REPRODUCTIVE SUCCESS ACROSS THE BLACK-CAPPED CHICKADEE (*POECILE ATRICAPILLUS*) AND CAROLINA CHICKADEE (*P. CAROLINENSIS*) HYBRID ZONE IN OHIO

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ABSTRACT.—Black-capped Chickadees (*Poecile atricapillus*) and Carolina Chickadees (*P. carolinensis*) hybridize in an east–west band from New Jersey to Kansas. Within the past century, the Ohio portion of this hybrid zone and the Carolina Chickadee range to the south have been moving northward, whereas the Black-capped Chickadee range has retracted. In Ohio, we characterized the genetic composition of the hybrid zone using five diagnostic molecular loci. Although there was no evidence of assortative mating in the center of the hybrid zone, we found a relative paucity of genetically intermediate breeding females as compared with breeding males. That suggests viability selection against female hybrids, in line with Haldane's rule. On the basis of reproductive variables (number of nestlings, reproductive success), we found a decrease in productivity of breeding pairs in the hybrid zone that is significantly and positively related to their probability of producing homozygous offspring at each autosomal or sex-linked locus. We also found that the decrease in productivity was significantly and positively related to the genetic composition of the male of the pair (i.e. pure male chickadees more productive). These data strongly suggest that hybrids are at a selective disadvantage. Because the zone of reduced reproductive success was considerably narrower than the zone of introgression, our results demonstrate that genetic introgression is occurring in the face of substantial selection against hybrids. *Received 16 April 2004, accepted 10 January 2005.*

Key words: Black-capped Chickadee, Carolina Chickadee, genetic indices, hybrid zone, *Poecile atricapillus*, *Poecile carolinensis*, reproductive success.

Éxito Reproductivo a través de la Zona de Hibridación de *Poecile atricapillus* y *P. carolinensis* en Ohio

RESUMEN.—Las especies *Poecile atricapillus* y *P. carolinensis* hibridan en una franja orientada de este a oeste desde New Jersey hasta Kansas. Durante el último siglo, la sección de Ohio de esta zona de hibridación y el rango de *P. carolinensis* al sur de ésta se han desplazado hacia el norte, mientras que el rango de *P. atricapillus* se ha contraido. En este estudio, caracterizamos la composición genética de la zona de hibridación en Ohio usando cinco loci moleculares diagnósticos. Aunque no existió evidencia de apareamiento asociativo en el centro de la zona de hibridación,

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encontramos una relativa carencia de hembras reproductivas genéticamente intermedias en comparación con los machos reproductivos. Esto sugiere la existencia de selección por viabilidad en contra de las hembras híbridas, lo que concuerda con la regla de Haldane. Con base en variables reproductivas (número de pichones, éxito reproductivo), encontramos una disminución en la productividad de las parejas en la zona de hibridación que está significativa y positivamente relacionada con su probabilidad de producir crías homocigóticas en cada locus autosómico o ligado al sexo. También encontramos que la disminución en la productividad estuvo significativa y positivamente relacionada con la composición genética del macho de la pareja (i.e. los machos puros fueron más productivos). Estos datos sugieren fuertemente que los híbridos se encuentran en desventaja selectiva. Debido a que la zona de éxito reproductivo reducido fue considerablemente más estrecha que la zona de introgresión, nuestros resultados demuestran que a pesar de que existe selección en contra de los híbridos, está sucediendo introgresión genética.

Hybrid zone dynamics is a fertile area for research on natural selection and speciation, because of the exchange of genes between distinct groups (Harrison 1990, 1993; Hewitt 1988). Within birds (see review in Grant and Grant 1992), the hybridization of many North American species has been studied (for review of Great Plains hybrid zones, see Rising 1983). For example, Black-Capped Chickadees (*Poecile atricapillus*) and Carolina Chickadees (*P. carolinensis*) are known to hybridize in many areas along their common border (e.g. Kansas: Rising 1968; Missouri: Braun and Robbins 1986, Sawaya 1990; Illinois: Brewer 1963; Ohio: Grubb et al. 1994; Virginia: Johnston 1971, Sattler 1996, Sattler and Braun 2000; West Virginia: Sattler 1996, Sattler and Braun 2000; Pennsylvania: Ward and Ward 1974, Cornell 2001). Because these species may not be sister taxa (Gill et al. 1989, 1993; but see Sattler and Braun 2000 for discussion), only limited hybridization might be expected.

In North America, except for a peninsular distribution in the Appalachian Mountains, the Black-capped Chickadee distribution abuts the north edge of the Carolina Chickadee distribution (Mostrom et al. 2002). In the southern Appalachians, Black-capped Chickadees are often found at high elevations, and Carolina Chickadees at lower elevations. In the early 1880s, Carolina Chickadees were described as permanent residents only within the southern portion of Ohio (Wheaton 1882). By the late 1930s, the hybrid zone was probably located across the middle of the state, approximately in the location of the east–west U.S. Interstate 70 (Trautman 1940). Currently, the zone is located ~100 km farther north, approximately along U.S. Highway 30 (Grubb et al. 1994, Peterjohn 2001). In other words, the Black-capped Chickadee distribution has been receding northward.

The chickadee hybrid zone is quite narrow, with genetic cline widths on the order of 20 to 30 km (Sattler 1996, Sattler and Braun 2000). Given the likely age of contact and the dispersal capabilities of chickadees (Weise and Meyer 1979), the narrow cline widths suggest that some sort of selection may oppose introgression across their hybrid zone (Barton and Gale 1993). In Illinois, Brewer (1963) found that hatching success was lower in the chickadee hybrid zone than for either parental species, but he had complete data on only four hybrid zone nests. He attributed the reduction to infertility and retarded development of eggs.

