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Extrapair paternity and mate choice in a chickadee hybrid zone

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The dynamics of hybrid zones are likely to be influenced greatly by patterns of mate choice, including "cryptic" choice mediated through extrapair copulations. To understand changes in hybrid zones over time and space, a detailed examination of mating patterns and correlates is needed. We studied the role of extrapair fertilizations (EPFs) in the breeding biology of hybridizing black-capped and Carolina chickadees in southeastern Pennsylvania over 4 years, using microsatellite DNA markers. We detected extrapair offspring (EPO) in 56% of 90 broods examined; these accounted for at least 26% of 477 offspring. Chickadees do not appear to use EPFs to reduce costs of heterospecific pairing: EPFs were no more likely to occur in genetically dissimilar (heterospecific) social pairs than in pairs where social mates were genetically similar. However, females paired with black-capped–like males were more likely to have EPO. Females that acquired EPFs did not obtain these from males genetically similar to themselves; instead, all females, regardless of their genotype or that of their social mate, tended to prefer Carolina-like males as extrapair partners. There was no relationship between the presence of EPO and hatching or fledging success. High rates of extrapair paternity and apparent female preference for Carolina-like males suggest that mate choice is an important influence in ongoing northward movement of this hybrid zone. *Key words:* chickadee, extrapair, hybrid, mate choice, paternity, poecile. *[Behav Ecol 17:56–62 (2006)]*

Within socially monogamous avian species, genetic studies have revealed that many birds pursue extrapair copulations (EPCs) as part of a mixed mating strategy (Griffiths et al., 2002; Petrie and Kempenaers, 1998; Spottiswoode and Møller, 2004; Westneat and Stewart, 2003; Westneat et al., 1990). Although extrapair paternity occurs widely, levels of inter- and intraspecific variation are high, and our understanding of the causes and consequences of pursuing EPCs is still limited. Much of the recent work on EPCs has focused on the role of the female because it now appears that in a majority of bird species, females control the success of copulations (Neff and Pitcher, 2005; Petrie and Kempenaers, 1998). In some bird species, females appear to actively pursue EPCs (Otter et al., 1998; Petrie and Kempenaers, 1998; Ramsay et al., 2000; Smith, 1988), though in most studies, detailed observations of EPCs are sparse or nonexistent.

While females could obtain direct benefits through extrapair mating, we focus here on mechanisms involving indirect benefits, that is, benefits mediated through the quality of offspring resulting from EPCs. Reasons for females pursuing EPCs generally fall under two hypotheses involving indirect benefits (Neff and Pitcher, 2005; Petrie and Kempenaers, 1998). First, the "good genes" hypothesis states that females pursue EPCs to obtain offspring of higher genetic quality than they would by mating with their social mate alone. According to this hypothesis, good genes exhibit additive genetic variation, and selection can be expected to favor choice by all females of the same high-quality males. Second, the "genetic compatibility" hypothesis states that females should choose extrapair mates whose genotypes, in combination with each female's own, result in offspring of higher fitness. Under this mechanism, females can be expected to differ in their choice of extrapair partners because of variation in their own genotypes.

Hybrid zones present ideal opportunities for testing the hypotheses involving indirect benefits because pairing with a heterospecific can result in substantial fitness costs (e.g., reduced reproductive success or offspring fecundity). In a study on hybridizing pied and collared flycatchers (*Ficedula* spp.), Veen et al. (2001) demonstrated that females paired with heterospecific males exhibited higher rates of extrapair fertilization (EPF) than females in conspecific pairings. Females obtaining EPFs did so primarily with conspecifics, indicating that the pursuit of EPCs may be an adaptive mating strategy aimed at reducing the costs of socially pairing with a male of the "wrong" species. Because females of the two species chose different partners, the flycatcher system can be viewed as a system consistent with the genetic compatibility hypothesis.

An open question is whether the findings of Veen et al. (2001) represent a general phenomenon in avian hybrid zones. In this study, we investigate the genetic compatibility and good genes hypotheses by examining social pairing and extrapair mating in a population of hybridizing black-capped chickadees (*Poecile atricapillus*) and Carolina chickadees (*Poecile carolinensis*) in southeastern Pennsylvania.

For a flycatcher-like adaptive mating strategy involving EPCs to develop in an avian hybrid zone, there must be reproductive costs to choosing the wrong mate. Consistent with this assumption, reproductive success of pairs from within the black-capped/Carolina contact zone, which stretches from Missouri to New Jersey (Mostrom et al., 2002), is indeed lower than that of pairs from nearby parental populations. Bronson et al. (2003a, 2005) showed that hatching and fledging successes are reduced by 50% or more in the Ohio portion of the hybrid zone. Within the southeastern Pennsylvania portion of the contact zone, hatching success and subsequent fledging production are also reduced, as a population average, relative to nearby parental reference populations, but only by about 15% (Cornell, 2001; Curry R, unpublished data). These studies together suggest that there can be sizeable fitness costs

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resulting from pairing with a heterospecific chickadee, although genetic analysis and direct observation have also indicated that F1 and subsequent generation chickadee hybrids can be viable and fertile (Curry RL, unpublished data).

