Behavioral Ecology
doi:10.1093/beheco/arr229
Advance Access publication 19 January 2012

Original Article

Interspecific dominance relationships and hybridization between black-capped and mountain chickadees

A. Grava,[a] T. Grava,[a] R. Didier,[a] L. A. Lait,[b] J. Dosso,[b] E. Koran,[b] T. M. Burg,[b] and K. A. Otter[a]
[a]Natural Resources and Environmental Studies, University of Northern British Columbia, 3333 University Way, Prince George, British Columbia V2N 4Z9, Canada and [b]Department of Biology, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta T1K 3M4, Canada

Black-capped chickadees (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*) are ecologically segregated due to differences in habitat preference. However, forestry practices in northwestern Canada have created a mosaic of coniferous (mountain chickadee habitat) and deciduous forest patches (black-capped habitat), which might explain cases of observed regional sympatry between these 2 closely related species. In *Poecile* species, social hierarchies amongst conspecific individuals influence life-history parameters such as mate choice. As a result, interspecific social hierarchies might drive hybridization between these 2 closely related species. By conducting field observations and aviary experiments, we demonstrated that black-capped chickadees are dominant over mountain chickadees. Using a combination of species-specific phenotypes (plumage), mitochondrial DNA (mtDNA) to assess maternal genotype, and microsatellite markers, we confirmed that genetic mixing occurs within our contact zone but that the pattern of parentage appears directional. All but one of the adult hybrids was phenotypically identified as mountain chickadee and had mountain chickadee mtDNA. Furthermore, all nestlings where microsatellites detected mixed-species ancestry were from mountain chickadee nests with both attending parents having mountain chickadee phenotypes. All mtDNA from these nestlings was mountain chickadee except for one individual, and in all cases, these nestlings showed genetic patterns of having arisen through extrapair copulations between female mountain and male black-capped chickadees. Our results suggest that hybridization may result from males of the mountain chickadees having lower expression of a preferred trait (dominance) than the black-capped chickadees. *Key words:* black-capped and mountain chickadees, dominance hierarchies, extrapair paternity, hybridization. *[Behav Ecol 23:566–572 (2012)]*

INTRODUCTION

Within species of the family Paridae (chickadees and titmice), dominance rank in winter flocks is known to drive mate choice (Otter et al. 1998; Mennill et al. 2004), breeding success (Otter et al. 1999), overwinter survival, and access to resources (Desrochers 1989; Ficken et al. 1990). Females paired with dominant males may also benefit from more secured and undisturbed foraging from other flock members under the protection provided by the female's mate (Hogstad 1988; Hogstad 1992; Lemmon et al. 1997). These benefits to females, both in winter resources and nesting success, may partially explain female preference within species for high-ranking males as social mates and/or extrapair partners (Otter and Ratcliffe 1996; Ratcliffe et al. 2007). Yet, the benefits from relative dominance relationships may not be restricted to within-species (intraspecific) interactions.

In Europe, up to 6 different species within the Paridae family can live in sympatric populations (Dhondt 2007). Relative dominance relationships between these species exist within mixed flocks and can result in not only competition over food but also nesting sites (Dhondt 1989). In contrast, many North American parids are parapatric, and it is rare to have more than 2 chickadee species (*Poecile* spp.) overlapping in the same zone (Dhondt 2007). North American parids form winter flocks where the intraspecific dominance relationships are both stable and linear (Ekman 1989), and this stability can also extend to interspecific relationships in regions where overlap occurs. For example, within the contact zone between Carolina chickadee (*Poecile carolinensis*) and black-capped chickadee (*P. atricapillus*) in eastern North America, aviary experiments showed that Carolina chickadees tend to be dominant over the black-capped chickadees (Bronson et al. 2003). In addition to potential competition for food and nesting resources, interspecific hierarchies may influence mate choice; Bronson et al. (2003) found female Carolina and black-capped chickadees tend to preferentially associate with the dominant males in aviary trials, regardless of their species relative to the female.

