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STRUCTURE AND DYNAMICS OF THE HYBRID ZONE BETWEEN BLACK-CAPPED CHICKADEE (*POECILE ATRICAPILLUS*) AND CAROLINA CHICKADEE (*P. CAROLINENSIS*) IN SOUTHEASTERN PENNSYLVANIA

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Abstract.—Analysis of the structure and stability of a hybrid zone can serve as a starting point for examining mechanisms that influence spatial and evolutionary relationships between species. Recent studies of the hybrid zone between Black-capped Chickadee (*Poecile atricapillus*) and Carolina Chickadee (*P. carolinensis*) have suggested that genetic introgression is limited to a narrow zone, while also reinforcing the conclusion that the line of contact between these parapatrically distributed species is now shifting northward. We investigated the structure, position, and recent movement of the chickadee hybrid zone in southeastern Pennsylvania. Using selectively neutral microsatellite DNA markers, along with mitochondrial DNA haplotypes, we documented large differences in genetic composition among three populations ≤65 km apart where we sampled large numbers of breeding residents during 1998–2003. Genetic results indicate that the three sites support a population of Carolina Chickadees (Great Marsh), a population in which most individuals exhibit evidence of hybridization (Nolde Forest), and a population comprising mostly Black-capped Chickadees but with evidence of hybridization now taking place (Hawk Mountain). The patterns within the Nolde Forest population suggest that selection against hybrids may not be as strong as has been concluded from studies in other parts of the chickadee hybrid zone. Comparison of mitochondrial and nuclear genotypes between samples collected ~15 years apart suggest that the northern edge of the hybrid zone shifted by ~20 km over this interval, with hybridization now occurring as far north as the Kittatinny Ridge and beyond, where only Black-capped Chickadee genotypes were previously detectable. Our data and historical accounts suggest that the hybrid zone, now ~50 km wide, may have become wider while also shifting northward. These results support the hypothesis that Carolina Chickadees enjoy a selective advantage during hybridization with Black-capped Chickadees, but both the proximate mechanisms and ultimate causes remain to be investigated. *Received 8 December 2004, accepted 5 April 2006.*

Key words: chickadee, contact zone, hybrid, introgression, *Poecile*.

Structure et dynamique de la zone d'hybridation entre *Poecile atricapillus* et *P. carolinensis* dans le sud-est de la Pennsylvanie

Resumen.—L'analyse de la structure et de la stabilité de la zone d'hybridation peut servir de point de départ pour examiner les mécanismes qui influencent les

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relations spatiales et évolutives entre les espèces. Des études récentes portant sur la zone d'hybridation entre la Mésange à tête noire (*Poecile atricapillus*) et la Mésange de Caroline (*P. carolinensis*) suggèrent que l'introgression génétique est limitée à une zone étroite, tout en renforçant l'idée que la zone de contact entre ces deux espèces parapatriques est en train de se déplacer vers le nord. Nous avons examiné la structure, la position et les mouvements récents de la zone d'hybridation des mésanges dans le sud-est de la Pennsylvanie. À l'aide de marqueurs microsatellites neutres de l'ADN et d'haplotypes d'ADN mitochondrial, nous avons documenté de grandes différences au niveau de la composition génétique de trois populations situées à 65 km l'une de l'autre, pour lesquelles nous avons échantillonné de grands nombres d'individus reproducteurs résidents entre 1998 et 2003. Les résultats génétiques indiquent que les trois sites supportent respectivement une population de Mésanges de Caroline (Great Marsh), une population dont la plupart des individus montrent des signes d'hybridation (Nolde Forest) et une population composée surtout de Mésanges à tête noire présentant des signes d'hybridation (Hawk Mountain). Les tendances dans la population de Nolde Forest suggèrent que la sélection envers les hybrides n'est peut-être pas aussi forte que ce qui avait été conclu à partir des études réalisées dans d'autres parties de la zone d'hybridation des mésanges. La comparaison des génotypes mitochondriaux et nucléaires entre les échantillons récoltés à environ 15 ans d'intervalle suggère que la limite nord de la zone d'hybridation s'est déplacée d'environ 20 km pendant cet intervalle. Des hybridations se produisent maintenant jusqu'à Kittatinny Ridge et même plus au nord, où seulement les génotypes de Mésange à tête noire étaient autrefois détectés. Nos données et les comptes-rendus historiques suggèrent que la zone d'hybridation, maintenant large d'environ 50 km, peut avoir élargie tout en se déplaçant vers le nord. Ces résultats supportent l'hypothèse que les Mésanges de Caroline profitent d'un avantage sélectif lors de l'hybridation avec les Mésanges à tête noire mais les mécanismes proximaux et les causes ultimes demeurent peu connus.

Hybridization is common in birds, occurring at least occasionally in >9% of species (Grant and Grant 1992). The causes and consequences of hybridization raise fundamental questions about avian evolution, particularly in regard to isolating mechanisms and species limits. Excellent opportunities exist for addressing these questions in hybrid zones, where otherwise allopatric species come into contact and interbreed (Barton and Hewitt 1985; Hewitt 1988, 1989; Harrison 1993). Among birds, hybrid zones may be broad or narrow, and they may be geographically stable or may shift in position over time (Grant and Grant 1992). The outcome for any particular pair of hybridizing species will depend on patterns of dispersal and selection, which will in turn be subject to extrinsic ecological influences, such as the presence of landscape ecotones, as well as "endogenous" mechanisms that affect the relative fitness of hybrids (e.g., Bronson et al. 2003a).

Hybridization between Black-capped Chickadees (*Poecile atricapillus*) and Carolina Chickadees (*P. carolinensis*) represents a longstanding challenge for ornithologists, in large part because the species are phenotypically very similar. Black-capped Chickadees are usually larger, with proportionately longer tails; they have more prominent white edging on flight feathers and, especially, on the greater secondary coverts. Also, they produce stereotyped two-note *fee bee* whistled song, in contrast to the songs of Carolina Chickadees, which are usually four notes long, with alternating high and low pitch (Smith 1991, 1993; Pyle 1997; Mostrom et al. 2002). Despite phenetic similarity, phylogenetic analysis (Gill et al. 2005) indicates that they are not sibling species: the Black-capped Chickadee is most closely related to the Mountain Chickadee, whereas the Carolina Chickadee is more distantly related; Black-capped and Carolina chickadees exhibit ~5% mitochondrial divergence in the cytochrome-*b* gene.

These two chickadee species exhibit parapatric overall distributions, with a long line of contact stretching from Kansas to New Jersey.

This line probably represents secondary contact following separation of the species during early Pleistocene glaciation or before (Brewer 1963, Gill et al. 1989), but biogeographic history of the pair has not yet been investigated in detail (Curry 2005). Major features of the present pattern were evident from early observations of morphology and songs, though surveys can be confounded by the ability of individuals to learn the songs of both species (reviewed in Curry et al. 2007). Tanner (1952) documented occupation of high-elevation habitats by Black-capped Chickadees in parts of the Appalachian Mountains as far south as North Carolina, and occupancy of lower elevations by Carolina Chickadees throughout the region. Tanner found no direct evidence for hybridization except for bilingual singing by at least one chickadee, but interbreeding was later reported from the mountains of Virginia (Johnston 1971). Brewer (1961, 1963) characterized a line of separation running from southwest to northeast across Illinois and discussed the occurrence and consequences of hybridization at length, but also noted apparent distributional gaps separating the species in some regions. Rising (1968) documented the presence of morphologically intermediate birds in southeastern Kansas, where the ranges appeared to be contiguous, as was evident from song patterns in southeastern Pennsylvania also (Ward and Ward 1974). The two species were separated in Indiana, however, by an unoccupied zone ≤30 km in width during the breeding season (Merritt 1981), though observations of bilingual singing and atypical songs (Merritt 1978) suggest that hybridization may have been occurring.