For birds to pursue a flycatcher-like strategy for avoiding costs of heterospecific pairing, EPCs must be part of their reproductive repertoire. Multiple studies in Ontario, Canada, have revealed that EPFs play a substantial role in the breeding biology of black-capped chickadees, with 29–50% of nests containing extrapair offspring (EPO), including 9–21% of all offspring (Doucet et al., 2005; Mennill et al., 2004; Otter et al., 1994, 1998). Preliminary evidence suggests that Carolina chickadees in a pure population in southeastern Pennsylvania also pursue EPFs at rates roughly comparable to those of black-capped chickadees (Curry R and Ruscica A, unpublished data). The possibility that chickadees within the black-capped/Carolina contact zone engage in EPCs has been assessed previously only in the two studies in Ohio, using multilocus fingerprinting methods (Bronson et al., 2003a, 2005); however, no EPO were detected.

Black-capped and Carolina chickadees nevertheless meet, potentially at least, both the requisite conditions for developing a mating strategy involving EPCs in response to costs of hybridization. To conduct the present study, it was necessary to (1) establish the genetic identity of breeders within the hybrid zone and (2) identify EPO, assigning actual paternity when possible. Because of the high degree of hybridization within our study area and the phenotypic similarity of the two species, we needed to employ genetic markers that could be used to "rank" hybrids within the contact zone. Recent studies have used microsatellite markers to establish hybrid indices for examining introgression in trout and salamanders (Hansen et al., 2000; Storfer et al., 2004). Microsatellite markers have also been used successfully for paternity analysis in many bird species, with highly polymorphic loci yielding high probabilities of paternity exclusion (e.g., Doucet et al., 2005; Mennill et al., 2004; Webster et al., 2001). In this study, we used microsatellite DNA analysis both to establish the genetic identity of chickadees in the contact zone and to assess the paternity of nestlings within the study site over a 4-year period.

To understand the role of extrapair paternity within our hybridizing black-capped/Carolina chickadee population, we determined the genetic identity of all breeders along a continuum of hybridization (0 = Carolina, 1 = black-capped). We then tested the following predictions: (1) females in genetically dissimilar pairs are more likely to have EPO, (2) females in genetically dissimilar pairs choose extrapair partners that are genetically similar to themselves, and (3) females in genetically dissimilar pairs that acquire EPO have nests with high hatching and fledging successes.

METHODS

Field methods

During four breeding seasons, 2000–2003, we monitored up to 151 artificial nest snags (made from plastic tubing, based on Grubb and Bronson, 1995) and 12 nest boxes in the black-capped/Carolina chickadee hybrid zone at the 269-ha Nolde Forest Environmental Education Center, Reading, Pennsylvania (40° 17′ N, 75° 58′ W). In each year, we studied an average of 29 nests in the tubes and nest boxes. Most chickadee pairs at Nolde use these structures for nesting. We also searched for nests in natural sites (dead trees) within the study area; data for both breeders and all nestlings from two such nests are included here. Nest tubes were monitored during the spring to determine residence of birds and to determine social pairings, laying date, clutch size, hatching success, and fledging success. We captured all adults either prior to the breeding season at feeders maintained during the winter using mist nets or walk-in treadle traps or during the nestling period using nets in front of each nest. We gave each adult a unique combination of a numbered Fish and Wildlife Service aluminum band and two or three plastic color bands. Identity of the social parents at each nest was determined from multiple observations of individuals excavating, egg laying, and feeding at the nest.

We collected blood samples from 114 broods and social parents during 2000–2003 by piercing the ulnar vein (Gaunt and Oring, 1999) and drawing 20–40 μl of blood into a microcapillary tube. We sampled blood from nestlings 9–12 days after hatching and from adults at the time of banding.

For genetic analyses, we used data from 90 families for which we obtained complete genotypes of both breeders and all nestlings. We used blood samples from 24 breeders at Hawk Mountain Sanctuary, Kempton, Pennsylvania (40° 38′ N 75° 59′ W; black-capped chickadees) and 45 breeders at Great Marsh, East Nantmeal Township, Pennsylvania (40° 08′ N, 75° 44′ W; Carolina chickadees), collected using similar methods during companion studies at these additional sites, to represent parental populations when assessing genetic identity of breeders at Nolde. Relative to Nolde, Hawk Mountain is 41 km NNW and Great Marsh is 24 km SE.

Molecular methods

DNA extraction

Blood taken from adults and nestlings was stored in lysis buffer for later extraction. Total genomic DNA was then extracted using the standard protocol (Qiagen DNeasy Tissue Handbook) for the Qiagen DNeasy Tissue extraction kit.

Mitochondrial DNA analysis

We used restriction fragment length polymorphism to determine mitochondrial haplotypes of breeders at Nolde Forest, Hawk Mountain, and Great Marsh. Because of a single base pair substitution, *Eco*RV cuts a black-capped cytochrome *b* fragment amplified by polymerase chain reaction (PCR), but not that of Carolina chickadee, whereas *Xmn*I cuts the Carolina cytochrome *b* fragment, but not that of black-capped chickadee (Kvist et al., 1996). Digested products were run through 3% agarose gels stained with ethidium bromide and visualized under ultraviolet light. We used information from mitochondrial DNA (mtDNA) analysis to examine the degree of concordance between nuclear (microsatellite) DNA results for genetic identity and mtDNA haplotype; this step was necessary particularly as a screen for the Hawk Mountain reference individuals because recent northward movement of the contact zone has brought a few hybrid or Carolina individuals into that population (Reudink M and Curry R, unpublished data; see later).

