexes but especially with juvenile males. When the adult and juvenile females were separated, the adults returned to baseline levels (see Experiment 5). Adult females may ignore juvenile males when they are housed without juvenile females, but seeing the juvenile females interacting with the juvenile males stimulated adult interest in them. When juvenile females are housed with adult females and juvenile males in late summer and early fall, juvenile females conform to adult female lack of interest in juvenile males (e.g., King et al., 2003b; White et al., 2002b). The data from this study suggest that by the late fall juvenile females have formed male preferences independent of adult females leading the adults to behave competitively with potential rivals.

### **Experiment 5: Return to baseline for age and gender mix**

We returned all the birds to the aviaries they were in just prior to the mix experiment and re-examined their assortment patterns. Did the mixing produce lasting effects?

#### *Method*

The females were returned to the Experiment 3.4 condition reversing the switch between Experiment 3.4 and 4.

#### *Results and discussion*

When the adult and juvenile females were re-separated, the behavioral changes observed in Experiment 4 generally reverted to the premix condition of Experiment 3.4. All 46 decreased their average number and proportion of NN points (Figures 5 and 6). The AJ and JJ flocks again displayed significant differences in the average number of NN points based on age class.

The two AJ flocks had fewer female-male associations than the two JJ flocks (Table 2). In addition, the two AJ flocks had a lower average proportion of female-male points than the two JJ flocks (Table 3).

In sum, the 11-day period when the two age classes were mixed did not appear to produce enduring effects. This finding is consistent with the King et al. (2003b) study that found the introduction and removal of approaching females could turn on but also turn off global flock activity (similar to the juvenile females in this study). One possible explanation for the dramatic changes that occurred in Experiment 4 was that the introduction of unfamiliar birds with different patterns of sociality produced the perturbations as opposed to differences in age and social preferences. To test this latter possibility, we proceeded to introduce a new set of unfamiliar adult females who had previously been housed with adult males and that frequently approached their male companions.

#### **Experiment 6: Introducing new adult females**

With the exception of Experiment 4 when the adult and juvenile females were mixed, the adult females generally interacted little with the juvenile males. From October 12 to November 14, we also recorded 1,176 APP in 35 7-minute blocks from another flock composed of adult males and females. This flock contained many pairs that had mated together in the previous summer. In this flock, the females were highly interactive with their males and thus displayed a level of female-male activity similar to what we observed with the juvenile females who were housed with juvenile males (overall assortment female-female: female-male 51:49 SE = 0.02 for both). Thus, we wanted to know how these new females who were currently very interactive with males would react if they were introduced to the AJ and JJ flocks. Furthermore these females represented a control for the effect of captivity, as these adults had been in the same cohort as the adults in Experiments 1-5.

We hoped to eliminate some possible explanations for the results of Experiment 4, the mixing round. When introduced to the AJ flocks, where they would be unfamiliar but accustomed to approaching males, it seemed possible they might interact with the juvenile males and then draw the resident adult females into the competition in a manner similar to the Experiment 4 age class mix. Alternatively, if they avoided the juvenile males, it would

appear that neither the fact that they were unfamiliar or accustomed to interacting with males was a deciding factor. But, if the females acted in a discriminating manner and avoided the juvenile males, it would seem to be due to the immature social and vocal behavior of the males. Finally, when these new adults were introduced to the JJ flocks we wanted to see if their prior experience with adult males might cause them to avoid the juvenile males unlike the prior adult introduction in Experiment 4.

### *Method*

From August to November 2004, the to-be-introduced adult females were housed in a large outdoor aviary measuring  $9.1 \times 20.75 \times 3.4$  m in a mixed-sex flock consisting of 20 adult females and 24 adult males. Five randomly chosen females from AJ1, AJ2, JJ1, and JJ2 were moved out into small outdoor flight cages. They remained separated by age class. Twenty adult females were moved in, five into each of the four flocks (AJ1, AJ2, JJ1, and JJ2).

### *Results and discussion*

The two AJ flocks had significantly fewer female-male associations than the two JJ flocks (Table 2; Figure 1). The average proportion of NN showed significantly different patterns based on condition (Table 3; Figure 2). Comparisons of the average and proportion of female-male points for resident versus introduced females found no significant differences.

