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2012

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Genetic Evidence Supports Boreal Chickadee (*Poecile hudsonicus*) × Black-capped Chickadee (*Poecile atricapillus*) Hybridization in Atlantic Canada

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Lait, Linda A., Randy F. Lauff, and Theresa M. Burg. 2012. Genetic evidence supports Boreal Chickadee (*Poecile hudsonicus*) × Black-capped Chickadee (*Poecile atricapillus*) hybridization in Atlantic Canada. *Canadian Field-Naturalist* 126(2): 143–147.

Both morphological and genetic evidence support a hybridization event between a Boreal Chickadee (*Poecile hudsonicus*) and a Black-capped Chickadee (*Poecile atricapillus*) in Atlantic Canada. Plumage of the hybrid was intermediate to both parental species, with buffy sides and a dark brown cap on the head. Mitochondrial DNA control region showed the female lineage to be from a Boreal Chickadee, while Z-linked markers showed mixed Boreal Chickadee × Black-capped Chickadee heritage, likely representing an F1 hybrid. This is the first documented case of hybridization between these species in eastern North America, and it adds to the increasing evidence supporting intrageneric avian hybridization.

Key Words: *Poecile hudsonicus*, Boreal Chickadee, *Poecile atricapillus*, Black-capped Chickadee, hybridization, mitochondrial DNA, Z chromosomes, nuclear introns, Nova Scotia, Atlantic Canada.

When reproductive isolating mechanisms are incomplete or break down, hybridization can occur (Schluter and Nagel 1995). The presence of hybrid individuals in a population or the presence of an established hybrid zone can confound conservation efforts (e.g., the Red Wolf, *Canis lupus rufus* (Reich et al. 1999), and the Imperial Pheasant *Lophura imperialis* (McCarthy 2006)) and phylogeny construction (Grant and Grant 1992). Due to genetic incompatibilities, hybrid offspring are often inferior to the parental species (i.e., low survival or sterility); the extent and directionality of the hybridization will in part determine whether a hybrid swarm is created and the reproductive capabilities of the hybrid offspring (Grant and Grant 1992; McCarthy 2006).

Hybridization is found in approximately 10% of non-marine avian species, and may be 50% or higher in waterfowl and game birds (see Cockrum 1952; Mayr and Short 1970; Grant and Grant 1992; McCarthy 2006). Hybrids are often found in areas where ranges overlap; examples of well-documented hybrid zones include those of the sapsuckers, *Sphyrapicus* spp. (Walters et al. 2002); the Northern Flicker, *Colaptes auratus* (Moore and Buchanan 1985); the Hermit Warbler, *Setophaga occidentalis* (formerly *Dendroica occidentalis*) × Townsend's Warbler, *Setophaga townsendi* (formerly *Dendroica townsendi*) (Morrison and Hardy 1983); the Golden-winged Warbler, *Vermivora chrysoptera* × Blue-winged Warbler, *Vermivora cyanoptera* (Gill 2004); and the Eastern Meadowlark, *Sturnella*

magna × Western Meadowlark, *Sturnella neglecta* (Lanyon 1966). Both intrageneric crosses (see examples above) and intergeneric crosses (e.g., Common House-Martin (also known as Northern House-Martin), *Delichon urbicum* × Barn Swallow, *Hirundo rustica* (McCarthy 2006)), are common in the literature, with intrageneric hybridization occurring more often in passerines, (i.e., 48 of 69 known crosses in Parulidae (Valender et al. 2009) and 19 of 28 in Paridae (McCarthy 2006)). Less frequent hybridization events such as interfamily crosses can also be found (McCarthy 2006).

In the Paridae (chickadees, tits, and titmice), two well-studied hybrids are the Black-capped Chickadee, *Poecile atricapillus* × Carolina Chickadee, *Poecile carolinensis* (Sattler and Braun 2000; Bronson et al. 2003; Bronson et al. 2005; Reudink et al. 2007), and the Tufted Titmouse, *Baeolophus bicolor* × Black-crested Titmouse, *Baeolophus atricristatus* (Braun et al. 1984; Curry 2005). A recent study has identified the presence of a Black-capped Chickadee × Mountain Chickadee, *Poecile gambeli*, hybrid zone in British Columbia (Grava et al. 2012). A number of other crosses, based primarily on morphology, have been suggested, both in the popular press and in research labs: Black-capped Chickadee × Tufted Titmouse (Chipper Woods Bird Observatory), Mountain Chickadee × Boreal Chickadee, *Poecile hudsonicus* (Teslin Lake Bird Banding Station 2006), and Black-capped Chickadee × Boreal Chickadee (Sergei Drovetski, unpublished data).