Microsatellite DNA analysis

We used six microsatellite loci isolated from black-capped chickadees (Otter et al., 1994) or blue tits (Dawson et al., 2000; Table 1) for genetic identity and paternity analysis. We analyzed 65 breeding adults from the Great Marsh population of Carolina chickadees (collected during 1998–2002) and 51 breeding adults from the Hawk Mountain black-capped chickadee population (collected during 1998–2000) for reference samples when setting up a hybrid index (I_H). We then analyzed DNA extracted from 145 breeding adults from Nolde Forest. Samples were amplified via PCR using fluorescently labeled forward primers. Amplified microsatellite loci were then run on an automated genetic analyzer (ABI 310) with

Table 1
Microsatellite primers used in this study, including the number alleles found in the original study and the species from which the microsatellites were isolated

Locus	Primer sequence (5′–3′)	Number of alleles in original study	Number of alleles at Nolde Forest	Isolated from species	Reference
Pca 2	F: GTT GGC CTT CTT GGC CCC R: TGT TGG AGG TTA GGA GGC CTC T	9	28	*Cyanistes caeruleus*	Dawson et al. (2000)
PCA 4	F: AAT GTC TTA CAG GCA AAG TCC CCA R: AAC TTG AAG CTT CTG GCC TGA ATG	10	24	*Cyanistes caeruleus*	Dawson et al. (2000)
Pca 8	F: ACT TCT GAA ACA AAG ATG AAA TCA R: TGC CAT CAG TGT CAA ACC TG	33	61	*Cyanistes caeruleus*	Dawson et al. (2000)
Pca 9	F: ACC CAC TGT CAA GAG CAG GG R: AGG ACT GCA GCA GTT TGT GGG	17	21	*Cyanistes caeruleus*	Dawson et al. (2000)
Pat 2–14	F: GAA CAG ATA AAA GCC AAA TTA C R: TAG TGA ATG CTT GAT TTC TTT G	13	28	*Poecile atricapillus*	Otter et al. (1998)
Pat 2–43	F: ACA GGT AGT CAG AAA TGG AAA G R: GTA TCC AGA GTC TTT GCT GAT G	19	21	*Poecile atricapillus*	Otter et al. (1998)

GeneScan Version 3.7 software (Applied Biosystems Inc., Foster City, California, USA). Scoring of peak sizes was conducted blind by a single observer (M.W.R.), with no indication as to the individual's identity, using Genotyper Version 3.7 software (Applied Biosystems). All homozygous peaks were scored at least twice and the raw data examined to ensure accuracy. All nonamplifying loci were reamplified via PCR and rerun on the genetic analyzer.

Genetic identity and hybrid index

To analyze the distinctiveness of the two "pure" species populations and to isolate individuals from Hawk Mountain that may in fact be Carolina or hybrid chickadees rather than pure black-capped chickadees, we performed an iterative maximum likelihood assignment test, following the methods of Storfer et al. (2004). All birds were originally assigned to a starting population at random; then, using the Doh! calculator (Brzustowski, 2002, adapted from Paetkau et al., 1995), we generated maximum likelihood scores for inclusion in each group (i.e., probability of being from Hawk Mountain or Great Marsh based on the multilocus microsatellite genotype). Each individual was then assigned to the group with the highest maximum likelihood score. With the individuals now reassigned according to the previous analysis, we repeated the process. Those individuals that were improperly assigned were reassigned into the group for which they now had the highest maximum likelihood score. We continued this procedure until most of the birds ceased switching groups between iterations. After 15 iterations, only 9 of the 116 birds continued to switch groups. Seven more iterations were conducted, and the same nine individuals switched groups between each iteration.

At the completion of the iterative assignment test, individuals were examined with respect to their known population (Hawk Mountain or the Great Marsh). Misassigned individuals ($n = 15$) and the nine individuals that continued switching during the iterative assignment test were excluded from subsequent analyses, as were birds from Hawk Mountain with a Carolina mtDNA haplotype ($n = 4$) and those individuals that were less than 100 times more likely to come from one group compared with the other ($n = 19$). After these exclusions, we were left with 24 birds from Hawk Mountain and 45 birds from the Great Marsh to be used as baseline/representative samples for analyzing individuals at Nolde Forest.

To further test the classifications of the iterative assignments and confirm the genetic distinctiveness of the two populations, we conducted an analysis in GeneClass 2.0 (Piry et al., 2004), whereby we ran a simulation to assess the probability of a genotype being derived from a source population (Hawk Mountain or Great Marsh). The simulation takes a bootstrap approach, simulating 10,000 random multilocus genotypes generated from the source population alleles and creating a frequency distribution of the marginal probability values that are generated. Once the frequency distribution is generated, the marginal probability of an individual's genotype belonging to one population or the other is compared with the random distribution of marginal probabilities, and a threshold is set at either the 5% or 1% level (i.e., there is a 95% or 99% probability of that individual's genotype coming from the given population), outside of which the individual is rejected from that population.