We wished to study the relationship between hybridization and reproductive success in greater detail. The objectives here were to employ genetic markers to map one segment of the hybrid zone in north-central Ohio and to examine the relationship between genetic composition of the parents and reproductive success.

Methods

Field methods.—The area of the hybrid zone studied within Ashland County, Ohio (40°50′N, 82°15′W) was bounded by County Road 700 on the north, State Route 95 on the south, State Route 89 on the west, and County Road 175 on the east (Fig. 1). The study area was 23 km from north to south and 6 km from west to east. The landscape was about equally divided among pasture, row crops, and woodlands. To limit the

overlap of points in Figures 2–4, the 21 sampling locations were condensed to 10 pooled samples based on similar latitudes. From north to south, the groupings were a–b, c, d, e, g–f, h, i–m, n–p, q–r, and s–u (Fig. 1).

In November of 1993 and 1994, we placed remote-controlled feeder traps (Pierce and Grubb 1979) filled with sunflower seeds within privately owned woodlands at all study sites within the zone. From December through
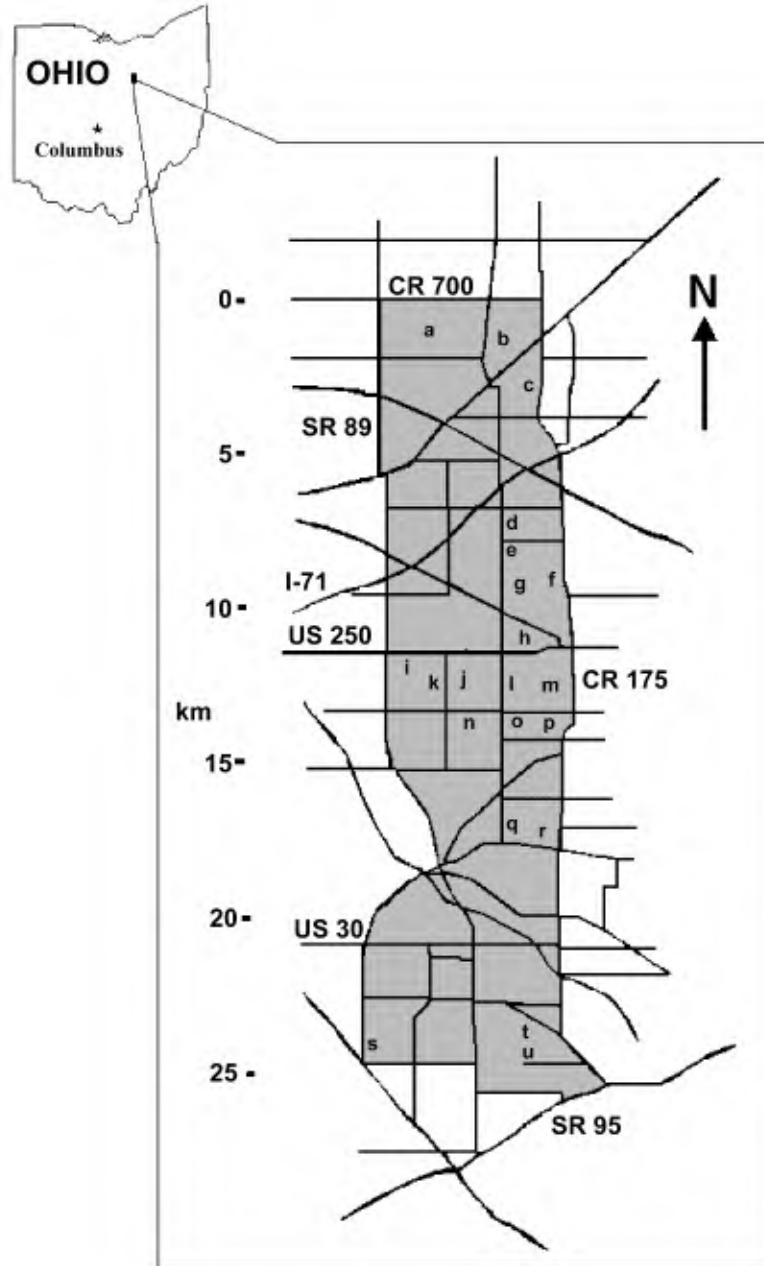

Fɪɢ. 1. The study transect (light gray area) in Ashland County, Ohio, indicating sampling points (letters) and major roadways.

February, we trapped or mist-netted chickadees visiting each feeder. In late February, we placed artificial nesting snags (Grubb and Bronson 1995) in the woodlands and monitored them through the chickadee breeding season (to the end of June).

At the time of capture, we banded each bird with a federal aluminum band and a colored leg streamer (Sullivan 1984) for individual identification from a distance. We weighed each bird to the nearest 0.1 g using a spring balance. Unflattened wing chord and tail length were measured to the nearest 0.5 mm, and tarsus length (from the bent "elbow" to the bent "wrist") to the nearest 0.1 mm. Sex was initially determined through behavioral observation subsequent to capture (i.e. males dominant to females) and relative size of the members of a pair (Desrochers 1990, Smith 1991). Sex was later verified for many individuals on the basis of vocalizations (e.g. singing males, begging females) and morphology (e.g. male cloacal protuberance, female brood patch). Finally, sex was determined through genetic techniques (see sex-linked marker below).

Molecular methods. — Methods for collecting blood, extracting DNA, genetic analysis, and parental analysis are detailed in Bronson et al. (2003). The genetic markers employed are diagnostic restriction-fragment-length polymorphisms (RFLP) of three types (Sawaya 1990, Sattler 1996, Sattler and Braun 2000). The enzyme/probe combinations of *Eco* RI/*ski*, *Bgl* II/RP104, and *Ava* II/RP7 detect autosomal loci. The combination of *Pst* I/C7 detects a sex-linked locus on the Z chromosome, and the combination of *Pst* I/mtDNA was used to genotype the maternally inherited mitochondrial DNA (mtDNA).