During banding of both Boreal Chickadees and Black-capped Chickadees in Nova Scotia in the 2010 field season, a morphologically intermediate specimen (hereafter referred to as Bird A; 45.7°N, 61.9°W) was identified (Figure 1). Although the distribution of these two chickadee species is generally sympatric, substantial differences exist in habitat preference (e.g., coniferous forest versus deciduous), and no cases of hybridization in eastern North America have been documented. A single hybrid specimen was found in Alaska (Sergei Drovetski, unpublished data) and confirmed using microsatellite analysis. In order to establish whether Bird A was of hybrid origin, a suite of molecular markers were used, including sex-linked, autosomal, and maternally inherited loci.

Methods

Birds were caught using mist nets and song playback. Birds were measured (mass, wing chord, tarsus length, and bill length), banded, and a blood sample (<100 µL) was taken from the brachial vein. In addition to Bird A, 109 Black-capped Chickadees and 18 Boreal Chickadees were banded over the 2007 and 2010 field seasons. A blood sample was taken from all birds and measurements were taken from a subset (see below). Photographs were taken of the potential hybrid bird (Bird A), and reference photos were taken of both parental species (Figure 1). A principal components analysis (PCA) was performed in JMP version 10.0 (SAS Institute Inc. 2012) on the morphological measurements, comparing the measurements of Bird A to those of Boreal Chickadees ($n = 12$) and Black-capped Chickadees ($n = 77$) from Nova Scotia and New Brunswick.

A combination of mitochondrial DNA (mtDNA), two Z chromosome loci, and a nuclear intron was used to determine the genetic composition of Bird A. DNA was extracted using a modified chelex extraction method (Walsh et al. 1991). Polymerase chain reaction (PCR) was used to amplify the DNA. Sexing primers P2 and P8 (Griffiths et al. 1998) were used to determine whether the specimen was male or female (important for Z chromosome analysis). The sexing PCR was run in a 10 µL reaction with 1.5 mM MgCl₂ and an annealing temperature of 51°C. PCR products were visualized on a 3% agarose gel.

The mtDNA control region was amplified in a 25 µL reaction (2.5 mM MgCl₂ and 54°C annealing temperature) with the primers LmochCR1 and H1015chCR (Lait et al. *in press*). PCR products were visualized on a 0.8% agarose gel. Samples from Boreal Chickadees ($n = 18$) (LAL, unpublished data) and Black-capped Chickadees ($n = 41$) (John Hindley, unpublished data) from Nova Scotia and New Brunswick were available as part of other studies. The Boreal Chickadee samples were amplified with LmochCR1 and H1015chCR; the Black-capped Chickadee samples were amplified with LbcchCR1 and HCRCbox (Grava et al. 2012).

The Z chromosome marker ALDB was amplified using the PCR primers AldB.6F (Cox et al. 2007) and AldB.8R (Hackett et al. 2008), and a second Z chromosome marker, SPIN, was amplified using Spin319F and Spin472R (Handley et al. 2004). The nuclear intron, β -fibrinogen intron 5, was amplified with primers Fib.5F and Fib.6R (Fuchs et al. 2004). Standard PCR protocols were used, with MgCl₂ concentrations of 1.5 mM, 2.0 mM, and 2.5 mM and annealing temperatures of 50°C, 60°C and 54°C, respectively. PCR products were visualized on a 0.8% agarose gel. Two representative samples each from the Boreal Chickadees and Black-capped Chickadees were sequenced at nuclear loci for comparison.

Results

The PCA of the measurements showed considerable overlap between the two parental chickadee species, particularly in tarsus length and bill length, with Bird A falling in the centre (data not shown). Morphological measurements of Bird A were intermediate to that of the Boreal Chickadee and the Black-capped Chickadee for mass and wing chord, and were similar for tarsus and bill length (Table 1). In Bird A, the crown was dark brown (rich brown in the Boreal Chickadee and black in the Black-capped Chickadee) and the sides were buffy-brown (buffy-brown in the Boreal Chickadee and white to pale buff in the Black-capped Chickadee) (see Figure 1).