Early work raised questions about the structure and dynamics of the chickadee contact zone that have since been addressed using molecular methods in three regions. In southwestern Missouri, morphological and vocal data revealed a line of contact with no obvious distributional gaps (Robbins et al. 1986). Associated analysis of allozymes showed high levels of protein similarity between the two species (Braun and Robbins 1986), resulting in poor resolution of the hybrid zone's position and width. Samples from the same study were subsequently examined by Sawaya (1990) using RFLP (restriction fragment length polymorphism) analysis of two species-specific nuclear markers and mitochondrial DNA (mtDNA), along with electrophoretic analysis of the allozyme guanine deaminase (GDA; after Gill et al. 1989). These methods permitted detection of hybrids mostly within a zone ~15 km wide, with evidence of introgression spanning ≥40 km for one autosomal locus. The same molecular methods used to examine a transect spanning the Appalachians in West Virginia and Virginia revealed extensive hybridization in zones ~25 km wide on both slopes, but with some introgression extending across much greater distances, particularly on the West Virginia side (Sattler and Braun 2000). Similarly, on comparatively flat terrain in north-central Ohio, a zone of hybridization 20–25 km wide was detected using an expanded set of species-specific markers (Bronson et al. 2003a), along with evidence of reduced reproductive success within the hybrid zone (Bronson et al. 2005).

Changes in geographic position of the chickadee contact zone over time may complicate analyses. In nearly all regions, the line of contact appears to be moving northward, with Carolina Chickadees and hybrids moving into areas previously occupied by resident Black-capped Chickadees; in Ohio, for example, the contact zone may now lie ~100 km north of its position as of the late 1930s (Bronson et al. 2005; see also Curry 2005).

The research cited above has focused predominantly on sections of the chickadee contact zone west of or within the Appalachian Mountains (see also Parkes 1987). Apart from the descriptive work of Ward and Ward (1974), the northeastern segment of the contact zone has received less attention. The area of contact in central New Jersey (Walsh et al. 1999, Mostrom et al. 2002) remains unstudied. In Pennsylvania, the two species are believed to exhibit parapatric overlap in Adams, York, Lancaster, Berks, Montgomery, and Bucks counties, but with evidence accumulating of a recent northward shift in the region (Ward and Ward 1974, Gill 1992, Uhrich 1997, McWilliams and Brauning 2000; see Fig. 1).

We investigated the genetic structure the hybrid zone between Black-capped and Carolina chickadees in southeastern Pennsylvania, with three goals. First, we sought to evaluate the current position and width of the contact zone, using data on mitochondrial haplotypes and nuclear markers, particularly microsatellite DNA. Microsatellites were appropriate for the present

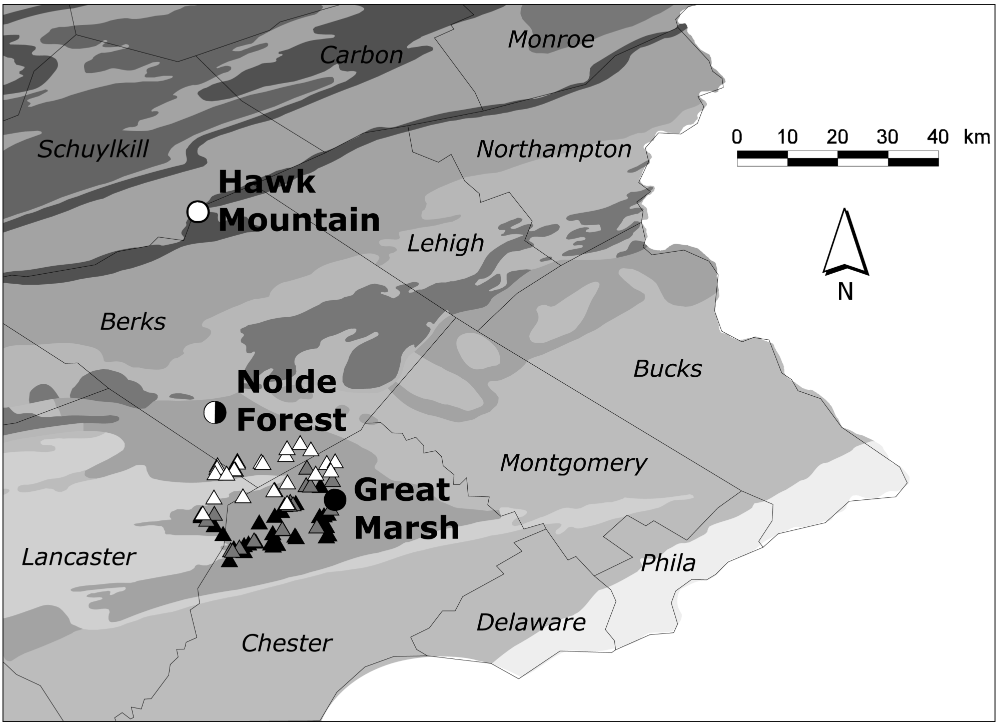

Fig. 1. Locations of three primary study areas in southeastern Pennsylvania: Hawk Mountain (HM, white dot), predominantly Black-capped Chickadees; Nolde Forest (NF, split dot), mixed–hybrid population; and Great Marsh (GM, black dot), Carolina Chickadees. Map includes boundaries for counties mentioned in the text; darkness of background shading indicates approximate relative elevation based on geological landforms (based on Pennsylvania Spatial Data Access maps); the Hawk Mountain site straddles the Kittatinny Ridge (~465 m elevation). Also shown are sites studied by Ward and Ward (1974), where chickadees observed in 1963–1969 sang exclusively Black-capped song (white triangles), both songs and variations (gray triangles), or exclusively Carolina songs (black triangles).

study because they are generally accepted to be selectively neutral and are highly polymorphic; consequently, they can be used as effective indicators of introgression (Chambers and MacEvoy 2000, Avise 2004, Grant et al. 2005). Second, we investigated the degree to which the contact zone in the focal region has shifted in position in recent decades. This component of the study was made possible by access to a series of specimens from the region, collected in the 1980s by F. B. Gill and colleagues, and comparison with data from our more recent field studies. Third, we sought to examine the extent of interbreeding within the contact zone. This component involved intensive analysis of genetic structure within three breeding populations in conjunction with research on behavioral and ecological aspects of hybridization (Reudink et al. 2006, Curry et al. 2007). The combined use of neutral markers and large within-population sample sizes represents an approach that differs from, and complements, recent work on hybridization of these species elsewhere; studies in Virginia, West Virginia, and Ohio have mainly relied on samples from across broad geographic regions, but with comparatively few birds examined at individual localities.