Data analysis. — On the basis of the statistical models of Boecklen and Howard (1997), only four or five markers might be adequate to coarsely categorize individuals in a hybrid zone. However, the models assume that no $F_1 \times$ backcross or backcross $\times$ backcross mating occurs within the zone. We were not willing to make that assumption for this chickadee hybrid zone, so we followed Boecklen and Howard's (1997) suggestion and created a genetic index based on "the percentage of loci that are characteristic of a pure species."

We combined the five molecular genotypes (*Eco* RI/*ski*, *Bgl* II/RP104, *Ava* II/RP7, *Pst* I/C7,

and *Pst* I/mtDNA) into a genetic index (GI) for each individual, calculated as the number of Carolina Chickadee alleles divided by the total number of alleles examined (Bronson et al. 2003). There are two alleles for each autosomal marker and one for the mtDNA haplotype. The Z-linked marker has two alleles in males and one in females (females are the heterogametic sex in birds). Thus, GI was based on up to eight marker alleles for females and nine marker alleles for males. For some of the nonparametric correlations, GI was transformed

$$GI' = |GI - 0.5|$$

to adjust for the potential underlying parabolic distribution of GI. Transformed GI' ranges from 0.5 for either pure Carolina Chickadee or pure Black-capped Chickadee to 0 for maximal intermediate birds.

For each set of parents, a compatibility index (CI) was calculated on the basis of the expected proportion of homozygous offspring they could produce at each autosomal or sex-linked locus, averaged across loci (Bronson et al. 2003):

$$CI = [(\sum_{i=1}^{3} \delta_i) + 2\varepsilon]/4$$

where δ_i are the autosomal loci ($\delta_1 = Eco$ RI/*ski*, $\delta_2 = Bgl$ II/RP104, $\delta_3 = Ava$ II/RP7; $\delta = 1$ if the parents are identical homozygotes, $\delta = 0.5$ if at least one is heterozygous, and $\delta = 0$ if they are opposite homozygotes) and ε is the sex-linked locus *Pst* I/ C7 ($\varepsilon = 0.5$ if the parents are identical homozygotes, $\varepsilon = 0.25$ if the male is heterozygous, and $\varepsilon = 0$ if they are opposite homozygotes). To allow for equal weighting of sex-linked loci in the CI, only the expected proportions of male offspring are considered for those loci (females cannot be heterozygous). It seems important to at least equalize the contribution of the sex-linked markers to CI because of the disproportionate involvement of sex chromosomes in reproductive isolation (Coyne and Orr 1989). Compatibility index ranges from 0 to 1, with higher values indicating more complementary genetic backgrounds.

MINITAB, version 13.1 (Minitab, State College, Pennsylvania) was used for generating nonparametric correlations based on the ranks, Spearman's rho (ρ). To reduce the chance of making Type I errors, we employed the sequential Bonferroni technique (Hochberg 1988) to correct for the number of similar tests.

We expected *a priori* that several variables (all reproductive measures and CI of a breeding pair) would have reduced values in the middle of the hybrid zone. Consequently, for figures involving the relevant comparisons, a second-order polynomial trend line was included (instead of a linear trend line), but both types of lines are shown, if only to facilitate visual assessment of the pattern.

RESULTS

The frequencies of alleles at marker loci consistently changed in a clinal fashion across the hybrid zone, from a low proportion of Carolina Chickadee alleles in the north to a high proportion in the south, with the midpoint in allele frequency between 10 and 15 km in all five cases (Fig. 2). Though coincident in position, some variation among markers in cline width was apparent. For example, the frequency of the *Pst* I/C7 marker allele changed from 0 to 1 between 7.4 and 16.2 km, whereas *Eco* RI/*ski* only varied from about 0.1 to 0.8 over the entire transect.

Across the hybrid zone, we obtained records of reproductive output for 29 pairs of chickadees. There was no significant correlation between genetic indices of the male versus female of each pair (Spearman's ρ = 0.310, P = 0.101, n = 29), which suggests that mating was nonassortative. Genetic confirmation of parentage for two nests could not be obtained, because of a laboratory accident that caused the loss of the DNA for the nestlings. We analyzed DNA fingerprints for the remaining 27 nests, finding no evidence of extrapair fertilizations. Of the 100 offspring tested, 14 had one or more unattributable bands. None of those 14 individuals could be excluded as the offspring of the putative parents on the basis of band-sharing scores (Wetton et al. 1987).

For all 29 pairs, there was no loss of offspring between hatching and fledging. Therefore, for those 29 pairs of chickadees, number of nestlings equaled number of fledglings, fledging success (ratio of fledglings to nestlings) was 100%, and reproductive success (ratio of fledglings to eggs) equaled hatching success (ratio of nestlings to eggs) (Table 1). Figure 3 places various reproductive measures within the study landscape (with a second-order polynomial trend line included for easier visualization). No trend was apparent in clutch size across the zone (Fig. 3A), but

both number of nestlings or fledglings (Fig. 3B) and reproductive success (Fig. 3C) displayed apparent troughs near the midway point of the zone. The effect on reproductive output in those troughs appears to be substantial; at least half the eggs failed to hatch in 14 of 22 nests between 5 and 15 km, whereas 0 of 7 nests outside that zone had hatching success <0.7. However, those troughs were also narrow; all nests with reproductive success <0.5 were found in the region from 7.4 to 13.0 km.

