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SHORT COMMUNICATION

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The reproductive choices of eavesdropping female black-capped chickadees, *Poecile atricapillus*

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Abstract In animals where males engage in signalling interactions, females might evaluate male–male contests to inform their reproductive choices. We used interactive playback to engage territorial male black-capped chickadees (*Poecile atricapillus*) of known dominance status in countersinging contests with an aggressive or submissive opponent. Previous analysis of these data showed that high-ranking males who received aggressive playback were more likely to be cuckolded. Here we describe the particular reproductive decisions of females whose partners received aggressive versus submissive playback. The proportion of extra-pair young per brood was higher for females paired to high-ranking males that received aggressive playback compared to submissive playback, and similar to levels in broods of females paired to low-ranking males. We found no strong predictors of whether high-ranking subjects lost paternity following aggressive playback. Females usually preferred extra-pair sires with high dominance status. When females had extra-pair fertilizations with low-ranking males, females chose males who had received submissive playback. We conclude that females mated to aggressive-playback, high-ranking males pursued mixed mating strategies similar to those of females mated to low-ranking males. Our results support the idea that male performance in song contests may influence multiple aspects of female reproductive choices.

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Introduction

Male song plays an important role in female choice of social partners and copulation partners in many different animals (Searcy and Yasukawa 1996), yet how male song influences female reproductive choices remains poorly understood. Within a communication network involving many singing males, females may extract information from signalling interactions between males without being directly involved in those interactions (McGregor and Dabelsteen 1996). Through such eavesdropping behaviour, females may make low-cost, direct comparisons between males to inform decisions about their mating behaviour (McGregor and Dabelsteen 1996; Otter et al. 1999).

Black-capped chickadees (*Poecile atricapillus*) provide a model system for investigating the relationship between male song contests and females' reproductive choices. Female mixed-mating tactics follow a predictable pattern based on the status of males in winter-flock dominance hierarchies; females paired to high-ranking males rarely engage in a mixed reproductive strategy whereas females paired to low-ranking males frequently target high-ranking neighbours as extra-pair partners (Smith 1988; Otter et al. 1998; Mennill et al. 2003). Male chickadees sing a two note song ("fee-bee") that they transpose across a frequency continuum of approximately 800 Hz (Horn et al. 1992). During countersinging contests, territorial males often frequency-match and overlap the songs of their opponents, behaviours that are both thought to be signals of directed aggression (Mennill and Ratcliffe 2003).

Previously we used interactive playback to engage territorial male black-capped chickadees in song contests with a simulated opponent. The simulated opponent either frequency-matched and overlapped the male (aggressive playback) or avoided frequency matching and overlapping (submissive playback). Females were more likely to engage in a mixed reproductive strategy after their high-ranking partner received aggressive playback (Mennill et al. 2002). However, females may alter multiple aspects of

their mixed strategy based on male song contest performance, including what proportion of the brood to devote to mixed matings and whom to choose as an extra-pair partner. Here we extend analyses of the experiments described in Mennill et al. (2002) to explore these female reproductive decisions. Our goal was threefold: (1) to compare the proportion of extra-pair young in the broods of high- and low-ranking playback subjects, (2) to compare the extra-pair partners chosen by females paired to manipulated high- and low-ranking males, and (3) to examine whether playback subjects' likelihood of being cuckolded could be predicted by any feature of the male, his partner, or their previous breeding experience.

Materials and methods

Each January of 1999–2001 we colour-banded all adult chickadees in a 2.0-km^2 study area at Queen's University Biological Station. We measured each bird's wing length, tarsus length, and mass. In February and March of each year, we assessed winter flock dominance hierarchies, which are stable across feeding sites (Smith 1991), by tabulating pairwise interactions between birds at feeders (see Mennill et al. 2003). Over 3 years we observed 8,886 pairwise dominance interactions between birds in 61 winter flocks. We used dominance interactions to assign males to high-ranking or low-ranking status (see Mennill et al. 2002). We also assigned each male a continuous rank score to facilitate comparisons between males across flocks; we calculated the total number of interactions won by each male divided by the total number of interactions in which he was involved.

