

A TEST OF THE ENDOGENOUS AND EXOGENOUS SELECTION HYPOTHESES FOR THE MAINTENANCE OF A NARROW AVIAN HYBRID ZONE

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Abstract.—The contributions of genetic and environmental factors to differential reproductive success across hybrid zones have rarely been tested. Here, we report a manipulative experiment that simultaneously tested endogenous (genetic-based) and exogenous (environmental-based) selection within a hybrid zone. We transplanted mated pairs of two chickadee species (*Poecile atricapilla* and *P. carolinensis*) and their hybrids into isolated woodlots within their hybrid zone and monitored their reproductive success. Although clutch sizes were similar, based on an estimate of the genetic compatibility of a pair, hybrid pairs produced fewer nestlings and fledglings than did pairs of either parental species. According to a linear model generated from the data, a pure pair of either parental species would be expected to produce 1.91–2.48 times more fledglings per nesting attempt, respectively, than the average or least compatible pair in the experiment. Our result of decreased reproduction for hybrid pairs relative to parental species pairs within same environment (the hybrid zone in this experiment) support the endogenous selection hypothesis for maintenance of this hybrid zone. Because the experiment was conducted entirely within the hybrid zone (i.e., the same environment for parental and hybrid pairings), our data do not support the exogenous selection hypothesis as it predicts either all pairings doing poorly or the hybrid pairs more successful than the parental pairs.

Key words.—Endogenous selection, exogenous selection, hybrid zone, *Poecile atricapilla*, *Poecile carolinensis*, transplant experiment.

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Stable hybrid zones have been observed in a variety of taxa (see reviews in Barton and Hewitt 1981a, 1985; Hewitt 1988; Harrison 1990, 1993; Arnold 1997). While such zones are commonly considered to reflect a balance between selection and dispersal (Haldane 1948; Fisher 1950), the nature of selection is not necessarily the same across all zones. Both exogenous and endogenous factors (Moore and Price 1993) have been advanced to explain barriers to gene flow across narrow hybrid zones (Barton and Hewitt 1985). Exogenous selection involves adaptation to local environments. In other words, relative fitnesses of individuals vary with the environment. For example, in a fern hybrid zone, the genetic composition across the hybrid population could be attributed to amount of soil moisture and light level (Kentner and Mesler 2000). While most studies have considered only the abiotic component of adaptation, Rohwer et al. (2001) correctly expanded the scope of exogenous selection to include social interactions. For example, in the hybrid zone between hermit (*Dendroica occidentalis*) and Townsend's (*D. townsendi*) warblers in the Pacific Northwest (USA), the aggressive superiority of the Townsend's warbler appears to be a primary influence on the zone (Rohwer and Wood 1998; Pearson 2000; Pearson and Manuwal 2000; Pearson and Rohwer 2000; Rohwer et al. 2001). In contrast, endogenous selection involves adaptation or coadaptation on the genomic level regardless of the external environment. Again, in other words, the relative fitnesses of individuals are independent of the

environment. Individuals of mixed ancestry are selected against because alleles are favored either within a complementary genetic background or at high frequency (Barton and Gale 1993). For example, in a study controlling for vegetation type, reduced density in the zone between chromosomal races of a grasshopper *Podisma pedestris* was attributed to hybrid inviability (Barton 1980; Barton and Hewitt 1981b).

Barton and Hewitt's (1981a, 1985) reviews of hybrid zones in numerous taxa concluded that endogenous selection is the important factor maintaining the majority, but exogenous selection has also been shown to function in some cases (Harrison 1990; Arnold 1997). In any given hybrid zone, both types of selection may be occurring with one or the other as the predominant factor. Few of the observational studies of hybrid zones now in hand (Harrison 1993) distinguish unambiguously between exogenous and endogenous selection. For example, individuals observed to have reduced fitness within a hybrid zone are of mixed ancestry (possibly endogenous selection), but are also located within a limited geographical area in which few or no parentals occur (possibly exogenous selection). An exception is the observational study of the pied (*Ficedula hypoleuca*) and collared (*F. albicollis*) flycatcher hybrid zones in Sweden in which extensive life-history data have been collected for more than 20 years (Veen et al. 2001). This long-term dataset shows no recruitment of offspring from female F₁ hybrids and slightly reduced fertility of male F₁ hybrids (Veen et al. 2001). Such a distinct genetic pattern over the long term, especially linked with sex, limits the likelihood of exogenous selection as being the primary influence.