For breeding individuals, GI of each sex had a positive and significant relationship with location in the study landscape (female GI: Spearman's ρ = 0.553, P = 0.002, n = 29, Fig. 4A; male GI: Spearman's ρ = 0.769, P < 0.001, n = 29, Fig. 4B). There was a paucity of breeding females of intermediate GI in comparison with breeding males (e.g. no females vs. 12 males in the GI range from 0.3 to 0.6; Fig. 4A–B). The CI of breeding pairs was lowest in the middle of the transect (Fig. 4C). That trough coincided with the trough in productivity (Fig. 3C).

Transformed GI of the female ranged from 0.12 to 0.50 (Fig. 5A–B) and was not significantly related to any reproductive measure (Table 2). The lack of females (only one) with a transformed GI <0.25 could have weakened the correlation. The transformed GI of the male, which ranged from 0.05 to 0.50 (Fig. 5C–D), was not significantly related to clutch size (Table 2), but was positively and significantly related to number of nestlings or fledglings (Table 2; Fig. 5C) and to reproductive success (ratio of fledglings to egg; Table 2; Fig. 5D).

The CI between the male and female of a breeding pair ranged from 0.25 to 1.0 (Fig. 6). Although CI was not significantly related to clutch size (Table 3), it was positively and significantly related to number of nestlings or fledglings (Table 3; Fig. 6A) and to reproductive success (ratio of fledglings to egg; Table 3; Fig. 6B).

DISCUSSION

Selection and hybrid zone maintenance.—Our results demonstrate that there is a narrow region of reduced reproductive success at the center of the chickadee hybrid zone in Ohio. We monitored a 23-km transect of the zone within which we observed substantially reduced reproductive output of populations in the center of the transect as compared with those at each end. On
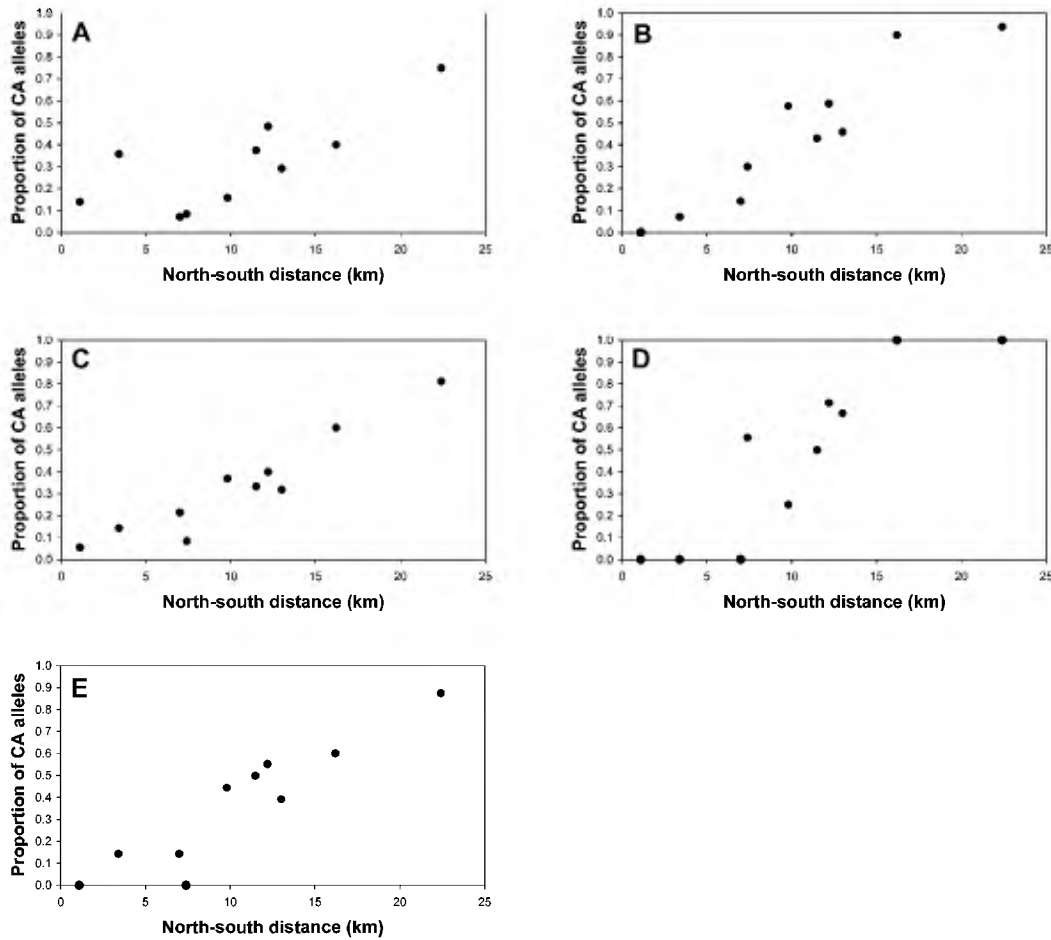
FIG. 2. Distribution of Carolina Chickadee alleles at each of five loci across the study site in north-central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. (A) *EcoR* I/*ski*, $n = 143$; (B) *Ava* II/RP7, $n = 121$; (C) *Bgl* II/RP104, $n = 142$; (D) *Pst* I/C7, $n = 60$; (E) *Pst* I/mtDNA, $n = 142$. Only adults, not nestlings, were used for the analysis to limit nonindependence of data points because of relatedness. All available adults (breeders and nonbreeders) were included. Varying sample sizes are attributable to (1) difficulty in scoring a few nonbreeding individuals, especially for *Ava* II/RP7; and (2) a need to know the sex of each individual to determine the number of alleles to be considered for *Pst* I/C7. Consequently, only individuals observed breeding were included for that locus to insure the correct attribution of sex.

the basis of geographic distributions of allele frequencies for five diagnostic genetic markers, the transect we monitored spanned the core of the hybrid zone. Brewer (1963) also provided anecdotal evidence of reduced reproductive success in the chickadee hybrid zone. Although comparable reproductive success data (fledglings per successful clutch) are not available for either Black-capped or Carolina chickadees in areas immediately adjacent to the study area or for Carolina Chickadees in general, the extremes of our transect had a similar number of Black-capped Chickadee fledglings to the southern peninsula of Michigan (5.5 vs. 6.6; Nickell 1956). Therefore, we are confident that the observed reduction in productivity is limited to the hybrid zone and is not a widespread chickadee phenomenon. Reduced reproductive success indicates that some form of selection is operative in the hybrid zone.