The population structure of the baseline sample birds was then analyzed by examining the assignment probabilities, where p_x is the likelihood of an individual belonging to population x and p_y is the likelihood of an individual belonging to population y, and then applying a formula to obtain an I_H score. The I_H (adapted from Hansen et al., 2000) was calculated as:

$$I_H = 1 - \ln(p_x)/[\ln(p_x) + \ln(p_y)].$$

This formula serves to assign each individual a score between 1 and 0. Ideally, individuals of one species should have scores close to 1, while the other species have scores close to 0; however, when a high number of alleles are shared between species, discriminatory power can be weakened (Hansen et al., 2000).

After determining the two baseline populations, the genotypes of individuals at Nolde Forest were analyzed to determine an I_H score. We conducted a maximum likelihood assignment test of the microsatellite genotype data to assign individuals at Nolde Forest to one of the baseline populations using the software program WHICHRUN (Banks and Eichert, 2000). Using this approach, "known" samples determined previously were used as the parental pools for assigning "unknown" individuals (i.e., individuals from Nolde Forest). This program was chosen because it allows unknown individuals to be assigned to a parental population without changing the parental populations (Storfer et al., 2004). Like the Doh! calculator, WHICHRUN generates likelihood ratios for belonging to one population relative to the other population. If an individual has a much higher likelihood ratio from one population compared with the other, the probability of it belonging to the assigned population is high. Again, the formula for calculating the I_H was applied to the values of

p_x and p_y to assign the individuals in the hybrid zone a value between 0 and 1.

The I_H ranges from 0 to 1, and ideally, parental populations should be closest to either end of the spectrum, while hybrids exhibit intermediate scores (Campton and Utter, 1985). I_H scores for individuals used to establish reference populations from Hawk Mountain ranged from 0.53 to 0.69 (average = 0.60 ± 0.04, $n = 24$), scores from Great Marsh ranged from 0.36 to 0.46 (average = 0.41 ± 0.03, $n = 45$). Birds from Nolde Forest had I_H scores ranging from 0.40 to 0.58 (average = 0.48 ± 0.03, $n = 145$). Of the individuals at Nolde Forest, 63.9% had an index score intermediate between the ranges of the Marsh and Hawk Mountain populations. Approximately 27.8% of the individuals at Nolde Forest fell within the range of index scores found at the Great Marsh, while 8.3% of the individuals at Nolde Forest fell within the range of index scores found at Hawk Mountain.

Hybrid classes

After establishing a I_H score for each breeder at Nolde Forest, we examined the distribution of hybrid indices to establish hybrid classes. We divided the distribution of I_H scores into 20% increments, resulting in five hybrid classes, numbered 1–5 (1 = black-capped; 2 = black-capped–like; 3 = intermediate; 4 = Carolina-like; and 5 = Carolina). These values were used to examine if social pairs at opposite ends of the spectrum (i.e., an individual from class 1 paired with an individual from class 5; $n = 9$) were more likely to engage in EPCs than individuals that were paired with an individual of the same species (i.e., both individuals from class 1 or both individuals from class 5; $n = 6$). Hybrid classes were necessary to examine if pure species pairs (rather than just genetically similar pairs) had lower incidences of extrapair paternity. This analysis allows us to distinguish pure species pairs from pairs that are genetically similar, yet are both hybrids (e.g., a pair with hybrid class scores of 3 and 3 cannot be distinguished from pure species pairs of 5 and 5).

Microsatellite DNA analysis—paternity exclusion and assignment

Employing the same microsatellite markers used to determine the genetic identity and allele frequencies of individuals within the hybrid zone, we determined the rate of EPFs for 90 broods at Nolde Forest and the actual paternity of a subset of extrapair nestlings. It was not possible to determine the actual paternity for all nestlings on the periphery of the hybrid zone because copulations may be occurring with individuals outside of Nolde Forest for whom no genetic information is available. We used the following equation of Webster et al. (2001) to estimate P_{ej} (probability averaged over all alleles at the jth locus):

$$P_{ej} = 1 - 2\sum(x_i)^2 + \sum(x_i)^3 + 2\sum(x_i)^4 - 3\sum(x_i)^5 + 2\left(\sum(x_i)^2\right)^2 + 3\sum(x_i)^2\sum(x_i)^3.$$

P_{ej} is the probability that a randomly chosen male other than the actual sire will not have the offspring's allele at the jth locus, where x_i represents the allele frequency. In order to estimate P_{et} (total probability of exclusion), we again used the following equation employed by Webster et al. (2001):

$$P = 1 - \prod(1 - P_{ej}).$$

While ideally all six microsatellite loci would be analyzed for every individual, technical considerations (e.g., lack of sufficient DNA, null alleles) in some cases limited the number of loci available for paternity analysis. Two previous paternity studies on black-capped chickadees employed only three microsatellite markers but still excluded sires with a high degree of confidence (0.995; Mennill et al., 2004; Otter et al., 1998). In this study, we employed at least four microsatellite loci for use in paternity analysis. In cases in which fewer than four loci were available, the nest was excluded from paternity analysis.