Interactive playback

We used interactive playback to engage territorial male chickadees in countersinging interactions with a simulated male opponent. We ran Syrinx-PC software (J. Burt, http://www.syrinxpc.com) on a laptop computer connected to a microphone and a loudspeaker (standardized to amplitude 90 dB at 1 m). Observers remained $\geq$18 m from the speaker during trials. Playback began with "chick-a-dee" calls to lure territorial birds to the speaker location, near the centre of each male's territory, and to incite the subject to sing. For each subject song, we gave exactly one song in response according to the appropriate treatment (see below). Trials lasted a maximum of 6 min (average trial length: 5.48±0.30 min). Stimulus design is described in Mennill and Ratcliffe (2003).

During playback, we simulated an aggressive or a submissive opponent. In aggressive trials we overlapped every song given by the subject (playback song began before subject's song was complete) and matched the frequency of the subject's songs with an accuracy of 50 Hz. In submissive trials we delayed playback responses (playback song began ~1.5 s after subject's song was complete) and avoided matching the frequency of the subject's songs by singing 300–400 Hz higher than the subject. In control playback sessions, we mimicked natural territorial encounters (D.J. Mennill, personal observation) to reinforce playback subjects' social status; we gave submissive playback to high-ranking males and aggressive playback to low-ranking males. In experimental playback sessions, we attempted to alter females' perceptions of their social partners; we gave aggressive playback to high-ranking males and submissive playback to low-ranking males.

We conducted playback trials between April 24 and May 8, a time period when birds have broken out of winter flocks and males routinely engage in territorial song contests. We gave playback to males between 0800 and 1200 hours on the 1st or 2nd day of their partner's fertile period (determined by observing nest lining, female vocal behaviour, or direct nest inspection; Smith 1991). Subjects were distributed across the study site and neighbours were not given playback in direct succession. Playback trials were repeated to each male on two successive mornings. In all treatments, we followed playback subjects and their partners for 30 min between 0600 and 0800 hours prior to the 1st day of playback and 30 min at the same time of day following the 2nd day of playback.

Paternity assignment

We analyzed paternity through PCR amplification using three highly variable microsatellite loci (combined exclusionary power of 0.995; full primer details in Mennill et al. 2003). We considered birds to be extra-pair young if they had two (n=22) or three (n=15) allelic mismatches with their social father. The inclusion of an additional (third) locus produced identical results to those reported in Mennill et al. (2002) except for one brood where a nestling mismatched his social father at only one of three loci and was thus considered within-pair in the present study.

To assign paternity, we compared the genotypes of extra-pair young to all known males. We re-ran putative extra-pair fathers next to extra-pair young at all three loci to confirm assignments. For 36 of 37 extra-pair young, only 1 extra-pair male matched each offspring at all three loci; for the remaining extra-pair young, from a nest at the edge of our study population, no known male matched at all three loci.

Statistical analyses

We gave experimental playback to 24 high-ranking males and 24 low-ranking males. We gave control playback to 12 high-ranking males and 12 low-ranking males. Nest predation and adult mortality restricted our paternity analyses to the broods of 23 experimental high-ranking males, 17 experimental low-ranking males, 10 control high-ranking males, and 6 control low-ranking males. We contrast the proportion of extra-pair young in experimental and control broods against unmanipulated broods (26 high-ranking males' broods, 21 low-ranking males' broods) from a concurrent study (Mennill et al. 2003). Values are reported as mean±SE. All tests are two-tailed.

Results

Proportion of extra-pair young

Playback manipulations influenced the proportion of extra-pair young per brood for high-ranking playback subjects but not low-ranking playback subjects. High-ranking males who received aggressive playback had a higher proportion of extra-pair young per brood than high-ranking males who received submissive playback or no playback (Fig. 1A). All low-ranking males had similar proportions of extra-pair young per brood (Fig. 1B).