Even though some clarifications are still needed, most studies suggest that mountain chickadees (*P. gambeli*) and black-capped chickadees are sister species within the black-headed chickadee clade (Gill et al. 1993, 2005). Although their geographic ranges overlap west of the Rocky Mountains, the 2 species are often allopatric at local scales due to ecological segregation: mountain chickadees prefer high elevation dry conifer forests, whereas black-capped chickadees are associated with lower elevations and mixed forests with much higher deciduous component (McCallum et al. 1999; Foote et al. 2010). Local areas of sympatry, though, do occur throughout the range of distribution overlap; these contact zones appear

Address correspondence to A. Grava. E-mail: roux@unbc.ca.
Received 31 March 2011; revised 19 December 2011; accepted 20 December 2011.

to occur at the interface between where the upper altitudes inhabited by black-capped chickadees meet the lower altitudes used by mountain chickadees, and within these zones where the habitats preferred by either species abut. Within species, both mountain chickadees and black-capped chickadees share a similar social hierarchy organization. They overwinter in flocks with stable and linear hierarchies: males are dominant over females and juveniles are typically subordinate to adult birds within these sex-classes (Minock 1972; Ekman 1989; Smith 1991). Previous studies in contact zones suggested that black-capped chickadees are dominant to mountain chickadees (Minock 1972; Hill and Lein 1989). Minock (1972) found that while black-capped chickadees typically dominate mountain chickadees at winter feeding stations, there were a number of cases where mountain chickadees dominated interactions (about 20% of observed encounters). Both studies were unable to control for the effect of age and sex of interactants, factors known to influence dominance relationships in several parids (Ekman 1989; Smith 1991; McCallum et al. 1999). If a stable and linear interspecific hierarchy does exist among these 2 sister species, the general preference of females for dominant males may extend to heterospecifics (Bronson et al. 2003) and could drive hybridization through cross-species mate choice.

Our objective was to observe interactions between wintering mountain and black-capped chickadees in a contact zone in northern British Columbia, Canada, to determine relative interspecific hierarchies within mixed winter flocks. We observed natural encounters at temporary winter feeders of both intraspecific and interspecific interactions among individually banded birds for which age and sex had previously been determined. Third-party effects, such as audience of observers or the presence of a dominant mate in the vicinity can influence the outcome of natural encounters: Hogstad (1992) showed that the female mated to the alpha male experienced less aggression from the other flocks members and had both increased foraging time and decreased vigilance rates when her mate was close by (less than 5 m). As a result, we also paired birds in aviaries to confirm our assessment of relative interspecific dominance relationships.

We then used plumage, mitochondrial DNA (mtDNA), and microsatellite analyses to distinguish between mountain and black-capped genotypes and phenotypes of both adults and nestlings from the study site in comparison with 2 single-species control populations to determine the amount of hybridization in our contact zone. As mtDNA is maternally inherited, it allowed us to determine the maternal genotype for all individuals tested, which we also compared with individuals' plumage, as these 2 species are dimorphic for plumage patterns. Individuals with plumage phenotype of one species, but mtDNA of the other species, might indicate introgression. Using genetic differences between the 2 species, microsatellite analysis allowed us to detect mixed-species parentage in adults. Furthermore, as all nests in our population had conspecific social pairs, microsatellite analysis allowed us to determine whether nestlings from these nests showed mixed-species parentage (arising from extrapair behavior).

MATERIALS AND METHODS

Study species and study site

In the fall and early winter of 2007–2008 and 2008–2009, we blood sampled and banded birds with a unique combination of one Canadian Wildlife Service numbered aluminium band and 3 plastic colored bands at the John Prince Research Forest (JPRF) in northern British Columbia, Canada (lat 54°40′N, long 124°24′W). Black-capped chickadees and mountain chickadees are typically distinguished based on plumage patterns, the main differences being the presence of a white supercilliary line in the mountain chickadee which is absent in the black-capped chickadee. Furthermore, black-capped chickadees have prominent white edges to the secondary feathers that are lacking on mountain chickadees. We classified birds as phenotypically mountain chickadee or black-capped chickadee based on species characteristic plumage. We determined the sex of the birds by using a combination of body measurements (wing chord, tail, tarsal length, and weight), males being larger than female in both species (McCallum et al. 1999; Foote et al. 2010), and confirmed these assessments during the breeding season with sex-specific behavior (e.g., male feeding its mate). Age was determined using the shape and the color pattern of the outermost rectrix (Pyle 1997). We also used long-term data sets; as most birds at the study sites are banded in their first fall/winter, multiyear banding records allow us to identify adults from juvenile birds in both species.

Field observations

Temporary feeding stations were set up in March–April 2008 and January–February 2009. We observed birds interacting at these feeders for periods lasting from 0.5 to 2 h, depending on the number of birds and/or number of interactions (observations were longer when more birds were present or more interactions were occurring). We recorded both interspecific and conspecific interactions to determine the social hierarchy within species and across species. We used 4 different behaviors to determine the relative rank of 2 interacting birds: 1) "chase"—the focal bird chases away its opponent, 2) "supplant"—the focal bird supplants its opponent, 3) "submissive posture"—the focal bird gives a display that elicits a submissive posture from an opponent, and 4) "wait"—the opponent waits for the focal bird to leave before approaching the feeder. These behaviors are often associated with each other (e.g., submissive postures often follow being supplanted), and the focal bird was considered dominant over its opponent if any of these behaviors were witnessed (Ficken et al. 1990; Otter et al. 1998; Ratcliffe et al. 2007). We also recorded the number of birds of each species and every visit to the feeder to control for frequency of interactions in relation to differential use of feeders by either species.