A 766 bp fragment of mtDNA from the control region was amplified and sequenced for Bird A (GenBank accession number JN654584) and >10 samples from each pure species (based on phenotype); a 501 bp fragment was aligned between the two species. The mtDNA from the control region from Bird A matched that of the Boreal Chickadee (Table 2). The sexing markers showed that Bird A was male (ZZ), and the Z chromosome markers amplified two fragments of equal length. A 520 bp fragment of ALDB and a 646 bp fragment of SPIN were amplified for Bird A and two females of each pure species. A two base pair insertion/deletion differentiated the parental species in both Z chromosome markers, allowing a direct comparison (Table 2). The Boreal Chickadee and Black-capped Chickadee sequences are distinct (1.7% sequence divergence for ALDB and 2.3% for SPIN), allowing easy separation. In each case, the hybrid sample produced two distinct sequences, one matching the Black-capped Chickadee sequence and one matching the Boreal Chickadee sequence. A 539 bp sequence for the β -fibrinogen intron contained three site variants between the two chickadee species (0.56% sequence divergence). Again Bird A contained two distinct sequences—one matching each species.

Discussion

Although the intermediate phenotype of Bird A was sufficient to suggest a hybridization event, genetic test-



FIGURE 1. Photographs of a Black-capped Chickadee, *Poecile atricapillus* (left), Bird A (middle), and a Boreal Chickadee, *P. hudsonicus* (right). The Black-capped Chickadee and Bird A photographs were taken on May 29, 2010 in Nova Scotia by RFL. The photographs of the Boreal Chickadee were taken on May 14, 2010 in New Brunswick by Kimberly Dohms.

TABLE 1. Mean morphological measurements (standard deviation in parentheses) for Boreal Chickadees and Black-capped Chickadees from Nova Scotia and New Brunswick and for Bird A.

	Mass (g)	Wing chord (mm)	Tarsus length (cm)	Bill length (cm)
Black-capped Chickadee ($n = 77$)	11.7 (0.9)	65.0 (2.5)	1.8 (0.1)	0.9 (0.1)
Boreal Chickadee ($n = 12$)	10.3 (0.9)	62.7 (3.2)	1.9 (0.1)	0.8 (0.1)
Bird A	11.0	63.0	1.6	0.8

TABLE 2. Variable sites found in the 501 bp fragment of the mtDNA control region, the 520 bp ALDB fragment, and the 646 bp SPIN fragment of the Z chromosome for samples from Boreal Chickadees, Black-capped Chickadees, and Bird A (a dash represents a deletion).

Sample	Control region	ALDB	SPIN
	1112222222222222333444	1223335	111122223344555
	2452271145677889999777139	551441580	500582562445679
	0006712406012161689578167	567342630	556938757801830
Black-capped Chickadee	-ACTTAGAGGATCACTCAGCCGACG	TGCCAAGTG	A--CGGTCAGGTGGG
Boreal Chickadee	ATTCGGTGAAGCTGA-YGAATCGTA	--TCCAACA	CCCAATCTTCACAGC
Bird A (sequence 1)	ATTCGGTGAAGCTGA-TGAATCGTA	TGCCAGGTG	A--CGGTCAGGTGGG
Bird A (sequence 2)	n/a	--TTCAGCG	CCCAATCTTCACAAC

Scenario	Female parent	Male parent	Offspring (Bird A)
1	Z^{BO} W	Z^{BC} Z^{BC}	Z^{BC} Z^{BO}
2	Z^{BO} W	Z^{BC} Z^{BO} (hybrid)	
3	Z^{BO} W (hybrid)	Z^{BC} Z^{BC}	
4	Z^{BO} W (hybrid)	Z^{BC} Z^{BO} (hybrid)	
5	Z^{BC} W (hybrid)	Z^{BC} Z^{BO} (hybrid)	
6	Z^{BC} W (hybrid)	Z^{BO} Z^{BO}	

FIGURE 2. Possible scenarios explaining the observed genetic patterns in Bird A. Bolded genotypes show the transmission of the Z chromosome (Black-capped Chickadee, *Poecile atricapillus*, Z^{BC}, and Boreal Chickadee, *P. hudsonicus*, Z^{BO}) from parent to offspring. Hybrid female parents would be the result of a female Boreal Chickadee mating with a male Black-capped Chickadee to retain the Boreal Chickadee mtDNA. As hybrids are not common, the first scenario is the most likely.