Extra-pair partner choices

For 11 of 23 high-ranking males who lost paternity following aggressive playback, we identified 11 extra-pair sires (1 male lost paternity to 2 extra-pair sires and 1 extra-pair young could not be assigned). Females paired to aggressive-playback high-ranking males chose extra-pair sires who had similar high rank scores to their social partners (Fig. 2A; Z=0.8, P=0.43) and similar high rank

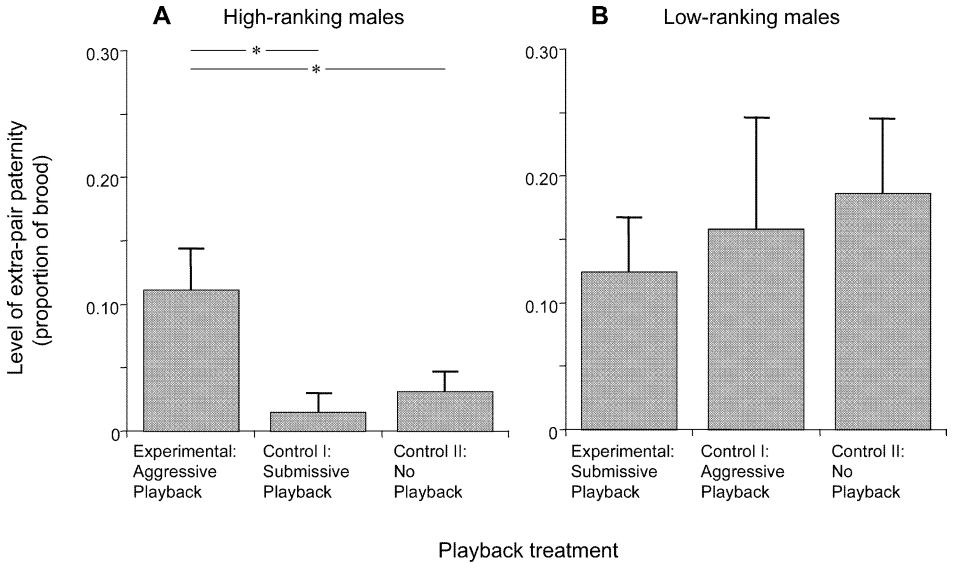

Fig. 1A, B Average proportion of extra-pair young per brood for playback-manipulated and control black-capped chickadees. **A** Females paired to high-ranking males who received aggressive playback (n=23) had a greater average proportion of extra-pair young per brood than females paired to high-ranking males who received submissive playback (n=10; Z=2.0, P=0.04) and females paired to high-ranking males who received no playback (n=26 broods from Mennill et al. 2003; Z=2.4, P=0.02). **B** Females paired to low-ranking males that received submissive playback (n=18) had similar average proportions of extra-pair offspring to females paired to low-ranking males who received aggressive playback (n=6; Z=0.5, P=0.62) and females paired to low-ranking males who received no playback (n=22 broods from Mennill et al. 2003; Z=0.4, P=0.67). *Asterisks* indicate significant differences at P<0.05. *Error bars* show standard error