Aviary experiment

We conducted aviary experiments in late winter (February and March 2009) to determine the interspecific social hierarchy. We paired the birds by sex and age to control for likely effects of these 2 parameters. One bird of each species was caught in its flock territory (using mistnet or potter trap) during the day and immediately transported to and released into the aviary. To ensure that birds had no previous contact with each other, we paired birds caught from territories at least 3 km apart. We kept the birds overnight to let them acclimate to the aviary conditions and ran the experiment the next morning.

The aviary was divided into 3 different compartments. Each outside compartment was provided with unlimited food (sunflower seeds) and shelters (tree and nest-box). The central compartment was used to run the experiment; sliding walls allowed us to open the 2 outside compartments housing either bird to allow them access to the central compartment and create visual contact between the 2 individuals. A feeder was set up in the middle of the central compartment prior to starting trials, with a mesh divider (1 × 1 cm plastic garden mesh) centered on the feeder to allow visual contact and close proximity over the resource but preventing physical contact.

The food source from the outer compartments was removed 1 h before the trials started. To start the trials, we opened the sliding barriers allowing the birds to interact around the central feeder. Each trial lasted 1 h, and we recorded the number of visits to the feeder and agonistic interactions (chases, supplants, waits, and submissive postures). After 1 h of observations, the birds were isolated in their respective compartment, caught, and released into their flock's territory (no bird was held more than 24 h).

Genetic analysis

We assessed evidence and origin of mixed parentage in both adult and nestlings from the studied contact zone using a combination of expressed phenotype (plumage) versus maternal genotype (mtDNA) and microsatellites markers.

We sampled individuals from 2 pure populations to identify species-specific genetic patterns for both mountain chickadees ($N = 26$ Riske Creek, BC, lat 51°57′N, long 122°30′W, 300 km from JPRF) and black-capped chickadees ($N = 30$ Prince George, BC, lat 53°53′N, long 122°48′W, 150 km from JPRF). These 2 populations were considered as pure populations: 95% of the chickadees were from one species only, with few incidental occurrence of the other. These totals are based upon at least 5 years of population monitoring in either population (Otter KA, personal communication and Martin K, personal communication). For either reference population, there were no phenotypic indications of mixing between the species.

DNA was extracted from 5 µl of blood–ethanol mix using standard chelex extraction (Walsh et al. 1991). For each individual, the mitochondrial control region was sequenced and genotypes obtained for 6 microsatellite loci. mtDNA sequences were used to assess the maternal lineage of each bird, including the distinction between our phenotypically assessed pure populations of either species (see above). In species where hybridization occurs, the phenotype may not always match the mtDNA sequences. For example, hybridization between hermit and Townsend's warblers has resulted in phenotypically pure Townsend's warblers outside of the hermit warbler range, but which contain hermit warbler mtDNA sequences (Rohwer et al. 2001). mtDNA for the control region was amplified using 2 µM each LbcchCR1 (CCA CCA CCC CAT AAT AAG GA) and HCRCbox (CCA CTT GTA TCT GTG ARG AGC) primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, and 2.5 U taq polymerase in Promega Flexi buffer. The thermal profile was 94 °C for 120 s, 50 °C for 45 s, 72 °C for 1 cycle, followed by 37 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 60 s, and a final step of 72 °C for 300 s and 4 °C for 20 s. Samples were sequenced on an ABI 3130 sequencer using a BigDye terminator kit following removal of unincorporated primers and dNTPs using Exo-SAP (exonuclease and shrimp alkaline phosphatase). Sequencing reactions were cleaned using sodium acetate precipitation prior to injection of the sequencing reaction.