Methods

Field methods.—Our study involved intensive investigation of breeding populations at three sites in or near the contact zone in southeastern Pennsylvania (Fig. 1). Nolde Forest

Environmental Education Center (269 ha, south of Reading, Berks County; 40°17′N, 75°58′W) fell within the hybrid zone, judging from overall species distributions in the region coupled with suggestions of recent northward movement of the zone (Gill 1992, Uhrich 1997), especially in relation to song patterns observed in the 1960s (Ward and Ward 1974; see Fig. 1). (At the beginning of our research, we believed the Nolde Forest population to contain only Black-capped Chickadees, because all resident males then produced, and still do, the song of that species; Rossano 2003, Curry et al. 2007.) In an effort to sample reference populations of each of the parental species, we investigated breeding adults from Hawk Mountain Sanctuary and 10 nearby woodlots (~150 ha in total area, centered on the Schuylkill and Berks county border, 40°38′N, 75°59′W, presumed to be entirely or predominantly Black-capped Chickadees), coordinated with an associated study of forest fragmentation (Guers 2006), and adults from Great Marsh (200 ha; East Nantmeal Township, Chester County, 40°08′N, 75°44′W; Carolina Chickadees). Hawk Mountain is 41 km north-northwest and Great Marsh 24 km southeast of Nolde Forest (Fig. 1).

We initiated field study at Great Marsh and Nolde Forest in 1998 and at Hawk Mountain in 2000; the bulk of the data reported here derive from all three sites during four breeding seasons, 2000–2003. We obtained demographic data and blood samples mainly from breeding adult chickadees using artificial snags for nesting (made from plastic tubing, based on Grubb and Bronson 1995); the maximum number of snags available each year was 120 at Hawk Mountain, 152 (plus 12 nest boxes) at Nolde Forest, and 54 at Great Marsh. We monitored snags to determine laying date, clutch size, hatching success, and fledging success (Cornell 2001, Reudink et al. 2006). We sampled breeding chickadees at 2–15 nests (n = 30) that reached at least the laying stage in each year at Hawk Mountain; an average of 37 nests per year (range: 18–54; n = 168) at Nolde Forest; and an average of 17 nests per year (range: 9–26; n = 105) at Great Marsh.

We captured all adults either before the breeding season at feeders, using mist nets or traps, or during the nestling period, using mist nets positioned in front of each nest snag. Each adult received a unique combination of a numbered federal aluminum band and two or three plastic color bands. We obtained blood samples by piercing the ulnar vein (Gaunt and Oring 1999) and drawing 20–50 μL of blood into a microcapillary tube; samples were then stored in lysis buffer (100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) at 4°C for later DNA extraction. Our samples include 51 unique breeding adults from Hawk Mountain, 180 from Nolde Forest, and 65 from Great Marsh.

Specimens.—We incorporated analyses of genetic data for 118 chickadee specimens from the southeastern Pennsylvania region to provide context for results from the breeding populations. F. B. Gill collected this specimen series in 1986–1989 from eight localities mainly along a northwest–southeast transect spanning the contact zone in Carbon, Berks, and Montgomery counties (Fig. 2); and from farther northeast and northwest at Stroudsburg, Monroe County, and Lock Haven, Clinton County, respectively (not shown). After collection, these specimens were stored at −70°C at the Academy of Natural Sciences, Philadelphia.

Molecular methods.—For analyses based on polymerase chain reaction (PCR), we extracted DNA from blood samples and from tissue (pectoral muscle) of thawed specimens using the DNeasy Tissue extraction kit (Qiagen, Valencia, California). For haplotype analyses using whole mtDNA, Gill and colleagues used standard chloroform-phenol extraction methods (see Gill et al. 1993).

We used PCR–RFLP analysis of the cytochrome-*b* gene to determine the mitochondrial haplotype of birds breeding at Hawk Mountain, Nolde Forest, and Great Marsh. This involved PCR amplification of the cytochrome-*b* gene (using primers developed by Kvist et al. 1996) followed by separate restriction enzyme digests using *EcoR* V and *Xmn* I. Digested products were run through 3% agarose gels stained with ethidium bromide and visualized under ultraviolet light. Because of a single base-pair substitution (Kvist et al. 1996), *EcoR* V cuts the Black-capped cytochrome-*b* fragment, but not the Carolina, whereas *Xmn* I cuts the Carolina cytochrome-*b* fragment, but not the Black-capped.

Restriction-fragment analyses were also used to determine haplotypes for a subset of the field-collected specimens. Haplotype results for 74 individuals, evaluated using digests of complete mtDNA extractions using the enzymes

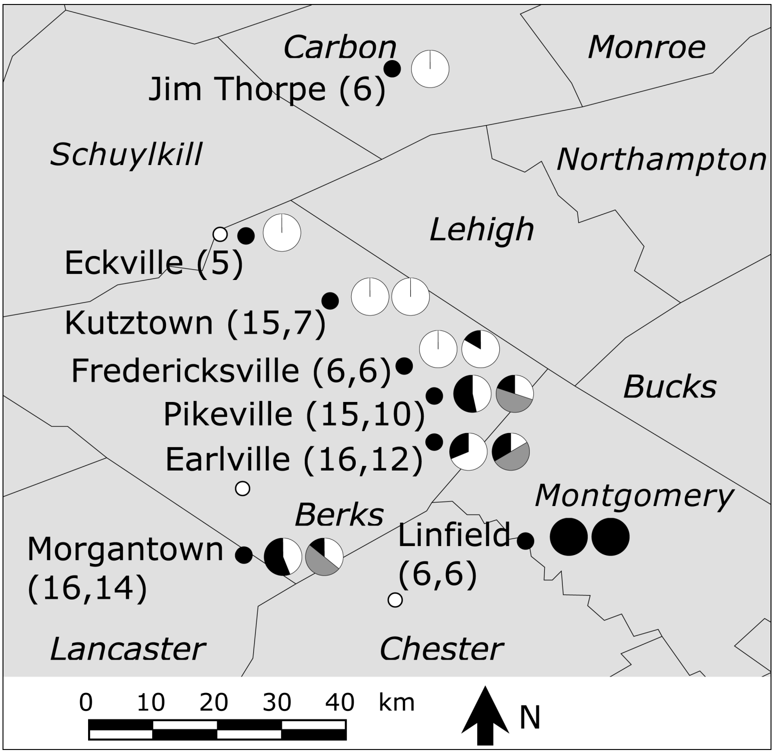

Fig. 2. Genetic patterns in chickadee specimens collected by F. B. Gill in 1986–1989 in southeastern Pennsylvania (county names in italics). For each named collection locality (filled dots), the left pie chart shows mtDNA percentages (white, Black-capped; black, Carolina) and the right pie chart shows nuclear allozyme (GDA) results where available (white, Black-capped; gray, hybrid heterozygotes; black, Carolina). Sample sizes are indicated in parentheses for mtDNA or for both analyses as applicable. Open dots refer to the three sites studied intensively since 1998 (see Fig. 1).

BamH I, *Hae* I, *Sal* I, and *Sma* I (following Gill et al. 1993), were provided by F. B. Gill and coworkers. S.P.M. analyzed cytochrome *b* from an additional 21 specimens from the series using the PCR–RFLP methods detailed above.

Carolina Chickadees exhibit a different allele for GDA than Black-capped Chickadees (Gill et al. 1989). Electrophoretic data for this locus were made available by F. B. Gill for 56 birds from the specimen collection (Fig. 2). Laboratory methods followed Braun and Robbins (1986) but used horizontal starch gel electrophoresis (Gill et al. 1989).