ing was required to confirm its genetic origin. The mtDNA, Z chromosome, and nuclear loci strongly support the hybrid origin of Bird A. The possibility exists that one of the parents (Figure 2) could have been a hybrid, but the presence of a Black-capped Chickadee and a Boreal Chickadee β -fibrinogen allele, as well as Z chromosomes from both species, suggest that it is more likely an F1 hybrid with a Boreal Chickadee mother and Black-capped Chickadee father. As Bird A is male (ZZ), it could have received the Black-capped Z chromosome either as the maternal contribution (Figure 2, scenarios 5 and 6) or as the paternal contribution (scenarios 1 to 4), and it could have received the Boreal Z chromosome either as the maternal contribution (scenarios 1 to 4) or as the paternal contribution (scenarios 5 and 6). As the mtDNA shows that the female lineage is of Boreal Chickadee (or hybrid) origin, the most parsimonious explanation is that Bird A is the result of a cross between a female Boreal Chickadee and a male Black-capped Chickadee. All of the other alternatives would require additional hybridization events, and this is unlikely given the lack of hybridization documented between these species.

As with many boreal birds, the Boreal Chickadee is undergoing a severe population decline, most likely due to a combination of habitat loss and climate change (Sauer et al. 2005). The combination of reduced breeding population and habitat availability may be pushing the Boreal Chickadee to increasingly share habitat and resources with the Black-capped Chickadee. Nova Scotia is near the southern edge of the Boreal Chickadee's range, and Boreal Chickadees are less common than Black-capped Chickadees in Nova Scotia; it may be that a lack of available Boreal Chickadee mates triggered the hybridization event.

This is in contrast to the hybridization between Black-capped Chickadees and Carolina Chickadees that occurs extensively along the contact zone between the two species running from Kansas to New Jersey in the United States (Bronson et al. 2005; Reudink et al. 2007). In that case, the two chickadee species are phenotypically indistinguishable, they reside in the same habitat, and, although their song is quite different, they are capable of plasticity in song learning. As many as two-thirds of the chickadees in the hybrid zone are of hybrid origin, suggesting that the only reproductive barrier between the Black-capped Chickadee and the Carolina Chickadee is physical isolation (Reudink et al. 2007).

In another example of a hybridization event involving the Black-capped Chickadee, Grava et al. (2012) found that hybrid offspring of Black-capped Chickadees and Mountain Chickadees were produced through pairing of a more dominant male Black-capped Chickadee with a female Mountain Chickadee.

Hybrids between Black-capped Chickadees and Carolina Chickadees show contrasting patterns: Bronson et al. (2005) found no evidence of assortative mating, yet aviary studies suggested male Carolina Chickadee dominance (Bronson et al. 2003). Reudink et al. (2007) also found evidence of male Carolina Chickadee dominance in Pennsylvania in hybrid crosses, and reciprocal matings were observed. Geographic differences between Black-capped Chickadee and Carolina Chickadee hybrid zones are also apparent (see Reudink et al. 2007 for discussion), although all studies support the notion of a moving hybrid zone, with the range of the Carolina Chickadee expanding at the expense of the Black-capped Chickadee range.

Additional work banding chickadees in Atlantic Canada would be useful to assess whether there are other hybridization events between Black-capped Chickadees and Boreal Chickadees, caused perhaps by the decrease in suitable habitat. In addition to Bird A, the authors banded 109 Black-capped Chickadees and 18 Boreal Chickadees in Nova Scotia and New Brunswick over two field seasons; none were of intermediate phenotype. While the current data strongly suggest that Bird A is an F1 hybrid, we are unable to show definitively whether it is an F1 or later hybrid. Our data do confirm the hybrid origin of this specimen and raise the possibility of the existence of additional hybrid birds.

Acknowledgements

Thank you to Kimberly Dohms and all other field assistants for their help with sample collection, John Hindley for the sequences of the Black-capped Chickadee samples, and two anonymous reviewers for their comments. This project was supported by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to TMB, Alexander Graham Bell Canada Graduate Scholarship to LAL) and Alberta Innovates Technology Futures (Graduate Scholarship to LAL and New Faculty Award to TMB).

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Received 7 May 2012

Accepted 10 July 2012