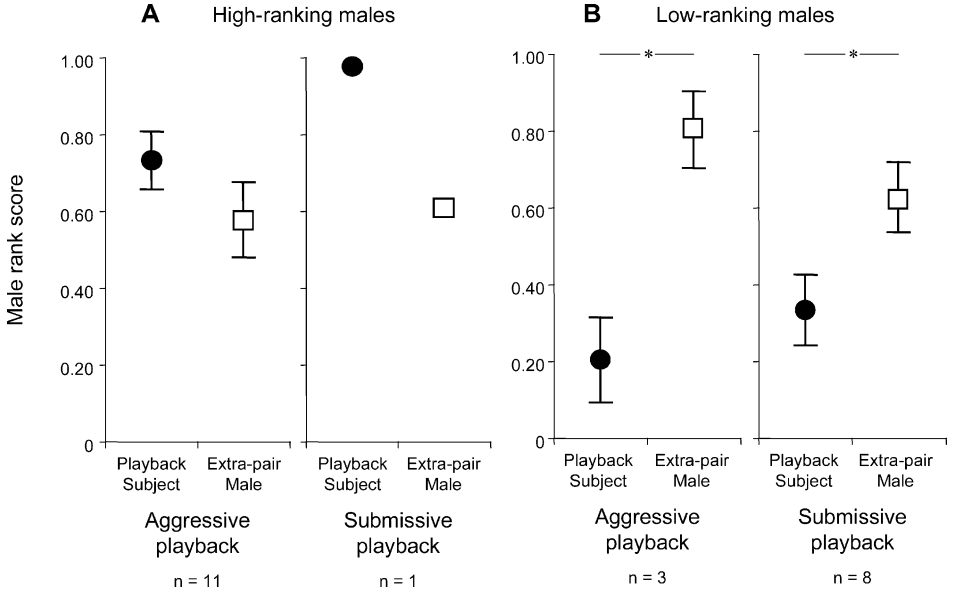

Fig. 2A, B Relative rank scores of playback subjects (*closed circles*) and the extra-pair males who cuckolded them following interactive playback sessions (*open squares*). **A** Females paired to high-ranking males chose extra-pair males with non-significantly lower rank scores, whether their partner had received aggressive or submissive playback (paired t-test for aggressive playback males: t=–1.1, P=0.32). **B** Females paired to low-ranking males chose extra-pair males with significantly higher rank scores, whether their partner had received aggressive or submissive playback (paired t-test for aggressive playback males: t=5.4, P=0.03; submissive playback males: t=2.4, P=0.05). *Asterisks* indicate significant differences at P<0.05. *Error bars* show standard error

scores to the extra-pair sires chosen by females paired to low-ranking males (Fig. 2; Z=0.9, P=0.36). In 9 of 11 cases, females chose extra-pair sires who belonged to a neighbouring flock during the previous winter. In the remaining 2 cases, females made very unusual mate-choice decisions; they had extra-pair young with a neighbouring low-ranking male from their own winter flock, a behaviour that has never been reported in black-

Table 1 Variables related to whether high-ranking male black-capped chickadees lost paternity following aggressive playback treatment. To maintain an experiment-wide error of 0.05, the alpha for each individual test must be lowered to 0.0015. Means and SE are presented

Variable	Male lost paternity following playback (n=11)	Male did not lose paternity following playback (n=12)	Test statistic	P
Male characteristics				
Rank score	0.73±0.06	0.83±0.05	Z=–0.6	0.56
Age (years)	2.9±0.4	2.6±0.3	$F_{1,22}$=0.4	0.52
Wing length (mm)	67.7±0.5	68.7±0.5	$F_{1,22}$=2.1	0.16
Tarsus length (mm)	14.8±0.3	14.7±0.3	$F_{1,22}$=0.0	0.83
Weight (g)	12.0±0.1	12.0±0.1	$F_{1,22}$=0.1	0.71
Male behavior during playback				
Proportion of playback <5 m from speaker (%)[a]	0.53±0.11	0.39±0.10	$F_{1,22}$=0.9	0.36
Proportion of playback >10 m from speaker (%)[a]	0.29±0.11	0.38±0.10	$F_{1,22}$=0.4	0.55
Closest approach distance to speaker (m)	4.5±2.4	1.9±2.3	$F_{1,22}$=0.6	0.44
Number of flights during playback	8.6±1.8	11.6±1.8	$F_{1,22}$=1.3	0.26
Number of passes over speaker during playback	1.9±0.8	2.4±0.8	$F_{1,22}$=0.2	0.66
Male singing performance during playback				
Number of songs sung by subject	51.6±8.3	64.4±7.6	$F_{1,22}$=1.3	0.27
Average pitch of subject songs (Hz)	3,395±61	3,359±55	$F_{1,22}$=0.2	0.67
Song length average (s)	0.96±0.02	0.98±0.02	$F_{1,22}$=0.6	0.45
Song length coefficient of variation (%)[a]	0.09±0.02	0.09±0.02	$F_{1,22}$=0.0	0.97
Intersong interval average length (s)	2.97±0.22	3.28±0.20	$F_{1,22}$=1.1	0.30
Intersong interval coefficient of variation (%)[a]	0.37±0.03	0.31±0.03	$F_{1,22}$=1.5	0.22
Changes in male behavior following playback				
Proportion of observation period male spent singing (%)[a, b]	0.12±0.19	0.18±0.17	$F_{1,19}$=0.1	0.82
Number of nuptial feeds male provided to female[b]	–0.25±0.25	–0.33±0.23	$F_{1,19}$=0.1	0.81
Number of male copulation solicitations[b]	0.01±0.66	0.48±0.59	$F_{1,19}$=0.2	0.63
Number of within-pair copulations[b]	0.05±0.72	0.13±0.68	$F_{1,19}$=0.1	0.82
Playback features				
Number of songs sung by playback	51.6±8.3	64.4±7.6	$F_{1,22}$=1.3	0.27
Average pitch of playback songs (Hz)[c]	3,379±58	3,382±53	$F_{1,22}$=0.0	0.97
Playback versus subject pitch difference (Hz)[c]	–15.2±12.8	22.0±11.7	$F_{1,22}$=4.6	0.04
Average playback overlap of subject songs (s)	0.34±0.05	0.33±0.05	$F_{1,22}$=0.0	0.92
Female characteristics				
Age (years)	2.5±0.4	2.0±0.4	$F_{1,22}$=0.7	0.42
Wing length (mm)	63.9±0.4	63.6±0.4	$F_{1,22}$=0.4	0.54
Tarsus length (mm)	14.8±0.2	14.0±0.2	$F_{1,22}$=14.7	0.001
Weight (g)	10.7±0.1	10.8±0.1	$F_{1,22}$=0.5	0.52
Brood size (number of nestlings)	5.8±0.6	6.7±0.6	$F_{1,22}$=1.0	0.33
Synchrony index[d]	0.46±0.07	0.46±0.07	$F_{1,22}$=0.0	1.00
Previous breeding experience				
Number of years pair together	1.3±0.2	1.2±0.2	$F_{1,22}$=0.2	0.64
Number of times male had bred previously	1.9±0.4	1.6±0.3	$F_{1,22}$=0.4	0.52
Number of times female had bred previously	1.0±0.4	1.5±0.4	$F_{1,22}$=0.7	0.42