The most convincing method for distinguishing the type

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of selection operating is to transplant parental taxa and their hybrids into the hybrid zone and measure the three groups' relative fitness (Moore and Price 1993). Since all three groups are within one common area, local environmental factors are not likely to contribute to any observed differences in reproduction. Furthermore, such local environmental factors, in all their variety, are not likely to be duplicated in the laboratory.

We present a manipulative test of the exogenous and endogenous selection hypotheses in a natural avian hybrid zone using reproductive variables as test criteria. We recorded the reproductive success of mated pairs of black-capped (*Poecile atricapilla*; BC) and Carolina (*P. carolinensis*; CA) chickadees and their hybrids (HY) transplanted into the hybrid zone. Our protocol eliminated both abiotic and social components of local environment as causal factors for any differential reproductive success. All three groups were relocated into the hybrid zone (similar ecological conditions) and, more specifically, into island woodlots devoid of other chickadees (similar social conditions). Exogenous selection predicts that within the hybrid zone either all three groups will have low reproductive success or HYs will have relatively higher reproductive success than either parental species. In contrast, endogenous selection predicts that hybrids will have lower reproductive success than either parental species. While endogenous selection also predicts that hybrids should have lower reproductive success than parental species within the range of each parental species, tests of that prediction were beyond the scope of this study.

METHODS

The Subjects and the Zone

Black-capped and Carolina chickadees 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). Although quite similar in appearance, the two species do have distinguishable phenotypes (Pyle 1997). In Ohio, a narrow hybrid zone between this species pair has existed for at least 120 years (Wheaton 1882) and, with respect to the parental populations on either side (Brewer 1963; Bronson 2002), reproductive success is substantially reduced within the hybrid zone.

The BC distribution abuts the north edge of the CA distribution. In the 1880s, CAs were described as permanent residents limited to the southern portion of the state (Wheaton 1882). Within Ohio in the late 1930s, the east-west oriented hybrid zone was apparently located across the middle of the state approximately along the line of U.S. Interstate 70 (Trautman 1940). Since then, the BC distribution has been receding northward. Currently, although still in an east-west orientation, the zone is located about 100 km farther north in the state, approximately along U.S. Highway 30 (Grubb et al. 1994; Peterjohn 2001) and is approximately 15 km wide based on genetic and 6 km wide based on reproductive success (Ashland County; Bronson 2002).

The Experimental Protocol

Our experiment was conducted in November through June from 1995–1996 to 1998–1999 within 35 isolated woodlots where the hybrid zone crosses the agrarian landscape of northern Crawford County, Ohio (40°50'N, 83°00'W). The woodlots were sufficiently small (2.16–5.87 ha) for a single chickadee breeding pair to defend. To facilitate breeding, we placed four or five artificial nesting structures (Grubb and Bronson 1995) within each woodlot.

At the beginning of each winter, we randomly assigned woodlots to one of three treatment groups, BC, CA, or HY. We restricted the assignment so that if a breeding record were obtained in a given year, the woodlot would not be assigned to the same pair type in subsequent years. Prior to the introduction of experimental chickadees, the resident chickadees in a woodlot were relocated. Experimental chickadees were captured either as a pair or small flock. BC and CA pairs were taken from well outside the morphologically defined hybrid zone, at distances ranging 25–50 km from its center. BCs were obtained from Seneca (41°4'N, 82°53'W), Geuga (41°20'N, 81°20'W), Medina (41°10'N, 81°50'W), and Huron (41°5'N, 82°45'W) Counties. CAs were obtained from Morrow (40°24'N, 82°49'W) and Union (40°14'N, 83°22'W) Counties. HYs were obtained within the hybrid zone from Crawford and Ashland (40°50'N, 82°10'W) Counties and moved 10–50 km to experimental woodlots. While it would be ideal to move the hybrids a similar distance to the parents, it was not logistically feasible. The shorter distance of movement for some of the hybrids could increase the likelihood of supporting the exogenous selection hypothesis. The likelihood of supporting the endogenous selection hypothesis should be unaffected.