Six avian microsatellites were used for genotyping: Ppi2 (Martinez et al. 1999), Titgata39 (Wang et al. 2005), Titgata02 (Wang et al. 2005), Pdo5 (Griffith et al. 1999), Escu6 (Hanotte et al. 1994), and Pat14 (Otter et al. 1998). Polymerase chain reaction (PCR) cocktail contained 0.05 µM of a fluorescently labeled M13 primer (700 or 800 nm wavelength), 2 µM of the forward and reverse primer, 200 µM dNTP, $MgCl_2$, 0.5 U of taq polymerase in a 1× PCR buffer. A 2 mM $MgCl_2$ concentration was used for 4 loci, the exceptions being Ppi2 (1.5 mM) and Escu6 (1 mM). The 5′ end of each forward primer was modified with the addition of M13 sequence (CAC GAC GTT GTA AAA CGA C) to allow for direct incorporation of a fluorescently labeled M13 primer (Burg et al. 2006). Three loci (Titgata39, Escu6, and Ppi2) were amplified using a 2-step annealing procedure: 1 cycle for 2 min at 94 °C, 45 s at 50 °C, 60 s at 72 °C; 7 cycles of 60 s at 94 °C, 30 s at 50 °C, 45 s at 72 °C; 31 cycles of 30 s at 94 °C, 30 s at 52 °C, 45 s at 72 °C; and 1 final cycle of 300 s at 72 °C. The other 3 loci (Titgata02, Pdo5, and Pat14) were amplified using a similar 2-step annealing process with 7 cycles at 50 °C and 25 cycles at 52 °C. PCR products were run on a 6% acrylamide gel on a Licor 4300 (Licor Inc.). Individuals of known allele sizes, negative controls, and a 50–350 bp size standard were included on each load/channel to ensure that alleles were sized consistently between gels. As alleles covered a range of sizes, alleles were sized using the size standard and a set of positive controls. All gels were scored manually by 2 different people.

Analyses

For the social hierarchy data, we conducted a combination of binomial tests and Fisher exact tests to compare numbers of observed interactions won by the different species and in different circumstances. Fisher exact tests were conducted with STATISTICA (version 6.0, StatSoft, Inc.).

mtDNA sequences were visually aligned using MEGA4 (Tamura et al. 2007) and assigned as either mountain chickadee or black-capped chickadee based on sequence similarity to birds from the pure populations. The mountain and black-capped chickadee sequences were highly divergent and easily assigned to 1 of the 2 species (Table 1). GenAlEx (Peakall and Smouse 2001) was used to test for deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium and to estimate standard diversity measurements for the microsatellite markers. STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) was used to estimate proportion membership of each individual to black-capped or mountain chickadee clusters ($K = 2$). STRUCTURE uses genetic data to assign individuals to clusters based on their genotype and determine the probability of recent ancestry from each cluster. We used prior sampling information (phenotype) for the birds from

Table 1

Variable sites in the mitochondrial control region of black-capped (BC) and mountain chickadees (MO)

		Variable sites
		11111 1222222223 3333333344 4444556666 67
		3468834669 9366667880 1222244513 3457161137 70
		9136838470 5723456091 1013779124 5975130373 69
BC_08	BC_08	CCCAGCTCCG TACTGGGTGT CCTACTCATA ATTGGCCTAA TT
BC_09	BC_09	....
BC_10	BC_10	....C T.CG..... ....
BC_11	BC_11	....
BC_12	BC_12	....A..... ....C T.CG..... C
BC_13	BC_13	....C.....C T.CG..... ....
MO_102	MO_102	TTTGATCTTA .GTCC.ACA. .TCGTCG.AG .AAAAGTCGG CC
MO_103	MO_103	TTTGATCTTA .GTCC.ACA. .TCGTCG.AG .AAAAGTCGG CC
MO_104	MO_104	TTTGATCTTA .GTCCAACA. .TCGTCG.AG .AAAAGTCGG CC
MO_105	MO_105	TTTGATCTTA .GTCC.ACA. .TCGTCG.AG .AAAAGTCGG CC
MO_106	MO_106	TTTGATCTTA .GTCCAACA. .TCGTCGGAG .AAAAGTCGG CC
MO_107	MO_107	TTTGATCTTA C.TCCAAC.. .TCGTCG.AG GAAAAGTCGG CC

Numbers represent the individual samples, and samples of both black-capped and mountain chickadees are derived from pure populations where >95% of birds in the area over multiple years of study were of one species only. A subset of samples are presented here to represent the general differences, and not all of the variable sites are contained within these 12 individuals. Sites are numbers on the H strand relative to the position of the sequencing primer. Nucleotide similarity to the reference sample BC _08 is indicated by a ".".

"pure" populations. For mixed populations and nestlings, we did not include any prior information. STRUCTURE uses this sampling information to help it assign individuals to each cluster, but the final assignment (i.e., ancestry coefficient) is based on the genetic data.