TABLE 1. Genetic and reproductive data for each pair of chickadees observed. All nestlings fledged. The location column gives the letter designation of each site (see Fig. 1) and its distance in kilometers from the northern end of the study transect.

Location		Genetic index		Compatibility index	Reproductive variables	
Km	Site	Female	Male		Clutch	Nestlings
1.1	a	0.125	0.111	1.000	4	4
1.1	a	0.250	0.222	0.750	7	5
3.4	c	0.125	0.111	0.875	8	8
7.0	d	0.000	0.111	0.875	8	6
7.4	e	0.750	0.333	0.625	7	3
7.4	e	0.000	0.444	0.500	7	2
7.4	e	0.125	0.778	0.250	7	6
9.8	f	0.250	0.000	0.625	9	1
9.8	f	0.250	0.222	0.875	5	4
9.8	g	0.000	0.333	0.625	7	2
9.8	g	0.000	0.444	0.625	6	2
11.5	h	0.125	0.333	0.625	8	1
12.2	j	1.000	0.333	0.375	8	5
12.2	j	0.125	0.556	0.375	8	3
12.2	j	0.875	0.556	0.625	8	2
12.2	j	0.125	0.778	0.500	7	5
12.2	k	0.750	0.222	0.500	8	4
12.2	l	0.625	0.333	0.375	7	1
12.2	l	0.875	0.333	0.375	7	1
13.0	n	0.750	0.444	0.500	7	3
13.0	n	0.000	0.667	0.375	6	1
13.0	n	0.000	0.667	0.375	8	7
13.0	o	0.750	0.556	0.625	6	3
13.0	o	0.250	0.667	0.375	4	4
13.0	p	0.750	1.000	0.750	6	5
16.2	q	1.000	0.889	0.875	7	7
16.2	r	1.000	1.000	1.000	9	9
22.4	t	1.000	1.000	1.000	7	7
22.4	u	1.000	1.000	1.000	7	6

What is the nature of selection in the chickadee hybrid zone? Reduced reproductive success was linked to genetic intermediacy of males and to the genetic compatibility of a breeding pair. Those links suggest that intrinsic genetic incompatibilities are responsible for the reduced reproductive success. However, although reproductive measures were related to genetic characteristics of the breeding pairs, both reproductive measures and genetic characteristics were also related to geographic position within the zone (Fig. 3). Thus, parental genotypes may not have been an exclusive cause for the reduced productivity in the middle of our sample transect. For example, environmental attributes (e.g. food availability, temperature,

precipitation) also could have been involved. Although we did not detect any gradients or other inconsistencies in environmental characteristics across our sample transect, such exogenous factors (Harrison 1990, Arnold 1997) could have existed and been causal. Because of its observational nature, our study cannot differentiate between intrinsic or extrinsic factors in the reduced reproductive success.

The best method for separating those causes is to perform a manipulative experiment (Moore and Price 1993). Therefore, as a result of the observations reported here, we relocated chickadees of both parental species and hybrids into isolated island woodlots within the hybrid zone and again observed
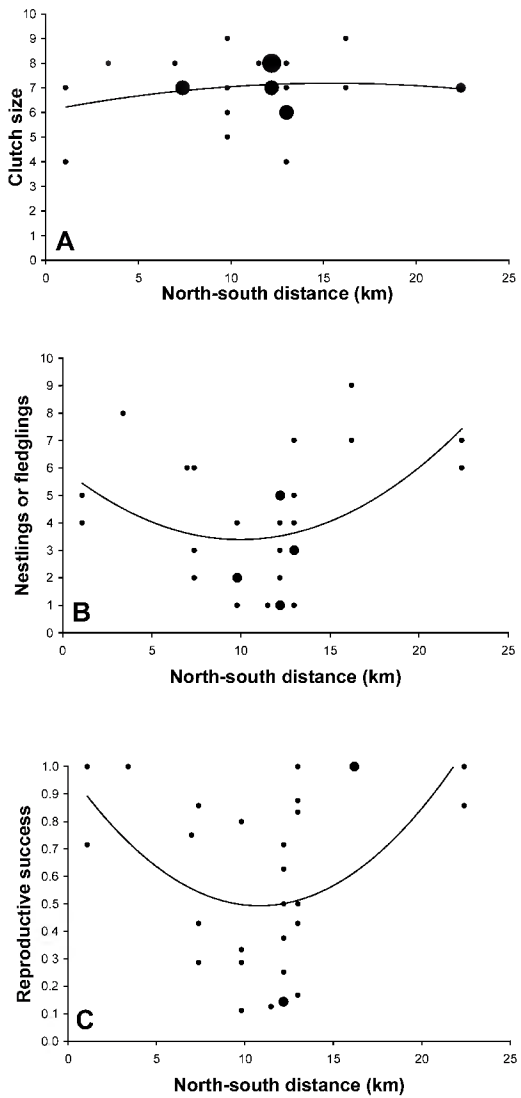
FIG. 3. Relationship of reproductive variables with location in the hybrid zone (with the second-order polynomial trend line for easier visualization). Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The four sizes of circles from smallest to largest indicate sample sizes of 1, 2, 3, and 4, respectively. (A) Clutch size, (B) number of nestlings or fledglings, and (C) reproductive success (ratio of fledglings to eggs).
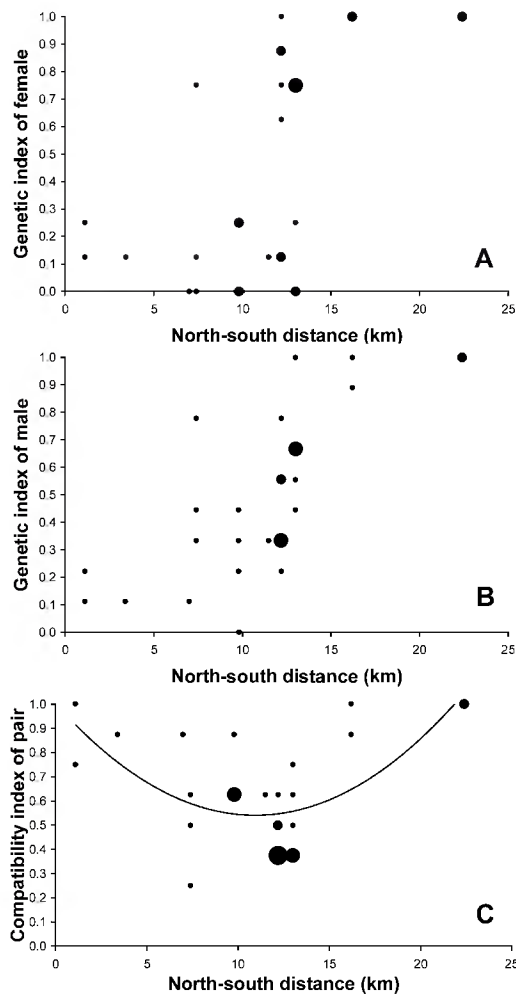