[a] Statistical analyses conducted on arcsin transformed data, actual values reported
[b] Difference between level of each behavior on the morning before 1st day of playback and the morning following 2nd day of playback are reported (n=20 males for whom we had 30-min behavior watches on both mornings)
[c] Effective resolution of 1 Hz for all frequency measures
[d] Synchrony index calculated according to Kempenaers (1993)

capped chickadees (Smith 1988; Otter et al. 1998; Mennill et al. 2003). In both cases, the low-ranking sire had received submissive playback.

Only 1 of 10 high-ranking males who received submissive playback lost paternity following playback (Fig. 2A). In this case, the female chose a high-ranking extra-pair sire from her own winter flock (the beta male in a flock of four males).

For 7 of 17 submissive-playback low-ranking males and 3 of 6 aggressive-playback low-ranking males who lost paternity following playback, we identified 11 extra-pair sires (1 male lost paternity to 2 extra-pair sires). Females paired to low-ranking males chose extra-pair sires with higher rank scores than their social partners, whether their partner had received aggressive or submissive playback (Fig. 2B). In 9 of 11 cases, females paired to low-ranking playback subjects had extra-pair young with high-ranking males from their own winter flock (n=3) or a neighbouring winter flock (n=6). In the remaining 2 cases, females chose low-ranking males from neighbouring flocks; in both cases the low-ranking sire had received submissive playback.

Correlates of playback effect on paternity

For the 23 high-ranking males who received aggressive playback, no characteristics of the playback subject, his behaviour during playback, his singing performance during playback, his behaviour following playback, or the playback itself showed a relationship with the presence of extra-pair young in the subject's brood (Table 1). Females with long tarsi were more likely to engage in a mixed mating strategy (Table 1) although the difference between the tarsus length of promiscuous and monogamous females was small (0.85 mm). No other female characteristic (Table 1) or any feature of the pair's breeding experience (Table 1) showed a relationship with female promiscuity.