To ensure the repeatability of microsatellite allele scoring, we amplified 12 individuals four times at six microsatellite loci and examined the amount of variation in base pair scoring. Base pair scoring was consistent within 2 bp 98.6% of the time; however, 11.5% of the scorings were off by more than 2 bp. In the few cases where the scores were off by 4 bp, both the maternal and paternal alleles were shifted 4 bp, which would be detected during analysis as a maternal mismatch and reanalyzed. Due to the lack of specificity in scoring dinucleotide repeat microsatellite alleles on the ABI 310 Genotyper, a conservative approach was taken to ensure that our estimate of the rate of extrapair paternity in this population was not artificially high. Mismatches were only called if they differed from the parental genotype by 4 bp, and offspring were only deemed extrapair if they did not match the paternal alleles at two or more loci. All scoring was conducted blind, with samples from different birds identified only by a catalog number assigned to each DNA extraction. All maternal and paternal mismatches were double-checked by reexamining the raw ABI output. After paternity exclusion analysis to detect extrapair paternity, we attempted to determine the actual paternity of EPO using software designed to evaluate patterns of shared alleles (NEWPAT; Amos, 2000) and double-checked all assignments by hand.

RESULTS

Social pairing

We focused our analyses on 90 nests for which we obtained genotype data on both social parents and all nestlings. We found no instances of double brooding in a single season; however, some individuals had offspring in more than one season. In only three instances were the same social pairs observed in more than 1 year. A total of 74 different breeding females accounted for these broods; 63 females (85.1%) had one brood, 8 (10.8%) had two broods, 1 (1.3%) had three broods, and 2 (3.7%) had four broods. Social mates comprised 71 different males; 54 (76.1%) were associated with one brood, 15 (21.1%) with two broods, and 2 (2.8%) with three broods. Hybrid index values for the breeding females ($\bar{x} = 0.486 + 0.0036$ SE, $n = 74$, range 0.403–0.576) did not differ from those for the socially paired males ($\bar{x} = 0.479 + 0.0322$ SE, $n = 71$, range 0.413–0.536; $t_{143} = 1.32$, $p = .189$). Hybrid index scores for the female and social mates in each unique pairing ($n = 81$) were uncorrelated ($r = -.044$, $p = .694$). Most pairings ($n = 73$) accounted for only a single brood that we analyzed, while seven pairings each were associated with two broods and one pairing was associated with three broods.

Paternity exclusion

We detected extrapair paternity at Nolde Forest during all 4 years of this study. (We found no clear cases of mixed maternity or "egg dumping" in the sample of broods considered here.) Of the 90 broods examined, 50 (55.6%) contained one or more EPO. Of the 477 nestlings analyzed, 126 (26.4%) were EPO. Exclusionary power based on the six-microsatellite loci was 0.99997. When we used only four loci in the analysis, exclusionary power was 0.9990. The percentage of broods containing one or more EPO did not vary year to year,

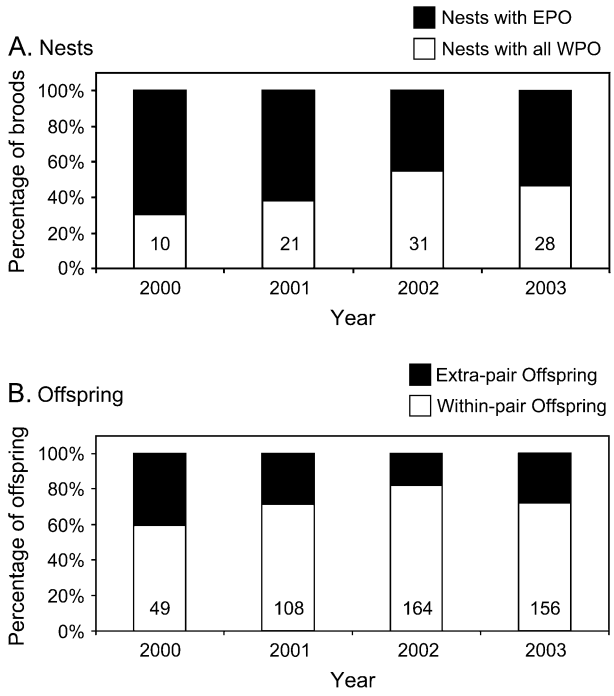

Figure 1
(A) Percentage of broods at Nolde Forest containing one or more EPO and all within-pair offspring (WPO). (B) Percentage of offspring at Nolde Forest that were extrapair and within-pair; sample sizes represent number of offspring. Numbers in bars represent sample sizes of (A) nests and (B) offspring for each year.

though the percentage of offspring that were extrapair did (broods: $\chi^2_3 = 4.38$, $p = .22$; offspring: $\chi^2_3 = 12.41$, $p = .006$; Figure 1).