FIG. 4. Relationship of genetic and compatibility indices of breeding individuals with location in the hybrid zone. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The four sizes of circles from smallest to largest indicate sample sizes of 1, 2, 3, and 4, respectively. (A) Female genetic index; (B) male genetic index; and (C) compatibility index of breeding pair, including the second-order polynomial trend line for easier visualization. The GI is calculated as the proportion of Carolina Chickadee alleles present in an individual (0 = Black-capped Chickadee; 1 = Carolina Chickadee). The CI was calculated using the average of the proportion of homozygous offspring a breeding pair could produce at each of the loci (0 = least compatible; 1 = most compatible).
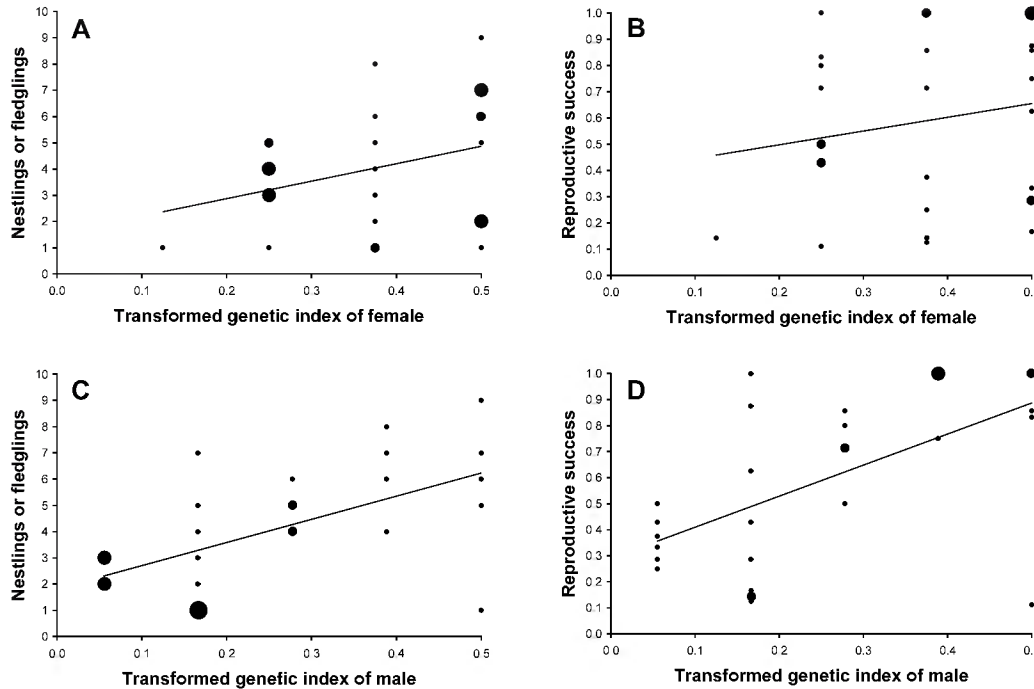
FIG. 5. Relationship of reproductive variables with transformed genetic indices, and linear trend lines for easier visual comparison. The four sizes of circles from smallest to largest indicate sample sizes of 1, 2, 3, and 4, respectively. (A) Number of nestlings or fledglings versus female GI', Spearman's ρ = 0.305; (B) reproductive success (ratio of fledglings to egg) versus female GI', Spearman's ρ = 0.229; (C) number of nestlings or fledglings versus male GI', Spearman's ρ = 0.579; (D) reproductive success (ratio of fledglings to egg) versus male GI', Spearman's ρ = 0.540. The GI' (GI' = |GI − 0.5|) is calculated from the proportion of homozygous allele pairings present in an individual (0.5 = Black-capped or Carolina Chickadee; 0 = maximal intermediate birds).