For genetic identity analysis, we used two microsatellite loci isolated from Black-capped Chickadees (Otter et al. 1994) and four from Blue Tits (*Cyanistes caeruleus*; Dawson et al. 2000). (For additional details about these loci, including primer sequences, see Reudink et al. 2006.) Samples were amplified via PCR using fluorescently labeled forward primers. Amplified loci were then run on an automated genetic analyzer (ABI 310) with GENESCAN, version 3.7 (Applied Biosystems, Foster City, California). Scoring of peak sizes was conducted blind, with no indication as to the individual's identity or source population, using GENOTYPER, version 3.7 (Applied Biosystems). All homozygous peaks were scored at least two times independently, and the raw data were re-examined to ensure accuracy. All nonamplifying loci were reamplified and rerun on the genetic analyzer. M.W.R. performed all genotype scoring.

STRUCTURE analysis.—To examine how many genetic groups can be recognized from the microsatellite data, we used STRUCTURE, version 2.1 (Pritchard et al. 2000). This program uses Bayesian methods to estimate simultaneously the number of populations and the allele frequencies in each population given the data. We analyzed 51 individuals from Hawk

Mountain, 180 from Nolde Forest, and 65 from Great Marsh. Following the suggestions of Pritchard et al. (2000), we ran the entire data set (without any prior information) to determine the most likely number of clusters. Using 750,000 estimation steps after 100,000 burn-in steps, we ran five replications of each value of K (number of clusters) from 1 to 6. Following this, we performed a second analysis using the two putative parental source populations (Hawk Mountain and Great Marsh) as priors (leaving the Nolde Forest population with unknown priors) for K = 2–5. We ran five replicates of the analysis with priors for each K.

RESULTS

Mitochondrial haplotypes.—All birds from the Gill specimen series produced diagnostic Black-capped or Carolina haplotypes, whether analyzed using whole mtDNA digested with *BamH* I/*Hae* I/*Sal* I/*Sma* I (n = 53) or PCR-RFLP analysis of cytochrome *b* (n = 21) or both methods (n = 21; no discrepancies depending on method). Haplotype data from the specimens indicate that the populations sampled in 1986–1989 spanned the contact zone (Fig. 2). All birds collected at Jim Thorpe (Carbon County), at Eckville (Berks County, 2 km east of Hawk Mountain Sanctuary), and at two sites farther south in Berks County (Kutztown, Fredericksville) exhibited exclusively Black-capped haplotypes, as did birds at the two sites farther northeast and northwest (Stroudsburg, n = 5; Lock Haven, n = 5). Mixed populations with respect to mtDNA occupied two sites in eastern Berks County (Pikeville, Earlville) and at Morgantown. All birds from Linfield, in the southeastern section of the study area, had Carolina mtDNA.

All birds in the three resident populations studied intensively since 1998 also exhibited cytochrome-*b* mtDNA that cut with one, and not the other, restriction enzyme. Chickadees exhibited almost complete replacement of haplotypes across the three sites (Fig. 3). At Hawk Mountain, 95% of males (n = 20) and 76% of females (n = 25 individuals) exhibited Black-capped haplotypes (likelihood-ratio χ^2 = 3.41, P = 0.065). At Nolde Forest, 96% of males (n = 83) and 99% of females (n = 65) exhibited Carolina mtDNA haplotypes. Black-capped haplotypes constituted a minority in the Nolde breeding population in every year, and none were found after 2000. At Great Marsh, all birds examined in all years of the study (n = 84 breeding adults, including 44 males and 40 females) had Carolina haplotypes.

Nuclear marker.—Analysis of GDA profiles permitted diagnostic categorization of individuals in the Gill specimen series from six sites (Fig. 2) as either Black-capped (homozygote), hybrid (heterozygote), or Carolina (homozygote). Consistent with prior work suggesting that GDA is sex-linked (Sawaya 1990, Sattler and Braun 2000), all birds heterozygous for GDA (n = 11) were males. Geographic variation in GDA largely matched the contemporaneous haplotype pattern: sites with individuals of both species based on GDA also included birds representing each of the two mtDNA haplotypes.

Comparison of GDA and haplotype data for individual birds yields additional resolution of the contact zone's position in the 1980s (Fig. 2). At Fredericksville, hybridization was evident from a single bird homozygous for Carolina GDA but with Black-capped mtDNA. To the southeast at Pikeville, the sample included two individuals with Black-capped GDA and mtDNA, one with Carolina GDA and mtDNA, and six hybrids (five GDA heterozygotes and one bird with mismatched GDA and mtDNA). Roughly 10 km southeast at Earlville, the sample suggested slightly greater Carolina influence, with two individuals having Black-capped GDA and mtDNA, four birds with Carolina GDA and mtDNA, and six hybrids (four GDA heterozygotes and two birds with GDA and mtDNA mismatch). The sample from Morgantown, southwest of the primary transect line, similarly exhibited a mixed population: four birds with Black-capped GDA and mtDNA, six with Carolina GDA and mtDNA, and four hybrids (two GDA heterozygotes and two birds with GDA and mtDNA mismatch).

Microsatellite analysis.—Using the data from breeding studies in 1998–2003, we genotyped 51 resident individuals from Hawk Mountain, 180 from Nolde Forest, and 65 from Great Marsh at six microsatellite loci. Across all populations, the average number of alleles per locus (mean ± SE) was 33.3 ± 16.91. Within-population allelic diversity at Nolde Forest was lower (21.1 ± 12.66). Departures from Hardy-Weinberg equilibrium were noted in all populations (Table 1), likely attributable to low heterozygosity along with hybridization at Nolde Forest and Hawk

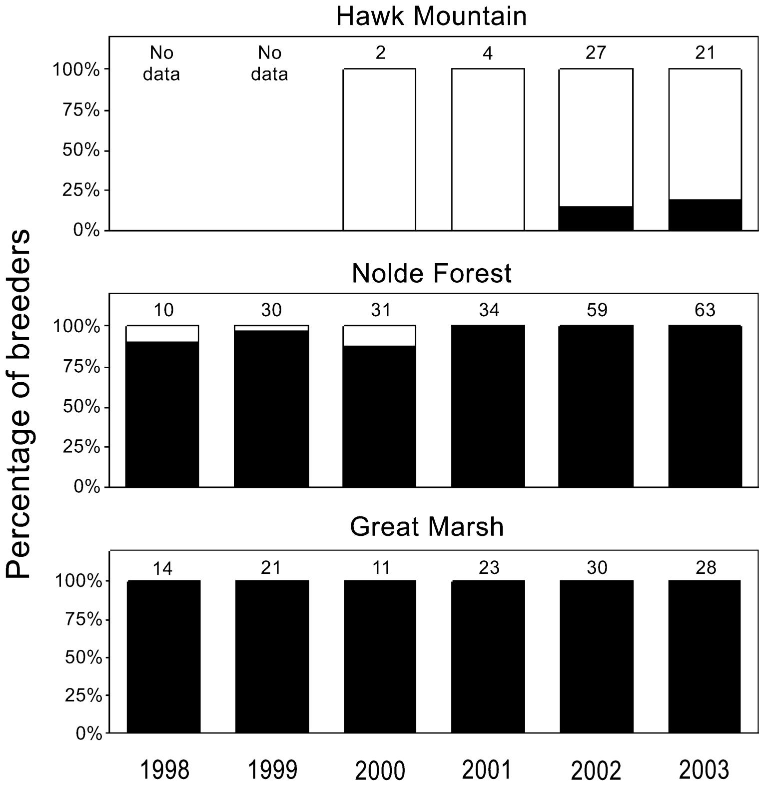

FIG. 3. Percentage of mtDNA haplotypes from breeders at Hawk Mountain, Nolde Forest, and Great Marsh. White shading indicates Black-capped haplotypes; black indicates Carolina haplotypes. Sample sizes for each site in each year are shown above bars; some birds bred in multiple years and are included for each year in which they bred.