The genetic identity of socially paired chickadees influenced the occurrence of extrapair paternity. Males that were more black-capped–like (i.e., hybrid index scores closer to 1) were more likely to lose paternity through EPO in their nests (logistic regression, Wald $\chi^2_1 = 8.80$, $p = .003$). Accordingly, average hybrid index scores were higher for males that lost paternity than for males with no EPO in their nests ($t_{88} = -3.26$, $p = .002$; Figure 2A).

However, the genetic similarity of the social pair (absolute value of female hybrid index score − social male hybrid index score) did not affect the likelihood that their brood contained EPO (logistic regression, Wald $\chi^2_1 = 3.13$, $p = .077$). To the extent that there was a trend in this analysis, the probability of having EPO did not increase but rather declined with the degree of genetic difference between the paired breeders. However, the average absolute difference in hybrid index between paired birds associated with broods containing EPO ($\bar{x} = 0.031 \pm 0.0249$ SE, $n = 50$) was not significantly lower than that of pairs whose broods did not include any EPO ($\bar{x} = 0.042 \pm 0.0298$ SE, $n = 40$; $t_{88} = 1.82$, $p = .079$, two-tailed).

We also examined pairings categorically to see if males in genetically similar pure combinations were less likely to lose paternity. Genetically dissimilar pairs (classes combinations of 1/5, 1/4, 2/5, 2/4; $n = 68$ pairs) were no more likely to have EPO than Carolina-like pairs (scores of 5/5 and 4/5) and black-capped–like pairs (1/1 and 1/2; $n = 22$ pairs, $F_{1,88} = 0.80$, $p = .78$). Within-pair offspring and EPO hybrid index scores were not significantly different (within-pair offspring: $n = 351$ offspring, mean $I_H = 0.477 \pm 0.0017$; EPO: $n = 126$ offspring, mean $I_H = 0.479 \pm 0.0028$; $t_{475} = 0.379$, $p = .705$).

Paternity assignment

We were able to assign paternity for nestlings in 26 of the 50 broods that contained EPO, accounting for 56 of the 125 EPO. For five of the 26 broods, two extrapair sires were responsible for EPO. The female's hybrid index score was no more similar to that of her extrapair mate than that of her social mate (repeated measures ANOVA, $F_{1,36} = 0.030$, $p = .86$), and the females own hybrid index value was uncorrelated with that of her extrapair partners ($r = -.016$, $p = .938$). However, when we compared hybrid index scores of social and extrapair males at each nest for which information was available ($n = 26$, with scores of both extrapair males averaged in the four cases where we detected two extrapair sires for the same brood), extrapair males had lower (more Carolina-like) hybrid index scores ($\bar{x} = 0.461 \pm 0.008$ SE) than did social males ($\bar{x} = 0.485 \pm 0.008$ SE; paired t test, $t_{25} = -2.47$, $p = .006$; Figure 2B). Correspondingly, hybrid index scores for extrapair sires were lower in comparison to those of all breeding males at Nolde ($z = -2.60$; $p = .009$), whereas the scores for social males at nests where EPO occurred did not differ from scores of all breeding males in the population ($z = 1.78$; $p = .070$).

Reproductive success

Average fledging success did not differ between nests containing EPO ($\bar{x} = 5.29$ offspring ± 0.24 SE, $n = 50$) and those with no EPO ($\bar{x} = 5.30 \pm 0.27$ SE, $n = 40$; $t_{88} = 0.037$, $p = .971$). In nests containing EPO, the average number of EPO was 2.58 ± 1.63 SE.

DISCUSSION

Extrapair paternity plays a substantial role for hybridizing black-capped and Carolina chickadees at Nolde Forest, with over half the nests containing EPO (accounting for roughly 26% of offspring). In pure populations of black-capped chickadees, EPO are present in approximately 30% of nests and account for approximately 10–15% of offspring (Mennill et al., 2004; Otter et al., 1994). While no studies have yet examined rates of extrapair paternity in Carolina chickadees, preliminary evidence (Ruscica A and Curry R, unpublished data) indicates that EPCs are occurring, though at lower rates than observed at Nolde Forest.

The tendency of chickadees to engage in extrapair mating does not appear to be influenced by the genetic distance between partners because females that were highly dissimilar from their social mates produce broods including EPO with the same frequency as females paired with similar males. Nevertheless, our finding that females socially paired to black-capped–like males were more likely to have EPO in their broods does support the more general prediction that the mating behavior of females is influenced, at least in part, by the characteristics of their social mate.

The tendency for females at Nolde to prefer Carolina-like males as extrapair sires, independently of their own genotype or that of their social mate, is more consistent with a good genes mechanism of mate choice than with a mechanism involving "compatible genes." In addition, our results are consistent with conclusions from a recent aviary study by Bronson et al. (2003b) involving the same two species collected from field populations in Ohio. In that study, Bronson and colleagues presented females of both species with dyads of black-capped and Carolina males to assess mate preferences, which they estimated from the relative amount of time the females spent on different sides of a choice cage. When able to interact through a screen barrier, Carolina males dominated