Only individuals with 3 or more genotypes were included, and most individuals (85%) had genotypes for 4 or more loci. As STRUCTURE is sensitive to the inclusion of kin groups, separate runs were done for nestmates. For each data set, all of the adults and a maximum of one nestling from each nest were run using 20 000 burnin, 50 000 Markov chain Monte Carlo runs, correlated allele frequencies, and admixture. An additional set of runs was done using an adult only data set. A total of 16 data sets were created as the maximum number of siblings was 16. Each data set was run 3 times, and results from all runs were averaged. As adults were run for each of the data sets, the ancestry coefficients (Q values) from all 51 runs were averaged. Using the ancestry coefficients from the pure populations, we determined a conservative threshold value for mixed ancestry. Black-capped chickadees from pure populations had an average Q value of 0.91 (range of 0.74–0.96), whereas mountain chickadees from the pure population had an average of 0.98 (0.97–0.99). The Q values of individuals in the data set were bimodal in distribution, with individuals having either >0.74 or <0.62 values. As the lowest value for an individual in a pure population was 0.74, we set the threshold for inclusion halfway between the minimum value of the upper and the maximum of the lower distributions; any individual with Q value of less than 0.68 assignment to one or the other species was investigated as being of mixed-species ancestry.

RESULTS

Field observations

At temporary winter feeding stations, black-capped chickadees were generally dominant over their mountain chickadees counterparts (159 of 190 interactions—83.7%; binomial test $P < 0.0001$) even though we observed some mountain chickadees dominating black-capped chickadees (31 interactions—16.3%). To control for the effect of sex and age on interspecific encounters, we compared only those interactions between birds of known age and sex (Table 2). Black-capped chickadees were dominant over their mountain chickadee counterparts when birds were matched by sex in 33 instances of 37 (binomial test $P < 0.0005$), by age in 26 of 27 encounters (binomial test $P < 0.0005$), and both sex and age in all cases (binomial test $P < 0.0005$, $n = 15$) (Table 2).

Table 2

Interspecific interactions in natural environment between black-capped chickadee (BC) and mountain chickadee (M); when birds were (1) of the same sex, (2) the same age, (3) matched both by sex and age, (4) female fighting off a male, and (5) a juvenile (second year—SY) bird dominant over an adult (after second year—ASY) bird (only interactions with birds of known sex and/or age are included in each comparison)

	BC dominant	M dominant
(1) Paired by sex	33	4
♂ versus ♂	18	0
♀ versus ♀	15	4
(2) Paired by age (adult birds only)	26	1
(3) Paired by sex and age	15	0
(4) ♀ dominant to ♂	21	1
(5) Juvenile dominant to adult	33	0

Furthermore, when interactions contravened the typical patterns in chickadees (i.e., females dominating males [$n = 22$] or juveniles dominating adults bird ($n = 33$), the dominant female (binomial test $P < 0.0005$) or the dominant juvenile (binomial test $P < 0.0005$) was a black-capped chickadee outranking a mountain chickadee competitor in all but one instance (Table 2).

Aviary experiment

Dyadic interactions in aviaries clearly revealed black-capped chickadees as the dominant species: of 11 dyads, black-capped chickadees were dominant over mountain chickadees in all 11 cases (winning a combined total of 81 of 82 interactions observed during the 11 dyadic trials, binomial test, $P = 0.02$, $n = 11$). Only one overt dominance display given by a mountain chickadee was observed in all trials. However, the black-capped chickadee involved in this dyad responded with both a chase and a supplant less than 1 min after this event, and it dominated the paired mountain chickadee in all additional interactions ($n = 5$) witnessed during this 1-h trial.

Genetic analysis

We obtained 734 bp of sequence from the mtDNA control region containing 73 variable sites of which 31, 6 transversions and 25 transitions, were fixed differences between the 2 species. All of the sampled individuals from either reference population (mountain chickadees—Riske Creek; black-capped chickadees—Prince George) had mtDNA profiles matching the other birds from their region, which also were divergent between the species phenotypes. Furthermore, all microsatellite loci were in HWE, and none showed evidence of linkage. The 6 loci were highly variable (Table 3), and each species contained unique alleles.

Within the overlap zone, all but one individual had mtDNA matching their species phenotype and nestlings from the same nest contained the same mtDNA haplotype. The single exception was one mountain chickadee nestling (N-09-75) that had mixed mtDNA sequence containing both black-capped and mountain chickadee sequence (i.e., possibly heteroplasmy). This sample was reextracted, reamplified, and sequenced a second time with similar results.