Discussion

Following playback to high-ranking male black-capped chickadees, females paired to males who "lost" song contests to aggressive opponents produced a greater proportion of extra-pair young per brood than females paired to males who "won" song contests with submissive opponents or males who received no playback. Females paired to low-ranking males had similar proportions of extra-pair young whether their partner received aggressive playback, submissive playback, or no playback. Choice of extra-pair partners by females paired to low-ranking playback subjects followed a species-typical pattern (Smith 1988; Otter et al. 1998), where females sought extra-pair partners with significantly higher rank scores than their social partner, either from their own winter flock or a neighbouring winter flock. Females paired to high-ranking males who received aggressive playback chose extra-pair partners with similarly high rank scores. In the few cases where females had extra-pair young with low-ranking males, including the first recorded cases of females cuckolding a high-ranking male for a low-ranking male from the same winter flock, females chose low-ranking males who had "won" song contests with submissive intruders.

Females paired to aggressive-playback high-ranking males responded to the apparent change in their partner's status in the same way that control females behaved when paired to a low-ranking male. First, the proportion of extra-pair young per brood was similar among females whose high-ranking partners received aggressive playback, females paired to unmanipulated low-ranking males (Mennill et al. 2003), and females paired to experimental low-ranking males (Fig. 1). Second, the average rank score of the extra-pair sires chosen by females paired to aggressive-treatment high-ranking males matched the average rank score of the extra-pair sires chosen by females paired to unmanipulated low-ranking males (Mennill et al. 2003) and experimental low-ranking males (Fig. 2). Previously we showed that aggressive playback to high-ranking males incited females to pursue a mixed mating strategy (Mennill et al. 2002). Here we demonstrate that these females did not adopt a radically different extra-pair mating tactic but instead made very similar reproductive choices to those made by females paired to low-ranking males.

No features of high-ranking playback subjects, their behaviour or singing performance during playback, their behaviour following playback, or their previous breeding experience could predict whether males lost paternity following aggressive playback treatments. Females who engaged in a mixed reproductive strategy following aggressive playback to high-ranking males had longer tarsi than females who remained monogamous. Given that tarsus length is a good indicator of skeletal body size (Senar and Pascual 1997), these females may have an advantage in physical encounters that appear to be common when female black-capped chickadees seek extra-pair copulations (D.J. Mennill, personal observation). Alternatively, this may be a spurious relationship given that female tarsus size shows no relationship with female mating strategy in unmanipulated nests (D.J. Mennill, unpublished data). Generally, we found no strong predictors of whether aggressive-playback high-ranking males were cuckolded following playback, suggesting that male performance in song contests is not the only cue used by females to inform their reproductive choices.

Previously we showed that the behaviour of high-ranking males did not change following aggressive playback (Mennill et al. 2002). Here we show that the behaviour of aggressive-playback high-ranking males did not vary with the mating strategy of their partners (Table 1). This suggests that females altered their reproductive choices by assessing the playback countersinging interactions directly, as opposed to assessing changes in their partner's post-playback behaviour. However, we cannot discount the alternative interpretation that playback manipulations influenced some aspect of male behaviour that we did not detect during our pre- and post-playback observations.

As a sexually selected ornament, male song has both an intrasexual competitive function and an intersexual courtship function (Berglund et al. 1996). Given that females revise their reproductive decisions based on male performance in countersinging interactions, male song can operate in an intrasexual context and intersexual context simultaneously; a signal that repels a male opponent may consequently attract an eavesdropping female, and, conversely, a signal that fails to repel a male opponent may consequently fail to attract an eavesdropping female. We suggest that this dual context of male song may influence female mating preferences in many different song systems. For example, in many songbirds females prefer males with large repertoires over males with small repertoires (Searcy and Yasukawa 1996). Given that song-type matching is an aggressive signal in many species (Vehrencamp 2001), and that males with large repertoires will be better able to match their opponents, female preference for large repertoire size may be a manifestation of female preference for males

who type-match their countersinging opponents. By shifting our focus away from the conventional dyadic model of information exchange towards a network-based model, we can include female eavesdropping on male–male countersinging contests as an important process in sexual selection. Given the fitness consequences of female eavesdropping that we have demonstrated, female assessments of male contest behaviour may be a very important selective force indeed.

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