TABLE 2. Spearman's rho (ρ) for chickadee reproduction on the transformed genetic indices (GI' = |GI − 0.5|) of the female and male of a pair. Sample size for all correlations is 29. Significant relationships based on the sequential Bonferroni technique ($p_{adj} = p \times$ [4 − Rank]; Hochberg 1988) are shown in bold.

Sex	Reproductive variable	ρ	p	Rank	p_{adj}
Female	Clutch size	0.076	0.696	3	0.696
	Nestlings or fledglings	0.305	0.107	1	0.321
	Reproductive success	0.229	0.231	2	0.462
Male	Clutch size	0.165	0.391	3	0.391
	Nestlings or fledglings	**0.579**	**0.001**	1	**0.003**
	Reproductive success	**0.540**	**0.002**	2	**0.004**

reproductive success (Bronson et al. 2003). The results of that experiment indicated that endogenous factors are primarily responsible for selection in the Ohio section of the chickadee hybrid zone. When pure pairs were moved into the hybrid zone, they still had greater reproductive success than hybrid pairs similarly moved within the zone. Thus, with the environment held relatively constant, the observed reproductive decline of hybrids in the hybrid zone must be mainly attributable to intrinsic genetic factors.
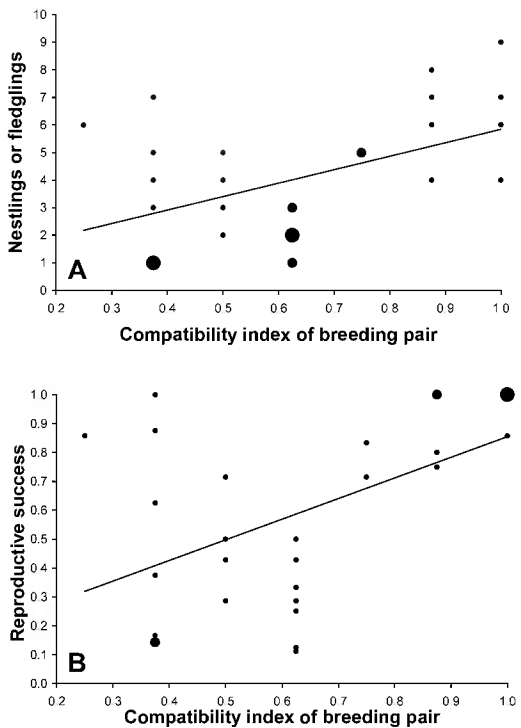
FIG. 6. Relationship of reproductive variables with CI of breeding pair, and linear trend lines for easier visual comparison. Small, medium, and large circles indicate sample sizes of 1, 2, and 3, respectively. (A) Number of nestlings or fledglings, Spearman's ρ = 0.425; (B) reproductive success (ratio of fledglings to egg), Spearman's ρ = 0.450. The CI was calculated using the average of the proportion of homozygous offspring a breeding pair could produce at each of the loci (0 = least compatible; 1 = most compatible).

Selection in the zone is probably balanced by inward dispersal of naïve parentals to create a stable, narrow hybrid zone (Barton and Hewitt 1989). A variety of evidence suggests that this hybrid zone is temporally stable (e.g. Tanner 1952, Rising 1968, Robbins et al. 1986, Grubb et al. 1994, Sattler and Braun 2000; but see Merritt 1981). The habitat in which these chickadees meet lacks obvious barriers and has been relatively unchanged since the last glacial maximum, so it is plausible that the hybrid zone existed long before it was detected. There is no evidence of assortative mating in our data or in previous studies (Robbins et al. 1986). Also, all genetic clines in this and previous studies of the hybrid zone (Sawaya 1990, Sattler 1996, Sattler and Braun 2000) were coincident in position, and cline widths were generally narrow with respect to the dispersal capabilities of chickadees (Weise and Meyer 1979). All these facts suggest that a narrow hybrid zone has existed between these chickadees for a relatively long period, and that a balance of selection and dispersal maintains the zone.

Both the demonstration of intrinsic selection against hybrids and the recent northward movement of the zone are indications that this portion of the chickadee hybrid zone functions as a "tension zone" (Key 1968, Barton and Hewitt 1985). In such cases, the location of the zone reflects a balance between dispersal from parental populations and selection against individuals of mixed ancestry, regardless of environment. The zone then moves until it reaches a location limiting either population density or dispersal (Harrison 1993). Tension zones are believed to be one of the most common kinds of hybrid zones in nature (Barton and Hewitt 1989).

Caveats.—The CI used here and by Bronson et al. (2003) is one of a suite of possible compatibility indices. Our CI considers each available

TABLE 3. Spearman's rho (ρ) for chickadee reproduction on the compatibility index of the breeding pair. Sample size for all correlations is 29. Significant relationships based on the sequential Bonferroni technique ($p_{adj} = p \times [4 - \text{Rank}]$; Hochberg 1988) are shown in bold.

Reproductive variable	ρ	p	Rank	p_{adj}
Clutch size	−0.027	0.888	3	0.888
Nestlings or fledglings	**0.425**	**0.021**	**2**	**0.042**
Reproductive success	**0.450**	**0.014**	**1**	**0.042**

locus independently and additively. A subset of alternative CIs would include epistatic interactions across loci and be nonadditive. Within this alternative subset, a pair would be categorized as pure only if all loci were homozygous for alleles representative of the same species (i.e. had only Carolina Chickadee alleles). By contrast, our CI will miscategorize some hybrid pairings as pure. For example, hybrid pairs that are homozygous and matching at all loci but do not have all alleles of one species will be miscategorized as pure. As a result, our conclusions based on this CI are conservative. We accepted that limitation primarily because it is conservative and, with nearly equal importance, because of the realization that our limited number of markers and families would likely cause some hybrid pairings to be miscategorized regardless.