A total of 15 individuals ($n = 264$) in the overlap zone showed evidence of mixed-species ancestry in microsatellite analysis (Table 4). Only 1 of the 65 adult black-capped chickadees from the mixed area had less than 60% assignment to black-capped chickadee via microsatellites, but this individual had black-capped mtDNA. The remaining 14 birds with evidence of mixed-species ancestry were all phenotypically mountain chickadees ($n = 97$) and had mountain chickadee mtDNA haplotypes (except nestling N-09-75 mentioned above) but also had black-capped chickadee ancestry based on nuclear microsatellites ranged from 32.3 to 90.6%. Seven of those birds were adult mountain chickadee ($n = 63$ mountain chickadee adults sampled in the contact zone), and 7 were nestlings ($n = 34$ mountain chickadee nestlings sampled). One nestling had inconclusive mtDNA (N-09-75 mentioned above) and also had mixed nuclear DNA (56.7% mountain and 43.3% black-capped ancestry). Genetic analysis for the social mother from this nest was not available, but none of the 6 other nestlings from that nest showed evidence of heteroplasmy. However, one other nestling from that same nest showed evidence of mixed-species ancestry through microsatellites analysis, indicating that mixed mtDNA for nestlings N-09-75 might be due to a rare phenomenon of paternal leakage (Kvist et al. 2003).

Table 3

Allelic variation at the 6 microsatellite markers (locus as named in original reference, see text)

Locus		Black-capped	Mountain
Ppi2	Size	308–536	318–544
	# alleles	37	24
	Ho	0.829	0.778
	He	0.926	0.921
	Private alleles	22	10
Titgata39	Size	224–260	220–252
	# alleles	10	10
	Ho	0.800	0.849
	He	0.764	0.859
	Private alleles	1	1
Titgata02	Size	216–272	220–260
	# alleles	14	10
	Ho	0.851	0.802
	He	0.858	0.800
	Private alleles	6	1
Pdo5	Size	250–336	240–290
	# alleles	16	19
	Ho	0.710	0.759
	He	0.810	0.825
	Private alleles	8	9
Escu6	Size	120–162	124–154
	# alleles	19	16
	Ho	0.893	0.914
	He	0.908	0.861
	Private alleles	6	2
Pat14	Size	137–165	135–169
	# alleles	15	16
	Ho	0.804	0.875
	He	0.835	0.878
	Private alleles	10	5

Size ranges for microsatellite alleles are given (size in base pairs) along with the number of alleles (# alleles) and observed (Ho) and expected (He) heterozygosities. Private alleles are number of species-specific alleles found in 1 of the 2 species.

Among the 6 mountain chickadee nests tested, 9/34 nestlings were determined to have genotypes consistent with being extrapair (e.g., being half siblings to the remaining nestlings within the same brood). Extrapair young were found in 4 of the 6 nests tested. Of the 9 extrapair nestlings, 7 were also classified by STRUCTURE as having DNA from both mountain and black-capped chickadees (Q values less than 0.70 assignment to either species) and were thus classified as hybrids. These hybrids were found in all 4 nests with extrapair young; in 2 nests, all extrapair nestlings were hybrids, and in the remaining 2 nests, there was 1 hybrid and 1 within-species extrapair nestling. In all but 1 of the 9 extrapair young, the mtDNA was mountain chickadee, suggesting that the attending mountain chickadee female at the nest was the genetic mother, and the extrapair sire was a black-capped male. The remaining case was the nestling classified as a hybrid based on microsatellite loci and showing heteroplasmy in the mtDNA (above).

Table 4

Number of individuals sampled in the study areas: adults phenotypically mountain chickadee and nestlings sampled in a nest where both parents were phenotypically mountain chickadee are classified as "M." Similarly, black-capped chickadee adults and nestlings are classified as "BC"

	Classified as M	Classified as BC
Adults nonhybrids	56	64
Genetically determined hybrids	7	1
Nestlings nonhybrids	27	102
Genetically determined hybrids	7	0

Genotyping was done using microsatellite analysis. All hybrids are from the area of overlap.

In contrast, 10 of 16 black-capped chickadee nests contained evidence of extrapair paternity, but in no instances did any of the black-capped nestlings ($n = 102$) have evidence of mixed-species ancestry. The combined phenotype, mtDNA, and microsatellite data suggest all hybridization detected among nestlings results from female mountain chickadees seeking extrapair copulations from male black-capped chickadees.

DISCUSSION

Black-capped chickadees were the clear dominant species in the contact zone. Not only was there a significant bias in dominance relationships between birds matched for age and sex in field observations, but all aviary dyads were won by black-capped chickadees. Within conspecific chickadee flocks, males typically dominate females and adults dominate juveniles (McCallum et al. 1999; Ratcliffe et al. 2007). In our study, we observed that female black-capped chickadees were consistently dominant over male mountain chickadees with whom they interacted. Furthermore, adult mountain chickadees were subordinate to juvenile black-capped chickadees in almost all interspecific interactions where the age of competitors was known. This would tend to increase the linearity effect across species: black-capped chickadees are always dominant to their mountain chickadee counterparts regardless of the sex and/or the age.