Mountain (see below). Linkage disequilibrium also was observed between sets of loci in each population, though the only population with no evidence of hybridization or introgression, Great Marsh, showed linkage disequilibrium only between loci with low heterozygosities, which suggests that the observed disequilibria at the other sites result from hybridization rather than chromosomal linkage. Average observed heterozygosity of the Nolde Forest population was 0.798, which was lower than the expected heterozygosity of 0.901. In both the Hawk Mountain and Great Marsh populations, average observed heterozygosity was again lower than the expected heterozygosity (Table 1).

Nei's (1978) unbiased genetic distance between the two baseline populations (Hawk Mountain and Great Marsh) was 0.337 (calculated using GENETIC DATA ANALYSIS, version 1.1; Lewis and Zaykin 2001). Pairwise distances for Nei's genetic distance and F_{ST} are listed in Table 2.

STRUCTURE analysis.—Without source population as prior information in the initial analysis, STRUCTURE identified five clusters (Fig. 4A). Hawk Mountain had the most pure population, and Nolde Forest had the most mixed, though the Great Marsh population was largely mixed as well.

When information about source populations was used as prior information, STRUCTURE identified three clusters as most probable (Figs. 4B and 5). In this analysis, both Hawk Mountain and Great Marsh showed almost entirely pure populations, whereas Nolde Forest demonstrated a clear mix between both the other populations and a third group that likely represents F1 and other hybrids. As without the priors, individuals from Hawk Mountain were most likely to be assigned to a single cluster ("Black-capped

Table 1. Number of alleles present in each population, including expected (H_E) and observed (H_{Obs}) heterozygosities. Asterisks indicate significant departures from Hardy-Weinberg equilibrium.

	Hawk Mountain			Nolde Forest			Great Marsh			All sites		
Locus	Number of alleles	H_E	H_{Obs}	Number of alleles	H_E	H_{Obs}	Number of alleles	H_E	H_{Obs}	Number of alleles	H_E	H_{Obs}
PCA 8	23	0.952	0.833	61	0.977	0.876*	39	0.975	0.837*	67	0.979	0.864
PAT 2-14	11	0.735	0.542*	21	0.799	0.727	14	0.826	0.778	24	0.827	0.718
PAT 2-43	16	0.934	0.917	28	0.895	0.815*	18	0.897	0.911	31	0.903	0.842
PCA 9	5	0.528	0.583	21	0.877	0.833	14	0.843	0.778*	21	0.865	0.799
PCA 4	14	0.923	0.750*	24	0.919	0.801*	18	0.930	0.933	27	0.926	0.823
PCA 2	13	0.840	0.625*	28	0.916	0.676*	12	0.821	0.750*	30	0.902	0.685
Average	13.7	0.819	0.778	30.5	0.897	0.780	19.2	0.882	0.874	33.3	0.900	0.789

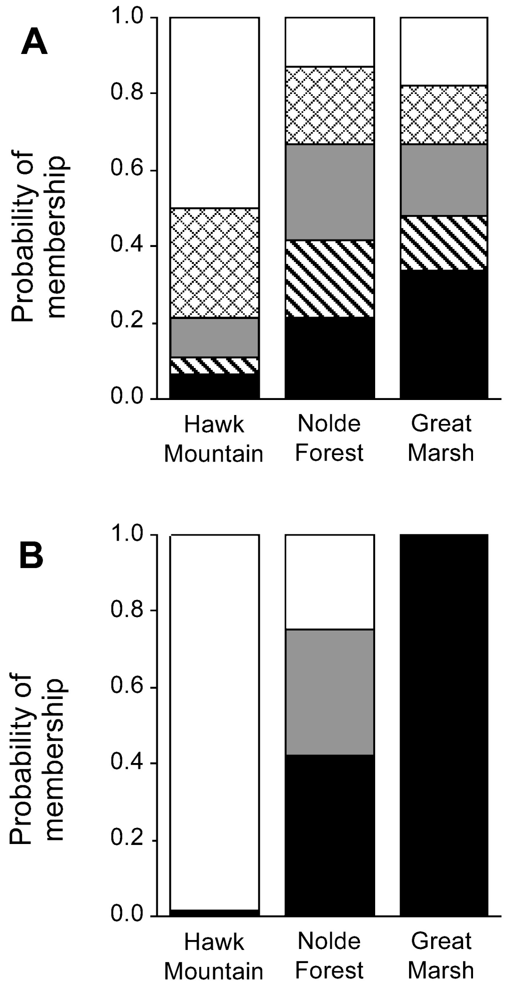

Fig. 4. Average posterior probabilities from STRUCTURE, version 2.1, for chickadees at Great Marsh, Hawk Mountain, and Nolde Forest. (A) Results for five clusters identified when no prior information was given. (B) Results for three clusters identified when Hawk Mountain and Great Marsh population information was designated as prior information. Shading distinguishes clusters that were later assigned to the tentative designation of "Black-capped cluster" (white), "Carolina cluster" (black), and cluster 3 (gray).

cluster"). Individuals from Great Marsh were much more likely to be assigned to the second cluster ("Carolina cluster"). Nolde Forest individuals were almost equally likely to be assigned to Black-capped, Carolina, or the third

Table 2. Calculated values for Nei's (1978) genetic distance between populations (upper cells); F_{ST} values between populations are in bold (lower half of the table). All F_{ST} values are significant after bootstrapping over loci.

	Hawk Mountain	Nolde Forest	Great Marsh
Hawk Mountain	—	0.514	0.337
Nolde Forest	**0.061**	—	0.091
Great Marsh	**0.048**	**0.009**	—

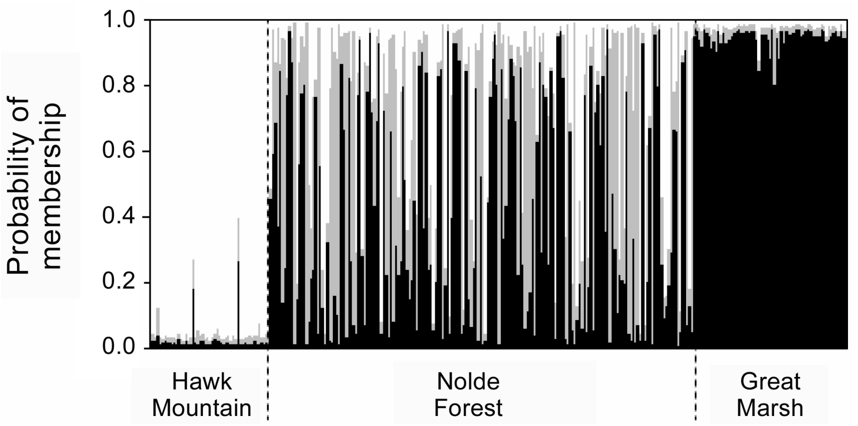

Fig. 5. Probability of membership in each of three clusters for each individual in sampled populations. White region of the graph represents the "Black-capped cluster"; black bars represent the "Carolina cluster"; gray bars, cluster 3.

cluster that likely represented backcrossed individuals.