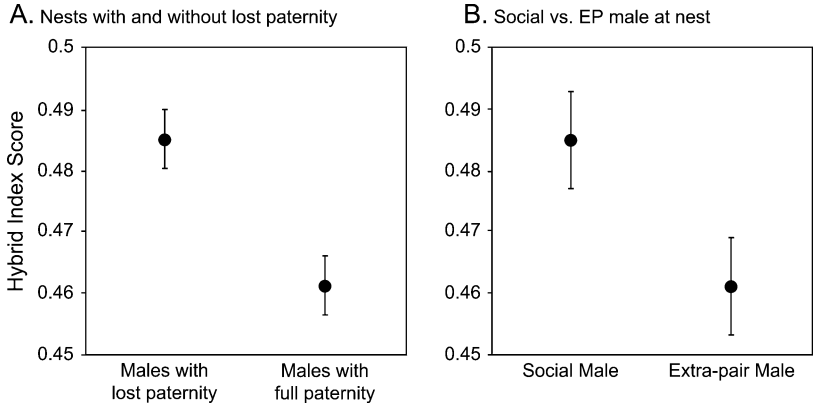

Figure 2
(A) Comparison of hybrid index scores (mean ± SE) for males whose broods contained EPO ($n = 50$ broods) and males whose broods contained only within-pair offspring ($n = 40$ broods). (B) Hybrid index scores (mean ± SE) for social males ($n = 26$) and extrapair sires for broods containing EPO ($n = 26$).

black-capped males (unless the Carolina male was much smaller). If females of either species witnessed the interaction, they preferentially associated with the dominant male, regardless of species; consequently, both Carolina and black-capped females gravitated toward Carolina males. Bronson et al. (2003b) hypothesized that this bias in mate choice, mediated by the outcome of dominance between the two species, may be playing a significant role in shaping the dynamics of the chickadee hybrid zone, including recent northward movement of the line of contact. Similarly, our data showing a bias in choice of extrapair partners that also favors Carolina-like males are consistent with the observed northward shift in the hybrid zone. Whether the bias in choice of extrapair mates that we detected is associated with asymmetry in dominance is a subject of our continuing field study in Pennsylvania.

Due to our as yet limited sample size, it is unclear whether the tendency for chickadee females at Nolde to engage in EPCs with Carolina-like males would be supported by additional assignments of extrapair sires. Because pure individuals of either species at Nolde Forest are rare, if present at all, it is unlikely that dyads of black-capped–like and Carolina-like birds are commonly observed by females. It is plausible that at the northern edge of the hybrid zone these patterns may be more robust and black-capped chickadee females are preferentially choosing Carolina or Carolina-like hybrids as social or extrapair mates, or both, and that mate choice is acting to expand the leading edge of the hybrid zone.

While we do not yet understand the reasons and consequences for the extrapair mating patterns we detected at Nolde, it seems clear that our chickadees do not exhibit the strategy proposed by Veen et al. (2001), in which females in genetically dissimilar pairings have a high proportion of EPO and pursue extrapair matings with conspecific males. One reason for different results in the two sets of birds may be that the costs of hybridization in the chickadee hybrid zone are much lower than in that of the flycatchers. In flycatchers, the fitness costs of hybridization are severe: F1 females are nearly sterile and F1 males have very low recruitment. In contrast, overall levels of hatching and fledging successes at Nolde are not drastically reduced relative to our reference black-capped and Carolina populations (Cornell, 2001) nor are they influenced by the genetic similarity of the pairings (Curry R and Reudink M, unpublished data). Given that the costs of mixed pairing appear to be more pronounced in Ohio (Bronson et al., 2003a, 2005), it is all the more intriguing that in the Ohio populations, extrapair mating has not as yet been shown to be a component of chickadee reproductive biology.

In the Pennsylvania segment of the chickadee hybrid zone, the extent of hybridization is extremely high, indicating relatively high fecundity of F1 and subsequent generation hybrids (Reudink M and Curry R, unpublished data). The phenotypic similarity of the two chickadee species (Sattler and Braun, 2000) and the frequent occurrence of bilingual birds and aberrant songs within the hybrid zone (Curry R et al., in preparation; Rossano, 2003) may make it difficult for females to discriminate between genetically similar and dissimilar mates.

If female chickadees were pursuing EPFs to reduce the costs of hybridization, genetically distant pairs that acquire EPO should have higher reproductive success than those that only produce offspring with their social mate. There was, however, no difference in reproductive success between females that acquired EPO and those that did not, indicating that EPFs are not confounding overall measures of reproductive success at the population level. In moving hybrid zones and populations that are only in the first stages of the hybridization process, the costs of hybridization may be more apparent (because only a small portion of the population is hybridizing). It will therefore be critical to examine the leading edge of the contact zone in Pennsylvania to determine if the costs of hybridization are more severe. If so, it may be that female mating strategies differ from those at the "center" of the hybrid zone, as examined in this study.

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REFERENCES

Amos B, 2000. NEWPAT, a general paternity program. http://www.zoo.cam.ac.uk/zoostaff/amos/newpat.htm.

Banks MA, Eichert W, 2000. WHICHRUN (Version 3.2) a computer program for population assignment of individuals based on multilocus genotype data. J Hered 91:87–89.

Bronson CL, Grubb TC Jr, Braun MJ, 2003a. A test of the endogenous and exogenous selection hypotheses for the maintenance of a narrow avian hybrid zone. Evolution 57:630–637.