This linearity effect seems to be particularly strong between these 2 closely related species when compared with other parids. Within the contact zone between black-capped chickadee and Carolina chickadee, an aviary study by Bronson et al. (2003) showed male Carolina chickadees were generally dominant to black-capped chickadees, but they did observed some reversals. Similarly, studies in Europe on tits showed a 2-way doubly asymmetric interaction: the great tit (*Parus major*) is dominant over the blue tit (*Cyanistes caeruleus*) during the nonbreeding season (Haftorn 1993), and the smaller blue tit is competitively dominant during the breeding season (Dhondt 1989).

Hybridization in Paridae has been reported within both New World and Old World species (reviewed by Curry 2005; Curry et al. 2007). As a result, the genetic analyses showing evidence of genetic mixing between black-capped and mountain chickadees within this contact zone is not surprising. As hybrid nestlings were only found in mountain chickadee nests that also showed evidence of extrapair paternity, the clear asymmetry in the relative dominance between these species in our contact zone may influence female choice and extrapair copulation. Indeed, black-capped chickadees tend to initiate extrapair mating with male of higher rank than their social mate (Smith 1988; Otter et al. 1994; Otter et al. 1998; Mennill et al. 2004). Similarly, if the social hierarchy with Carolina chickadee being dominant over black-capped chickadees in aviary trials (Bronson et al. 2003) held in the field; it might explain the asymmetry in mating patterns between these 2 species. Indeed, Reudink et al. (2006) found that individuals that were more black-capped-like tended to lose more paternity in their nest than did the Carolina-like males (Reudink et al. 2006).

Randler (2002) proposed 3 different hypotheses that might cause females to mate with a heterospecific male: 1) 1 of the 2 species involved is less abundant, resulting in females breeding with a heterospecific partner rather than not breeding at

all, 2) females fail to recognize conspecifics versus heterospecifics, and/or 3) heterospecific males may have subnormal, or lower, expression of sexually selected signals than do conspecific males. Even though mountain chickadees are less common than black-capped chickadee in our study area (Grava A, personal observation), both species are relatively abundant in this overlap zone. Also mountain chickadees and black-capped chickadees are easily distinguishable phenotypically through both plumage and vocal cues, so it is unlikely females are unable to distinguish between species. As all the social partners chosen by either species are conspecific, it also suggests that females do discriminate to species level. Even if assortative mating by species is the frequent mode of reproduction for both species, hybridization may arise through extrapair matings if: 1) females base decisions about engaging in extrapair behavior on a signal that is common to both species, and 2) there is an asymmetry between males of either species in expression of those signals (Hartman et al. 2011). If females of either species in our study area tend to seek extrapair copulations from dominant males, mountain chickadee females might be more likely to engage in mixed-species mating than black-capped chickadee females. As a result, hybridization would be expected to result from extension of extrapair behavior across species. Our genetic data on nestlings confirm that such directional extrapair copulations may be driving hybridization.

We did observe adults that had evidence of mixed-species ancestry, and all but one was phenotypically mountain chickadee. However, none of these hybrids bred within our study site. This indicates that black-capped/mountain chickadee hybrids are viable, but whether or not they are fertile remains to be addressed.

FUNDING

Natural Science and Engineering Research Council of Canada Discovery (Grant to K.A.O., 227580-2009).

We thank Andrea Norris for providing access to the pure mountain chickadee population in Riske Creek. Dexter Hodder and the John Prince Research Forest team for their assistance in the field during this study. We also thank Robert L. Curry and an anonymous reviewer for their comments and suggestions on the manuscript. Birds were captured under Canadian Wildlife Service banding permit no 22806, and all experiment were run under the Animal Care and Use approval from the University of Northern British Columbia's Animal Care and Use Committee.

REFERENCES

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

Burg TM, Gaston AJ, Winker K, Friesen VL. 2006. Effects of Pleistocene glaciations on population structure of North American chestnut-backed chickadees. Mol Ecol. 15:2409–2419.

Curry RL. 2005. Hybridization in chickadees: much to learn from familiar birds. Auk. 122:747–758.

Curry RL, Rossano LM, Reudink MW. 2007. Behavioral aspects of chickadee hybridization. In: Otter KA, editor. Ecology and behavior of chickadees and titmice: an integrated approach. Oxford: Oxford University Press. p. 95–110.

Desrochers A. 1989. Sex, dominance, and microhabitat use in wintering black-capped chickadees: a field experiment. Ecology. 70:636–645.

Dhondt AA. 1989. Ecological and evolutionary effects of interspecific competition in tits. Wilson Bull. 101:198–216.

Dhondt AA. 2007. What drives differences between North American and Eurasian tit studies? In: Otter KA, editor. Ecology and behavior of chickadees and titmice: an integrated approach. Oxford: Oxford University Press. p. 299–310.