Discussion

Our results support four major conclusions. First, our methods were effective for evaluating the genetic structure of the hybrid zone. Second, the contact zone in the focal region of southeastern Pennsylvania is currently situated between southeastern Schuylkill County and central Berks County. Third, our data support the hypothesis of rapid northward movement of the southeastern Pennsylvania contact zone in recent decades. Fourth, the composition of the Nolde Forest population indicates that hybridization is extensive within the contact zone. We will discuss these conclusions, before considering the broader implications of the study.

Hybrid zone structure.—As expected, RFLP analysis of mtDNA revealed contact between Black-capped and Carolina chickadees in southeastern Pennsylvania. Haplotype data from the specimen series indicate that as of the 1980s, populations north and west of central Berks County comprised Black-capped Chickadees only, whereas populations in southern Montgomery County (and, presumably, all areas farther south and east) contained Carolina Chickadees. Mixed populations were detectable in southeastern Berks County and northeastern Lancaster County. Haplotype data from the three sites studied more intensively since 1998 revealed mixed populations at Hawk Mountain, where Black-capped haplotypes predominated, and at Nolde Forest, where Black-capped haplotypes have recently dropped out. Consistent with the specimen data set, chickadees at Great Marsh all carry Carolina mtDNA.

Analysis of allozyme variation for the specimen series prompts only minor modification of the preceding conclusions. These data provide further support for mixed populations at the same sites indicated by the mtDNA data, as well as one additional site (Fredericksville) just northwest of the zone suggested by the haplotype data alone. The allozyme data also provide direct evidence of hybridization, and not just the existence of mixed populations, at all sites where both haplotypes were evident.

Results from recent intensive study at Hawk Mountain, Nolde Forest, and Great Marsh indicate that microsatellites can be used to uncover structure and position of the hybrid zone and to identify hybridizing chickadees. Distinct populations at Hawk Mountain and Great Marsh, consistent with the recent haplotype data showing predominance of Black-capped Chickadees at the former and exclusively Carolina Chickadees at the latter, were supported by STRUCTURE when source population was used as prior information: the data suggest that Hawk Mountain and Great Marsh populations were relatively (99%) pure, whereas Nolde Forest was clearly mixed. Unique alleles in both the Hawk Mountain and Great Marsh populations at each locus support the distinctness of each population, though larger sample sizes from the two populations may reduce the number of private alleles (alleles unique to each population), particularly given the high degree of allelic variation in the two populations. Minor lack of resolution may indicate that the population at Great Marsh includes a small percentage of birds with "residual" Black-capped alleles, as a result of relatively recent hybridization.

Hybrid zone position and breadth.—The data presented here support different conclusions about the location and breadth of the hybrid zone in southeastern Pennsylvania, depending on the period. Information derived from the specimens collected in 1986–1989 indicates that the hybrid zone was then centered between Pikeville and Earlville in Berks County. Contemporaneous data from Morgantown suggest that the hybrid zone formed a band running southwest to northeast roughly parallel to the Berks–Chester county line (Fig. 2). At that time, the overall width of the zone, assessed along a line perpendicular to the southwest–northeast axis, was ≥13 km in width, the distance between the extreme localities where hybridization was definitively detected (Fredericksville–Earlville). Additional mixing and hybridization undoubtedly was occurring beyond this zone in areas not sampled, so a more realistic minimal estimate of zone width in the 1980s is ~20 km.

Results from our more recent work (1998–2003) support the hypothesis that the zone is now positioned farther north and may be wider than indicated by the earlier data. Detection of Carolina mtDNA haplotypes at Hawk Mountain since 2000 shows that some Carolina Chickadees, or hybrids with Carolina mtDNA, are now resident on and near the Kittatinny Ridge (see Fig. 1). This result contrasts with the specimen data in which only Black-capped Chickadees were evident at Eckville (just east of Hawk Mountain) and Kutztown (14 km farther southeast) before 1990. Meanwhile, our data from Nolde Forest suggest that although chickadees in that population now exhibit exclusively Carolina mtDNA, many are hybrids. This conclusion is also supported by other Nolde Forest data, including morphological variation and intermediacy (Mullen 2001; R. Curry unpubl. data) and persistence of Black-capped song (Rossano 2003, Curry et al. 2007). We postulate that Nolde Forest supports a population in the final stage of a process of transition, and that Black-capped genetic and behavioral traits will gradually disappear at this site over the next few years. Presently, though, the data support an estimate of hybrid zone width of ≥40 km, the distance from Hawk Mountain to Nolde Forest. However, Nolde Forest lies almost due south of Hawk Mountain (Fig. 1). If the main axis of the contact zone runs from southwest to northeast, there may be populations on the Kittatinny Ridge directly northwest of Nolde Forest with structure like that of Hawk Mountain; if so, the zone would be ~35 km wide.

Data regarding the width of the Black-capped and Carolina hybrid zone are available from past studies in Missouri, Virginia, West Virginia, and Ohio. In Missouri, the primary hybrid zone was estimated to be ~15 km wide but with some evidence of introgression ≤40 km away (Sawaya 1990). In Virginia and West Virginia, hybrid zone width suggested by most markers was <30 km (with the Virginia section slightly narrower than the West Virginia section), but with one marker having introgressed across 50 km or more on both slopes of the Appalachians (Sattler and Braun 2000). In north-central Ohio, transition from Black-capped to Carolina genotypes was evident across a zone 10–25 km wide (Bronson et al. 2005).

Therefore, our data suggest that the hybrid zone in southeastern Pennsylvania is at least as broad currently as in some other sections of the overall line of contact between these chickadees and is broader than in Ohio. Current investigations are now addressing whether these discrepancies are the result of differences in methodology, regional landscapes, mating interactions, or all three (Curry 2005). That landscape factors may cause differences in zone width seems likely, because the regions discussed differ markedly, from the flat ground and agricultural matrix of the Ohio study to the comparatively steep altitudinal gradient examined in Virginia. Behavioral differences among regions have also been suggested, including high levels of extrapair paternity (involving both conspecific and heterospecific mates) in Pennsylvania (Reudink et al. 2006) but not in Ohio (Bronson et al. 2003a, 2005).

To further assess the position and breadth of the Black-capped and Carolina chickadees in southeastern Pennsylvania, it would be advantageous to employ more microsatellite loci and to sample individuals along a transect extending farther northwest and farther southeast of Hawk Mountain and Great Marsh, respectively. More thorough sampling of individuals from Hawk Mountain, at the leading edge of the contact zone, may reveal the degree to which, and mechanisms by which, Carolina and hybrid chickadees are invading that predominantly Black-capped population. Analysis of birds from farther south would help indicate whether there is a threshold distance at which Black-capped alleles entirely drop out.

Northward shift.—Three lines of evidence support the conclusion that the hybrid zone in southeastern Pennsylvania is moving northward rapidly. First, our data collectively contrast with earlier field observations from the region. Second, evidence from our intensive studies contrasts with data from the specimens collected a decade previously. Third, we detected change over time within the relatively brief span of our recent work.