Bronson CL, Grubb TC Jr, Sattler GD, Braun MJ, 2003b. Mate preference: a possible causal mechanism for a moving hybrid zone. Anim Behav 65:489–500.

Bronson CL, Grubb TC Jr, Sattler GD, Braun MJ, 2005. Reproductive success across the black-capped (*Poecile atricapillus*) and Carolina chickadee (*P. carolinensis*) hybrid zone in Ohio. Auk 122:759–772.

Brzustowski J, 2002. Doh assignment test calculator. http://www.biology.ualberta.ca/jbrzusto/Doh.php.

Campton DE, Utter FM, 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound Streams. Can J Fish Aquat Sci 42: 110–119.

Cornell K, 2001. Do hybridizing chickadees in Pennsylvania follow Haldane's Rule? Hatching success and nestling sex ratio in black-capped and Carolina chickadees (MS thesis). Villanova, Pennsylvania: Villanova University.

Dawson DA, Hanotte H, Greig C, Stewartand IRK, Burke T, 2000. Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Mol Ecol 9:1919–1952.

Doucet SM, Mennill DJ, Montgomerie R, Boag PT, Ratcliffe LM, 2005. Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. Behav Ecol 16:218–222.

Gaunt AS, Oring LW (eds), 1999. Guidelines to the use of wild birds in research, 2nd ed. Washington, DC: The Ornithological Council.

Griffiths SC, Owens IPF, Thuman KA, 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212.

Grubb TC Jr, Bronson CL, 1995. Artificial snags as nesting sites for chickadees. Condor 97:1067–1070.

Hansen MM, Ruzzante DE, Nielsen EE, Mensberg KLE, 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). Mol Ecol 9:583–594.

Kvist L, Ruokonen M, Orell M, Lumme J, 1996. Evolutionary patterns and phylogeny of tits and chickadees (genus *Parus*) based on the sequence of the mitochondrial cytochrome b gene. Ornis Fenn 73:145–156.

Mennill DJ, Ramsay SM, Boag PT, Ratcliffe LM, 2004. Patterns of extra-pair mating in relation to male dominance status and female nest placement in black-capped chickadees. Behav Ecol 15:757–765.

Mostrom AM, Curry RL, Lohr B, 2002. Carolina chickadee (*Poecile carolinensis*). In: The birds of North America, no. 636 (Poole A, Gill F, eds). Philadelphia, PA: The Birds of North America, Inc.

Neff BD, Pitcher TE, 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol Ecol 14:19–38.

Otter KL, Ratcliffe LM, Boag PT, 1994. Extra-pair paternity in the black-capped chickadee. Condor 96:218–222.

Otter KL, Ratcliffe LM, Michaud D, Boag PT, 1998. Do female black-capped chickadees prefer high-ranking males as extra-pair partners? Behav Ecol Sociobiol 43:25–36.

Paetkau D, Calvert W, Sterling I, Strobeck C, 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4:347–354.

Petrie M, Kempenaers B, 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends Ecol Evol 13:52–58.

Piry S, Alapetite A, Paetkau D, Cournet J-M, Baudouin L, Estoup A, 2004. GeneClass2: a software to assign or exclude individuals to populations and detect first generation migrants. J Hered 95:536–539.

Ramsay SM, Otter KA, Mennill DJ, Ratcliffe LM, Boag PT, 2000. Divorce and extrapair mating in female black-capped chickadees (*Parus atricapillus*): separate strategies with a common goal. Behav Ecol Sociobiol 49:18–23.

Rossano LA, 2003. Vocal patterns of hybridizing black-capped and Carolina chickadees in southeastern Pennsylvania (MS thesis). Villanova, Pennsylvania: Villanova University.

Sattler GD, Braun MJ, 2000. Morphometric variation as an indicator of genetic interactions between black-capped and Carolina chickadees at a contact zone in the Appalachian mountains. Auk 117: 427–444.

Smith SM, 1988. Extra-pair copulations in black-capped chickadees: the role of the female. Behaviour 4:15–23.

Spottiswoode C, Møller AP, 2004. Extrapair paternity, migration, and breeding synchrony in birds. Behav Ecol 15:41–57.

Storfer A, Mech SG, Reudink MW, Ziemba RE, Warren J, Collins JP, 2004. Evidence for introgression in the endangered Sonora tiger salamander, *Ambystoma tigrinum stebbinsi* (Lowe). Copeia 4:783–796.

Veen T, Borge T, Griffith SC, Sætre GP, Bures S, Gustafsson L, Sheldon BC, 2001. Hybridization and adaptive mate choice in flycatchers. Nature 411:45–50.

Webster MS, Chuang-Dobbs HC, Holmes RT, 2001. Microsatellite identification of extrapair sires in a socially monogamous warbler. Behav Ecol 12:439–446.

Westneat DF, Sherman PW, Morton ML, 1990. The ecology and evolution of extra-pair copulations in birds. Curr Ornithol 7: 331–370.

Westneat DF, Stewart IRK, 2003. Extra-pair paternity in birds: causes, correlates, and conflict. Annu Rev Ecol Syst 34:365–396.