The results indicated that the adult females introduced to the AJ flocks behaved similarly to the resident adult females and generally showed a low level of female-male interactivity. By contrast, the females introduced to the JJ flocks interacted with the juvenile males as much as the juvenile females thus replicating the previous adult introduction of Experiment 4. It seems unlikely that the results of this experiment reflect a 'home court effect' where the introduced birds simply adopted the behavior of the residents. Throughout the four titrations (Experiment 3), there was no evidence of such an effect and in the age class mix (Experiment 4), the counterbalanced resident, and introduced birds behaved similarly. Thus, it appears that when the adults were introduced to the AJ flocks, they assessed the juvenile males to be socially immature and interacted at a low level. This is not surprising given that the unpredictable rough-and-tumble play deterred the adults from

remaining in proximity to their young companions. For example, if a young male jumps on an adult female's back, she will depart the vicinity. The same scenario with a juvenile female will cause her to move away but remain in the area affording further female-male interactions. By contrast, the fact that the females introduced to the JJ flocks interacted with the juvenile males at a high rate, as much as the juvenile females, indicates that female social competition is a better predictor of flock social activity than the presence of particular male traits.

### **Experiment 7: Switching juvenile males across age class**

To follow up on Experiment 2, we again moved the juvenile males, but this time across conditions so that the males with juvenile females were now with adult females and vice versa. We wanted to know whether the behavior of the juvenile males was contributing to the different patterns of sociality we were observing across the flocks.

#### *Method*

Because the introduced adult females had adopted the sociality of the aviary they joined, they were left in the flocks instead of the original females for this experiment. Now, all males were rotated across condition. The AJ1 males were switched with the JJ1 males and the AJ2 males were switched with JJ2 males.

#### *Results and discussion*

We observed no change in NN patterns, and hence no effect attributable to the switch in males. The two AJ flocks had fewer female-male associations than the two JJ flocks. There was a significant difference across age class for the average number of female-male points (Table 2; Figure 1). The average proportion of NN points showed significantly different patterns based on age class. The two AJ flocks had fewer female-male associations than the two JJ flocks (Table 3; Figure 2).

As in Experiment 2, the identity of the juvenile males did not affect the female pattern of female-male interactivity which again replicates King et al. (2003b), who found that overall flock social patterns were regulated by female behavior.

## Discussion

We manipulated and perturbed four flocks of adult and juveniles females, each housed with juvenile males from September to December. Housing the juvenile females apart from adult females but with juvenile males revealed low sex segregation among young conspecifics, a condition we had not previously seen perhaps because adult females were always present. In numerous flocks, we have found juvenile females avoid juvenile males when housed with adult females during the summer and fall (e.g., Smith et al., 2002; White et al., 2002b; King et al., 2003b). Thus, the current study suggests that juvenile females learn to decrease their interactivity with juvenile males by observing and interacting with adult females if they are with them in the first 100 days of life.

These data, suggesting a mentoring role for older females, seem to be at odds with Experiment 4 when the juvenile and adult females were mixed. We recorded a huge increase in activity by all of the females but the adult females appeared to adopt the pattern of sociality of the juveniles. Perhaps by 150 days of age, juvenile female social preferences are sufficiently stable to resist copying adult female social preferences. Thus, differences in early experience of juvenile females with adults may explain the wide range of variation observed in adult females' responsiveness to males. This variation in female reactivity to males appears to be a major controlling variable of overall flock activity: interactions between juvenile females and young males may serve via local enhancement to draw other females into parallel interactions even if the quality of the males is low. We saw such an effect in Experiment 6 when a new set of adult females was introduced to the AJ and JJ flocks. These females avoided the juvenile males in the AJ flocks but not in the JJ flocks thereby showing an enhancement effect of the juvenile females on the adults. Experiment 3 found that when females were exchanged between flocks within the same age class the flocks remained relatively stable. In this manipulation, there was very little variation in sociality within the groups. This again points to the importance of understanding the basis of individual variation in sociality because such variation appears to be predictive of overall flock activity. Finally, in Experiments 2 and 7, the males were exchanged across the flocks. The male exchange produced no significant changes in female-male activity, replicating the findings of King et al. (2003b). The finding of a period of sensitivity for the formation of female

preferences is intriguing as it mirrors the early sensitivity of young birds to adult male song. Finally, this study indicates that young females may play an important role influencing how older females interact with males which parallels findings that young males can have both positive and negative effects on the courtship behavior of adult males (White et al., 2002c; Gros-Louis et al., 2006).