Our observation of no extrapair fertilizations is remarkable and deserves some attention. Extrapair fertilizations have been documented in many songbirds, including Black-capped Chickadees in continuous habitat (Otter et al. 1998), and we expected to see some extrapair offspring. Although we do not have a definitive answer for our lack of extrapair offspring, we doubt that it is a consequence of our parental analysis method. We used multilocus fingerprinting (Jefferys' probes 33.15 and 33.6; Jefferys et al. 1985a, b) conducting the standard paternity and maternity analyses (Parker and Burley 1998). Although alternative methods now exist (e.g. microsatellite markers) that might be considered more powerful, the multilocus fingerprinting method has been successful in other avian populations with similar population band-sharing levels and failed to be effective only in highly inbred populations. Another possible explanation is that fragmentation of the breeding habitat in our study area limited opportunities for chickadees to seek extrapair fertilizations. However, although our study area was fragmented, chickadees are known to cross habitat gaps daily in areas of greater fragmentation (Grubb and Doherty 1999).

A more likely cause for the lack of extrapair offspring is that we may have primarily sampled dominant breeding pairs. In the winter flocks, these species form within-sex dominance hierarchies (Hartzler 1970). Females will desert their mate (in the winter) or obtain extrapair fertilizations (in the breeding season)

in favor of a male that ranks higher than their mate (Smith 1991, Otter and Ratcliffe 1996, Otter et al. 1998). Consequently, few extrapair offspring are observed in the dominant pair of a winter flock. Additionally, the dominant pair generally obtains a large breeding territory that encompasses most of the winter flock's territory (Smith 1991). We initially captured the birds in winter at temporary feeding stations and then focused our artificial nest placements in the vicinity of those feeders to obtain breeding data on the birds banded at those stations. As a result, we likely sampled the dominant breeding pairs while the subordinate pairs were forced to breed in unsampled areas. Although this sampling bias may result in detection of few extrapair fertilizations, we do not believe it would unduly bias our data with respect to reproductive success. Our genetic analyses of the zone indicate quite a hybrid swarm, and no pattern was observed in the relationship between the genetic indices of the observed breeding pairs (i.e. dominance status was not creating assortative mating based on genetics). We also do not believe our results regarding extrapair fertilizations should be generalized to all chickadee populations.

Variation in cline widths.—All genetic clines examined here were generally narrow, on the order of 20–30 km or less, which is consistent with other studies of the chickadee hybrid zone in Missouri and Appalachia (Sawaya 1990, Sattler 1996, Sattler and Braun 2000). However, there was some interesting evidence of variation in cline width. The cline for the autosomal marker *Eco* RI/*ski* cline was the broadest of the diagnostic markers in Ohio, as also observed in Appalachian transects (Sattler 1996, Sattler and Braun 2000). The cline for the sex-linked marker *Pst* I/C7 was the narrowest, again as observed in Appalachia by Sattler (1996). The variation in cline width suggests that selection against hybrids is stronger at some loci than at others. Limited introgression of sex-linked markers has been observed in other hybrid zones (e.g. Dod et al. 1993, Ferris et al. 1993) and is consistent with the expectation that selection will tend to be stronger on sex-linked loci (e.g. Charlesworth et al. 1987).

Haldane's Rule.—Haldane's rule holds that in hybridization events, reduced viability or fertility should be more evident in the heterogametic sex—the female in birds (Haldane

1922, Orr 1997, Turelli 1998). An analysis of the nestling sex ratio in the chickadee hybrid zone in Pennsylvania yielded a lack of significant support for Haldane's rule (Cornell 2001). Our data relating to fertility also show no support for the rule; the observed reduction in productivity was related to male genetic composition, not female. It should be noted, however, that the small number of genetic markers employed could have contributed to the difference in the relationships of female GI and male GI with reproductive measures. Male GI had a larger number of possible values because of the inclusion of a sex-linked marker.

Haldane's rule may not directly apply to comparisons of parental genetics with reproductive measures in all cases. Reproductive variables such as hatching success should be compared to the sex of the offspring themselves. Unfortunately, we do not know the sex of nestlings or unhatched eggs. If viability is affected, Haldane's rule would predict an increased ratio of females to males in unhatched eggs with a compensatory decrease in the ratio for viable offspring.

Other data from the present study indicate that there may be a viability effect on females. For example, a distinct gap between 0.3 and 0.6 exists in the distribution of adult breeding-female GI (Fig. 4A). By contrast, 12 males had GIs between 0.3 and 0.6 (Fig. 4B). Apparently, highly heterozygous females had been removed from our population samples before reproduction, the sampling point of this study. Therefore, Haldane's rule might well have been manifested by reduced viability of females prior to reproductive age.

Conclusion.—Our results indicate that the width of the zone based on reduced reproductive success (~6 km) is less than half that based on the genetic indices (>15 km). Furthermore, this relationship of reproductive and genetic indices of hybridization is likely conservative, because we know that two components of GI (*Eco* RI/*ski* and *Bgl* II/RP104) show introgression of Black-capped Chickadee alleles in southern Ohio (Sattler 1996). The pattern of reproductive success across a hybrid zone provides information about the first stage of hybridization and its effect, the actual barrier to the passage of genetic material between two taxa. Possibly, the width of the zone of decreased reproductive success could indicate the strength of that barrier. By

comparison, the patterns of introgression in the genetic markers across a zone also provide information about the strength of the barrier, because they represent the long-term effect of hybridization. When hybridization is effective (i.e. backcrosses are present), a hybrid zone is less analogous to a wall and more like a semipermeable membrane that allows alleles to pass through at various rates, depending on allele-specific selection factors (Barton 1983).

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