Before application of genetic methods to the study of these chickadees, assessment of the ranges of the species and their degree of interbreeding was based mainly on field observations of songs and plumage patterns. Birds identified as Black-capped Chickadees were breeding residents in southern Berks County in 1954–1956. Although there are occasional records dating back to the 1890s of Carolina Chickadees in the area around Reading in Berks County (which we suspect probably represents postbreeding wanderers), an increase in breeding Carolina Chickadees in south-central Berks County since 1960 has been clearly documented (Uhrich 1997). Observations from the 1960s by Ward and Ward (1974) suggested that the hybrid zone then ran from southwest to northeast ~10 km from the Berks–Chester county line (see Fig. 1). By the early 1980s, when most of the field data for the Pennsylvania Breeding Bird Atlas were gathered, vocal and morphological observations suggested the presence of both species or hybrids across a fairly wide zone in eastern Lancaster, western Chester, southern Berks, and central Montgomery counties (Gill 1992). Christmas Bird Count data, though subject to errors by observers unfamiliar with the difficulties of identifying the two chickadees and their hybrids, show similar trends (P. Hess unpubl. data).

Chickadee songs, however, can be particularly misleading: when we began our work at Nolde Forest in 1998, we heard no birds singing Carolina song (Rossano 2003), yet most males by then had Carolina mtDNA (present study). Nevertheless, the mixed song patterns that the Wards observed in Chester and Lancaster counties are now evident much farther north (Curry et al. 2007). Furthermore, the population at Great Marsh, which now appears to be composed only of Carolina Chickadees, lies directly within the zone of vocal intermediacy described by the Wards; however, we have heard no Black-capped Chickadee songs from resident males at this site since initiating our work in 1998. Also, the mix of Black-capped, Carolina, and aberrant songs currently exhibited by chickadees at Nolde Forest (Curry et al. 2007) closely resembles the vocalizations recorded by the Wards 30 years earlier in Chester County, ~20 km away.

Evidence of northward movement is as strong or stronger at the northern edge of the focal region. Prior to our work in 1998–2003, there was no indication of resident Carolina Chickadees or hybrids at Hawk Mountain (Gill 1992), apart from one individual heard giving Carolina song during the breeding season in 1986 (Uhrich 1997). The absence of Carolina haplotypes in the sample of specimens from both Eckville and Kutztown is consistent with the predominance of Black-capped Chickadees at Hawk Mountain until very recently. Detection of Carolina as well as Black-capped haplotypes at Hawk Mountain since 2002 shows that mixing is occurring there now. Furthermore, we have at least one case of certain hybridization at Hawk Mountain: a female with Carolina mtDNA hatched in the study area subsequently mated with a male with the Black-capped haplotype; the pair successfully fledged one brood.

Our evidence for northward expansion of the Carolina Chickadee range, and the corresponding northward shift in the position of the hybrid zone, is consistent with similar reports from Ohio, Indiana, Illinois, and elsewhere (reviewed in Bronson et al. 2005, Curry 2005). Experimental evidence involving captive birds suggests that male Carolina Chickadees may assert dominance over Black-capped males and, thereby, be attractive as mates to females of both species; this result provides a hypothesized proximate mechanism for northward shift of the hybrid zone (Bronson et al. 2003b). Whether social dominance operates the same way in mixed chickadee populations in the field remains to be determined and leaves the question of ultimate causes for the asymmetry in success unresolved (Curry 2005). That the geographic pattern and its movement reflect ecophysiological factors and, possibly, climate trends has been suggested (Bronson et al. 2005, Curry 2005), but evidence for this possibility is not yet available.

Composition of the Nolde Forest population.— Our results, using genetic markers presumed to be selectively neutral, indicate that hybridization can be extensive in contact-zone populations of these chickadees. STRUCTURE results showed that one-third of the Nolde Forest population does not cluster with either of the parental populations; this suggests a high degree of introgression, resulting in a population that appears to be a hybrid swarm. Birds at Nolde Forest exhibited a broad range of genotypes, but with the average individual having a higher probability of exhibiting a Carolina-like genotype (Fig. 4). Along with the prevalence of Carolina mtDNA haplotypes at Nolde Forest (Fig. 3), this further supports the conclusion that this site is currently at the southern edge of the contact zone.

Because of the large range of genotypes at Nolde Forest, we were unable to discern F1, F2, and backcrossed individuals with any degree of certainty, and it is unclear whether there are any individuals at Nolde Forest that are not the result of hybridization at some level. This pattern could be the result of isolation by distance (Wright 1943), which would result in the distribution of genes seen at Nolde Forest, with the genetic contributions of the reference populations being inversely proportional to their geographic distance as seen in the STRUCTURE results. However, the presence of a large number of individuals from a third cluster cannot be easily explained by a pure isolation-by-distance model and is indicative of a true hybrid population or hybrid swarm. To verify this, more populations would need to be sampled and tested for isolation by distance.

Previous conclusions about the extent of hybridization between Black-capped and Carolina chickadees and resulting introgression have been equivocal. Early observational reports questioned whether chickadees hybridized frequently, noting instead that competition and other ecological factors might keep the two species from interbreeding or even from coming into contact (Brewer 1963); the reports of gaps between the range limits of the species in some regions were consistent with this view (Tanner 1952, Merritt 1981; see also Grubb et al. 1994). By contrast, morphological and vocal patterns in Missouri led Robbins et al. (1986) to argue that hybridization was extensive and that genetic analysis might reveal high levels of introgression. Data from Virginia and West Virginia, obtained using the same genetic markers as Sawaya, revealed extensive interbreeding, with more than half of the birds sampled at some sites reflecting mixed ancestry (Sattler and Braun 2000). Work in Ohio resulted in detection of individuals with intermediate genotypes consistent with extensive mixing (Bronson et al. 2003a, 2005), but the same studies, through a combination of experimental manipulation and geographic analysis, yielded evidence for strong selection against hybrids. Inconsistencies among the various studies suggest the possibility of real biological differences in the degree to which introgression is slowed by selection among geographic regions. Consistent with this interpretation, we have found a comparatively small drop in reproductive success in the Pennsylvania hybrid zone in relation to comparison sites outside the zone (R. Curry and K. Cornell unpubl. data), which could help explain why such a high proportion of birds at sites such as Nolde Forest appears to be hybrids.

Conclusion.—Our study advances our understanding of hybridization in Black-capped and Carolina chickadees by clarifying patterns near the northeastern end of their line of contact. Use of neutral markers may account for the high percentage of individuals identified as hybrids in our contact-zone population; intensity of selection against hybrids may be less than has been argued elsewhere. Evidence that Carolina Chickadees are gaining ground at the expense of Black-capped Chickadees reinforces conclusions from other regions. These findings highlight the need for further study of ecological and genetic mechanisms that may provide Carolina Chickadees with an adaptive advantage during hybridization with their congener at their range interface.

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Literature Cited

Avise, J. C. 2004. Molecular Markers, Natural History, and Evolution, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113–148.

Braun, M. J., and M. B. Robbins. 1986. Extensive protein similarity of the hybridizing chickadees *Parus atricapillus* and *P. carolinensis*. Auk 103:667–675.

Brewer, R. 1961. Comparative notes on the life history of the Carolina Chickadee. Wilson Bulletin 73:348–373.

Brewer, R. 1963. Ecological and reproductive relationships of Black-capped and Carolina chickadees. Auk 80:9–47.