At the time of capture, each bird was banded with a U.S. Fish and Wildlife Service aluminum band and given a colored leg streamer (Sullivan 1984) for individual identification from a distance. To encourage introduced chickadees to remain until they bred, we maintained a sunflower-seed feeder (Pierce and Grubb 1979) in each woodlot.

Morphological Hybrid Index

At the time of capture, each experimental bird was scored on four plumage characters to produce a morphological hybrid index (MI): whiteness of secondary wing coverts, whiteness of secondary feather margins, whiteness of tail feather margins, and straightness of the lower margin of the "bib." The first three were scored similarly: 0 if white (BC-like), 1 if tan, 2 if gray (CA-like). The last was scored 0 if jagged (BC-like) and 1 if straight (CA-like). Based on a highest possible score of 7, pure BC and CA phenotypes would yield 0 and 7, respectively. All BC individuals included in the experiment had at most a score of 1 and all CA individuals at least a score of 6.

Blood and DNA Protocols

For genetic and parental analysis, a brachial vein of each chickadee was punctured using a 25-gauge needle, and a 50–75- μ l blood sample was taken in a heparinized capillary tube. Each blood sample was placed in 1 ml of lysis buffer (100

mM Tris at pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; Longmire et al. 1988) and stored at 4°C.

In preparation for DNA extraction, each blood sample had 200 µg of Proteinase K added and was then incubated at 55°C overnight. Five extractions were performed; two with phenol, two with 25:24:1 phenol:chloroform:isoamyl alcohol, and one with 24:1 chloroform:isoamyl alcohol. After the remaining aqueous phase of each sample had been dialyzed extensively against TNE₂ (10 mM Tris at pH 7.4, 10 mM NaCl, 2 mM EDTA), the extracted DNA samples were stored at 4°C for later genetic analysis.

The concentration and purity of each extracted DNA sample were assessed spectrophotometrically by comparing optical densities at 260 and 280 nm. The concentration estimate and integrity of each sample were assessed by running (at 80 V) 1.0 µg of undigested DNA through a 0.8% agarose gel, staining the resulting gel with ethidium bromide and viewing it on an ultraviolet light box. All samples were determined to be unsheared.

Genetic Index

Genetic analyses were performed following the methods of Sattler and Braun (2000). Five probes were used to identify on Southern blots restriction fragment length patterns diagnostic for CA and BC (Sambrook et al. 1989). First, *EcoRI* digests were probed with a 1200-bp fragment of the domestic chicken (*Gallus gallus domesticus*) oncogene *ski* (Li et al. 1986; Sawaya 1990; Sattler and Braun 2000). Second, *BglIII* digests were probed with a randomly cloned fragment of CA DNA designated RP104 (Sattler 1996). Third, *AvaII* digests were probed with a randomly cloned fragment of CA DNA designated RP7 (Sattler 1996). Fourth, *PstI* digests were probed with a randomly cloned 4000-bp fragment of tufted titmouse (*Baeolophus bicolor*) DNA designated C7 (Sawaya 1990; Sattler and Braun 2000). Fifth, *PstI* digests were also probed with house finch (*Carpodacus mexicanus*) mitochondrial DNA (mtDNA; Mack et al. 1986; Sawaya 1990; Sattler and Braun 2000) purified by subcellular fractionation and CsCl equilibrium-gradient centrifugation (Dowling et al. 1990).

Based on the statistical models of Boecklen and Howard (1997), as few as 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 such an assumption for this chickadee hybrid zone, so we chose the alternative of creating a hybrid index based on the percentage of loci characteristic of a pure species. Such an index has been strongly recommended by Boecklen and Howard (1997).

We combined the five molecular genotypes (*EcoRI/ski*, *BglIII/RP104*, *AvaII/RP7*, *PstI/C7*, and *PstI/mtDNA*) into a genetic index (GI) calculated as the proportion of CA alleles present in an individual. This is complicated slightly by the presence of mitochondrial and sex-linked loci among the assayed markers. The combination of *PstI/mtDNA* has only one variant because mtDNA is haploid. The combination of *PstI/C7* has only one allele in females because it is a sex-linked marker and females are the heterogametic sex in birds.