We recorded the songs of the males in the four flocks in October because female cowbird social behavior has been shown to influence male vocal behavior even though females do not sing (West & King, 1988; Smith et al., 2002; King et al., 2005). Miller (personal communication) found significant differences in the repertoire sizes of the males residing in the AJ and JJ flocks. Thus, while the males had little measurable effect on the female social activity, the females had a measurable effect on male vocal activity, as we have seen in sound-attenuating chambers where males were housed with pairs of females.

We studied two independent groups of juvenile females and two independent groups of adult females. We recognize that individuals may affect one another making the responses within flocks non-independent. That, indeed, is the whole point of looking at flocks: to gauge interactivity when birds are given broad degrees of freedom to organize their own social lives. But such individual analyses raise the issue of pseudoreplication (Hurlbert, 1984). Thus, we must be cautious in our interpretations based on comparisons of two aviaries of juveniles or adults, or four flocks when birds were exchanged across all four aviaries. For almost all comparisons of adult to juvenile aviaries, we obtained nonoverlapping values as shown in the tables and figures. In every round, juveniles were more active and affiliative than adults. In the female mixing experiment (Experiment 4), every female, juvenile and adult, increased interactions with the juvenile males. Thus, the data are robust and consistent.

Female songbirds, like females in many vertebrate systems, bring with them new methodological and theoretical issues. Lack of recognition of these differences has delayed integration with knowledge of males limiting the scope of theories and mechanisms of development and evolution (Gowaty, 1997; West et al., 2003). Gowaty places special emphasis on the lack of data in intraspecific variation among females and neglect of the view that females are active, not passive, participants in their own development. Our data identify a major source of variation, access of adult females during a

fledgling female's first summer and fall. We already know this course of variation affects male social skills (West et al., 1996; White et al., 2002b). Our data also indicate that females are active agents of ontogenetic change. Their movements and gestures affect song development directly in males and indirectly in young females (King et al., 2005). DNA analyses also now allow us to match eggs to parents so that we can use offspring viability instead of only playback song responsiveness to understand female mate choice. In this study, we were able to observe some of the earliest forms of social affiliation in this species to look for developmental variation in sociality. This new capability brought with it the surprising finding that female sociality must be influenced by early experience. This finding also fits with data from a study of early experience on hand-reared cowbirds: very young females, with no adults present, spent approximately equal time with male and female companions (Miller et al., in press). If young female behavior was simply random movement, one would expect to see these same patterns in the young males; however, very young males showed same sex assortment by the third week of life. Thus, early adult-juvenile interactions may facilitate juvenile females' acquisition of species-typical social categories, of which gender is the strongest (Smith et al., 2002). Males may pick up on the 'male' categories as soon as rudimentary song begins, starting as early as 40 days of age, allowing them to self-phenotype (Hauber & Sherman, 2003).

The habitat and conspecifics that an organism encounters on a regular basis shape its social development, setting it on a track that can affect its behavior for the rest of its life. We now know that regularly occurring small events early in development may escalate into large differences later on through a statistical learning mechanism (Aslin et al., 1998). The statistical regularities in language phonology were always thought to be an innate module because it was believed that infants could not learn subtle phonologic speech units (Pinker, 1991; Locke, 1995). Statistical regularities occur in the social realm as well (Saffran, 1996). In the same way, we need to understand how small differences early on in the female cowbirds' experience escalate into large differences in abilities later on in life. Having a better understanding of what motivates a female's behavior is key to understanding the social ecology: the reliable, predictable and dependable social events that assemble to form an animal's world.

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