Bronson, C. L., T. C. Grubb, Jr., and M. J. Braun. 2003a. A test of the endogenous and exogenous selection hypotheses for the maintenance of a narrow avian hybrid zone. Evolution 57:630–637.

Bronson, C. L., T. C. Grubb, Jr., G. D. Sattler, and M. J. Braun. 2003b. Mate preference: A possible causal mechanism for a moving hybrid zone. Animal Behaviour 65:489–500.

Bronson, C. L., T. C. Grubb, Jr., G. D. Sattler, and M. J. Braun. 2005. Reproductive success across the Black-capped Chickadee (*Poecile atricapillus*) and Carolina Chickadee (*P. carolinensis*) hybrid zone in Ohio. Auk 122: 759–772.

Chambers, G. K., and E. S. MacEvoy. 2000. Microsatellites: Consensus and controversy. Comparative Biochemistry and Physiology B 126:455–476.

Cornell, K. L. 2001. Hatching success and nestling sex ratio in Black-capped and Carolina chickadees: Do hybridizing chickadees follow Haldane's rule? M.Sc. thesis, Villanova University, Villanova, Pennsylvania.

Curry, R. L. 2005. Hybridization in chickadees: Much to learn from familiar birds. Auk 122: 747–758.

Curry, R. L., L. M. Rossano, and M. W. Reudink. 2007. Behavioral aspects of chickadee hybridization. Pages 95–110 *in* Ecology and Behavior of Chickadees and Titmice: An Integrated Approach (K. Otter, Ed.). Oxford University Press, Oxford, United Kingdom.

Dawson, D. A., O. Hanotte, C. Grieg, I. R. K. Stewart, and T. Burke. 2000. Polymorphic microsatellites in the Blue Tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Molecular Ecology 9:1941–1944.

Gaunt, A. S., and L. W. Oring, Eds. 1999. Guidelines to the Use of Wild Birds in Research, 2nd ed. Ornithological Council, Washington, D.C. [Online.] Available at www.nmnh.si.edu/BIRDNET/GuideToUse.

Gill, F. B. 1992. Carolina Chickadee. Pages 240–241 *in* Atlas of Breeding Birds in Pennsylvania

(D. W. Brauning, Ed.). University of Pittsburgh Press, Pittsburgh, Pennsylvania.

Gill, F. B., D. H. Funk, and B. Silverin. 1989. Protein relationships among titmice (*Parus*). Wilson Bulletin 101:182–197.

Gill, F. B., A. M. Mostrom, and A. L. Mack. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. Evolution 47:195–212.

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

Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. Science 256:193–197.

Grant, P. R., B. R. Grant, and K. Petren. 2005. Hybridization in the recent past. American Naturalist 166:56–67.

Grubb, T. C., Jr., and C. L. Bronson. 1995. Artificial snags as nesting sites for chickadees. Condor 97:1067–1070.

Grubb, T. C., Jr., R. A. Mauck, and S. L. Earnst. 1994. On no-chickadee zones in Midwestern North America: Evidence from the Ohio Breeding Bird Atlas and the North American Breeding Bird Survey. Auk 111:191–197.

Guers, S. L. 2006. Effects of habitat fragmentation on the reproductive success of chickadees in southeastern Pennsylvania. M.S. thesis, Villanova University, Villanova, Pennsylvania.

Harrison, R. G., Ed. 1993. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York.

Hewitt, G. M. 1988. Hybrid zones—Natural laboratories for evolutionary studies. Trends in Ecology and Evolution 3:158–167.

Hewitt, G. M. 1989. The subdivision of species by hybrid zones. Pages 85–110 *in* Speciation and Its Consequences (D. Otte and J. A. Endler, Eds.). Sinauer Associates, Sunderland, Massachusetts.

Johnston, D. W. 1971. Ecological aspects of hybridizing chickadees (*Parus*) in Virginia. American Midland Naturalist 85:124–134.

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

Lewis, P. O., and D. Zaykin. 2001. GENETIC DATA ANALYSIS: Computer program for the analysis of allelic data, version 1.1. [Online.] Available at hydrodictyon.eeb.uconn.edu/people/plewis/software.php.

McWilliams, G. M., and D. W. Brauning. 2000. The Birds of Pennsylvania. Cornell University Press, Ithaca, New York.

Merritt, P. G. 1978. Characteristics of Black-capped and Carolina chickadees at the range interface in northern Indiana. Jack-Pine Warbler 56:171–179.

Merritt, P. G. 1981. Narrowly disjunct allopatry between Black-capped and Carolina chickadees in northern Indiana. Wilson Bulletin 93:54–66.

Mostrom, A. M., R. L. Curry, and B. Lohr. 2002. Carolina Chickadee (*Poecile carolinensis*). *In* The Birds of North America, no. 636 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

Mullen, S. P. 2001. Genetic and morphometric variation across a chickadee hybrid zone in southeastern Pennsylvania. M.S. thesis, Villanova University, Villanova, Pennsylvania.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Otter, K., L. Ratcliffe, and P. T. Boag. 1994. Extra-pair paternity in the Black-capped Chickadee. Condor 96:218–222.

Parkes, K. C. 1987. Sorting out the chickadees in southwestern Pennsylvania. Pennsylvania Birds 1:105–106.

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

Pyle, P. 1997. Identification Guide to North American Passerines, Part I: Columbidae to Ploceidae, 2nd ed. Slate Creek Press, Bolinas, California.

Reudink, M. W., S. G. Mech, and R. L. Curry. 2006. Extrapair paternity and mate choice in a chickadee hybrid zone. Behavioral Ecology 17:56–62.

Rising, J. D. 1968. A multivariate assessment of interbreeding between the chickadees *Parus atricapillus* and *P. carolinensis.* Systematic Zoology 17:160–169.

Robbins, M. B., M. J. Braun, and E. A. Tobey. 1986. Morphological and vocal variation across a contact zone between the chickadees *Parus atricapillus* and *P. carolinensis.* Auk 103: 655–666.

ROSSANO, L. M. 2003. Vocal patterns of Black-capped and Carolina chickadees hybridization in southeastern Pennsylvania. M.S. thesis, Villanova University, Villanova, Pennsylvania.

SATTLER, G. D., AND M. J. BRAUN. 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.

SAWAYA, P. L. 1990. A detailed analysis of the genetic interaction at a hybrid zone between the chickadees *Parus atricapillus* and *P. carolinensis* as revealed by nuclear and mitochondrial DNA restriction fragment length variation. Ph.D. dissertation, University of Cincinnati, Cincinnati, Ohio.

SMITH, S. M. 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Comstock Publishing, Ithaca, New York.

SMITH, S. M. 1993. Black-capped Chickadee (*Parus atricapillus*). *In* The Birds of North America, no. 39 (A. Poole, P. Stettenheim, and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

TANNER, J. T. 1952. Black-capped and Carolina chickadees in the southern Appalachian mountains. Auk 69:407–424.

UHRICH, W. D., ED. 1997. A Century of Bird Life in Berks County, Pennsylvania. Reading Public Museum, Reading, Pennsylvania.

WALSH, J., V. ELIA, AND T. HALLIWELL. 1999. Birds of New Jersey. New Jersey Audubon Society, Bernardsville.

WARD, R., AND D. A. WARD. 1974. Songs in contiguous populations of Black-capped and Carolina chickadees in Pennsylvania. Wilson Bulletin 86:344–356.

WRIGHT, S. 1943. Isolation by distance. Genetics 28:114–138.

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