Thus for males, GI was based on nine alleles: one for the combination of *PstI/mtDNA* and two for each of the other enzyme/probe combinations. For females, GI was based on eight alleles: one each for *PstI/mtDNA* and *PstI/C7* and two for each of the other enzyme/probe combinations. Hence the index was calculated as:

$$GI = \frac{\left(\sum_{i=1}^3 \alpha_i\right) + \beta + \gamma}{8 + \sigma}, \quad (1)$$

where σ is the sex ($\sigma = 1$ if male and 0 if female), α_i are the autosomal loci ($\alpha_1 = EcoRI/ski$, $\alpha_2 = BglIII/RP104$, $\alpha_3 = AvaII/RP7$; $\alpha = 2$ if homozygous CA, 1 if heterozygous, and 0 if homozygous BC), β is the sex-linked locus *PstI/C7* ($\beta = 1 + \sigma$ if homozygous CA, 1 if heterozygous, and 0 if homozygous BC), and γ is the mtDNA locus *PstI/mtDNA* ($\gamma = 1$ if CA and 0 if BC). For linear regressions involving measures of reproductive success, GI was transformed:

$$GI' = |GI - 0.5| \quad (2)$$

to adjust for the potential underlying parabolic distribution of fitness versus GI. Thus, GI' was 0.5 for either CA or BC and 0 for maximal HY.

Compatibility Index

For each set of parents, a compatibility index (CI) was calculated based on the average of the proportion of homozygous offspring they could produce at each of the three autosomal (0, 0.5 or 1) and one sex-linked (0, 0.25 or 0.5) loci

$$CI = \frac{\left(\sum_{i=1}^3 \delta_i\right) + 2\epsilon}{4}, \quad (3)$$

where δ_i are the autosomal loci ($\delta_1 = EcoRI/ski$, $\delta_2 = BglIII/RP104$, $\delta_3 = AvaII/RP7$; $\delta = 1$ if the parents are identical homozygotes, 0.5 if at least one is heterozygous, and 0 if they are opposite homozygotes) and ϵ is the sex-linked locus *PstI/C7* ($\epsilon = 0.5$ if the parents are identical homozygotes, 0.25 if the male is heterozygous, and 0 if they are opposite homozygotes). For each locus, a higher proportion indicates a greater genetic complementarity between the parents. For example, for the *EcoRI/ski* marker, a pair of chickadees composed of a pure BC and a pure CA would have all heterozygous offspring and a score of 0. To allow for equal weighting of all four loci in the CI, the percentage for the sex-linked *PstI/C7* marker was doubled (female offspring only have one allele so cannot be heterozygous). An additional reason for at least equalizing the contribution of the sex-linked marker to CI is the disproportionate involvement of sex chromosomes in reproductive isolation (Coyne and Orr 1989).

Parental Analysis

While only one pair of chickadees occupied each woodlot, extrapair fertilizations are not uncommon in chickadee populations in continuous habitat (Otter et al. 1998) and chickadees are known to cross gaps between woodlots on a daily

TABLE 1. The morphological, genetic and reproductive data for each experimental pair of chickadees. Fledging success is the proportion of nestlings that fledge. BC indicates black-capped, and CA, Carolina, chickadees. HY indicates their hybrids.

Species	Morphology and genetics of experimental pair					Reproductive variables of experimental pair			
	Morphological index		Genetic index		Compatibility index	Clutch	Nestlings	Fledglings	Fledging success
	Female	Male	Female	Male					
BC	0.000	0.000	0.500	0.000	0.500	7	6	6	1.000
BC	0.143	0.000	0.250	0.000	0.750	5	3	3	1.000
BC	0.000	0.000	0.000	0.125	0.875	7	6	6	1.000
BC	0.000	0.000	0.000	0.125	0.875	8	8	8	1.000
BC	0.000	0.143	0.000	0.125	0.875	8	1	1	1.000
BC	0.000	0.000	0.125	0.000	1.000	8	8	8	1.000
BC	0.000	0.000	0.000	0.000	1.000	8	8	8	1.000
CA	1.000	0.858	0.500	0.625	0.625	6	5	4	0.800
CA	1.000	1.000	0.750	0.875	0.875	9	9	9	1.000
CA	1.000	1.000	0.875	1.000	0.875	6	5	5	1.000
CA	1.000	0.858	1.000	1.000	1.000	7	7	7	1.000
CA	1.000	1.000	1.000	1.000	1.000	9	5	5	1.000
HY	1.000	0.429	0.875	0.250	0.375	6	3	3	1.000
HY	1.000	0.429	0.625	0.000	0.375	7	5	5	1.000
HY	0.000	0.000	0.250	0.250	0.500	6	3	3	1.000
HY	0.571	0.286	0.500	0.125	0.500	8	2	2	1.000
HY	0.429	0.429	0.000	0.500	0.500	6	3	3	1.000
HY	0.429	0.143	0.000	0.500	0.625	9	2	2	1.000
HY	0.286	0.714	0.125	0.375	0.625	8	2	2	1.000