Ekman J. 1989. Ecology of non-breeding social systems of *Parus*. Wilson Bull. 101:263–288.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 164:1567–1587.

Ficken MS, Weise CM, Popp JW. 1990. Dominance and resource access in winter flock of black-capped chickadees. Wilson Bull. 102:623–633.

Foote JR, Mennill DJ, Ratcliffe LM, Smith SM. 2010. Black-capped chickadee (*Parus atricapillus*). In: Poole A, Gill F, editors. The birds of North America [online]. Philadelphia (PA): Academy of Natural Sciences.

Gill FB, Mostrom AM, Mack AL. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. Evolution. 47:192–212.

Gill FB, Slikas B, Sheldon FH. 2005. Phylogeny of titmice (Paridae): II. Species relationships based on sequences of the mitochondrial cytochrome-b gene. Auk. 122:121–143.

Griffith SC, Stewart IRK, Dawson IRK, Owens IP, Burke T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? Biol J Linn Soc. 68:303–316.

Haftorn S. 1993. Is the coal tit *Parus ater* really the most subordinate of the Scandinavian tits? Ornis Scand. 24:335–338.

Hanotte O, Zanon C, Puga A. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting Emberiza schoeniclus. Mol Ecol. 3:529–530.

Hartman P, Wetzel D, Crowley P, Westneat D. 2011. The impact of extra-pair mating behavior on hybridization and genetic introgression. Theor Ecol. doi:10.1007/s12080-011-0117-1.

Hill BG, Lein MR. 1989. Natural and simulated encounters between sympatric black-capped and mountain chickadees. Auk. 106:645–652.

Hogstad O. 1988. The influence of energy stress on social organization and behaviour of willow tits *Parus montanus*. Fauna Norv Ser C. 11:89–94.

Hogstad O. 1992. Mate protection in alpha pairs of wintering willow tits, *Parus montanus*. Anim Behav. 43:323–328.

Kvist L, Martens J, Nazarenko AA, Orell M. 2003. Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). Mol Biol Evol. 20:243–247.

Lemmon D, Withiam ML, Barkan CPL. 1997. Mate protection and winter pair-bonds in black-capped chickadees. Condor. 99:424–433.

Martinez JG, Soler JJ, Soler M, Møller AP, Burke T. 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). Evolution. 53:269–278.

McCallum DA, Grundel R, Dahlsten DL. 1999. Mountain chickadee. In: Poole A, Gill F, editors. The birds of North America, No. 453. Philadelphia (PA): Academy of Natural Sciences. p. 1–28.

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

Minock ME. 1972. Interspecific aggression between black-capped and mountain chickadees at winter feeding stations. Condor. 74:454–546.

Otter KA, McGregor PK, Terry AM, Burford FRL, Peake TM, Dabelsteen T. 1999. Do female great tits (*Parus major*) assess males by eavesdropping? A field study using interactive song playback. Proc R Soc Lond B Biol Sci. 266:1305–1310.

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

Otter KA, 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.

Otter KA, Ratcliffe LM. 1996. Female initiated divorce in a monogamous songbird: abandoning mates for males of higher quality. Proc R Soc Lond B Biol Sci. 263:351–354.

Peakall R, Smouse PE. 2001. GenAlEx v5.1: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research, version 5.1. Canberra (Australia): Australian National University.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945–959.

Pyle P. 1997. Identification guide of North American birds. Bolinas (CA): Slate Creek Press.

Randler C. 2002. Avian hybridization, mixed pairing and female choice. Anim Behav. 63:103–119.

Ratcliffe L, Mennill DJ, Schubert KA. 2007. Social dominance and fitness in black-capped chickadees. In: Otter KA, editor. Ecology and behavior of chickadees and titmice: an integrated approach. Oxford: Oxford University Press. p. 131–146.

Reudink MW, Mech SG, Curry RL. 2006. Extrapair paternity and mate choice in a chickadee hybrid zone. Behav Ecol. 17:56–62.

Rohwer S, Bermingham E, Wood C. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. Evolution. 55(2):405–422.

Smith SM. 1988. Extra-pair copulation in black-capped chickadees: the role of the female. Behaviour. 107:15–23.

Smith SM. 1991. The black-capped chickadee: behavioural ecology and natural history. Ithaca (NY): Comstock Publishing.

Tamura T, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596–1599.

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for PCR based typing from forensic material. Biotechniques. 10:506–513.

Wang MT, Hsu YC, Yao CT, Li SH. 2005. Isolation and characterization of 12 tetranucleotide repeat microsatellite loci from the green-backed tit (*Parus monticolus*). Mol Ecol Notes. 5:439–442.