basis (Grubb and Doherty 1999). To confirm that extrapair parentage did not confound results, we conducted standard paternity and maternity analyses (Parker and Burley 1998).

For parental analysis, 5 μ g of DNA from each chickadee were digested with 5 \times *Hae*III at 37°C for 3 h. The DNA fragments were separated through a 1.0% agarose gel at 20 V for 65 h (all fragments smaller than 1600 base pairs were run off the gel). The DNA was transferred to nylon membranes by Southern blot in 10 \times SSC buffer and UV cross-linked to the nylon membrane. Jeffreys' multilocus minisatellite probe 33.6 (Jeffreys et al. 1985a,b) was radiolabeled by primer extension. The hybridizations were run overnight at 62°C in 1.5 \times SSC, 0.1% SDS, 5 \times Denhardt's solution, and 6% w/v dextran sulfate. After hybridization, the nylon membranes were washed four times for 30 min at 62°C in 1.5 \times SSC, 0.1% SDS, and then exposed to X-ray film at -20°C for approximately one week.

Statistical Analyses

Minitab version 13.1 (Minitab Inc. 2000) was used for regression, Kruskal-Wallis, and pairwise binomial comparison analyses. Because various measures of reproductive success of the same birds are not statistically independent, we used the sequential Bonferroni technique (Hochberg 1988) to reduce the chance of Type-I errors.

RESULTS

Analysis of genetic markers of the birds from experimental woodlots confirmed the result from plumage scores that all experimental woodlots were within the hybrid zone (see Table 1, Fig. 1). Additionally, the MI and GI were significantly and positively related (see Fig. 2; $r^2 = 0.778$, $df = 69$, $P < 0.001$) for individuals from the hybrid zone, CA population, and BC population. When GI was considered more closely, the distributions of GIs for individuals from the hybrid zone,

the CA population, and the BC population were significantly different (see Table 1; Kruskal-Wallis $H = 45.76$, $df = 2$, $P < 0.0001$). However, the molecular markers revealed greater introgression of foreign alleles into parental populations (see Fig. 1) than was evident from the morphological hybrid index, a result that has been obtained in independent studies of this hybrid zone at other locations (Sattler and Braun 2000).

Over the course of four years, we made 175 introductions to the study woodlots. After losses due to disappearance (140), nest-site competition (9), nest depredation (3), nest flooding (1), and inaccessible nest sites (1), 19 pairs (7 BC, 5 CA and 7 HY) remained to produce records adequate for determining reproductive success. BC (88.7%), CA (87.1%), and HY (84.4%) did not differ significantly in the proportion of introductions lost prior to determining reproductive suc-

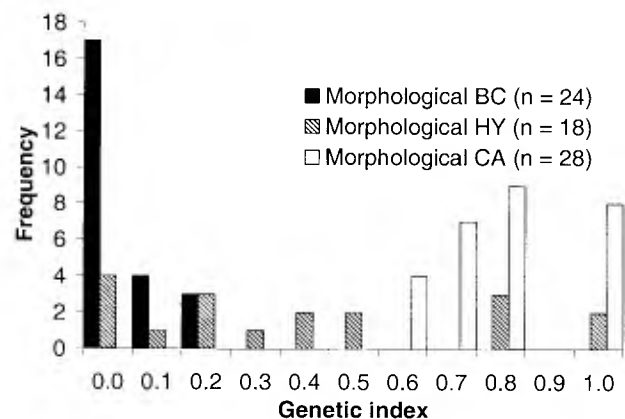


FIG. 1. Genetic indices of Ohio chickadees taken from within the morphological black-capped (BC), morphological hybrid (HY), and morphological Carolina (CA) distributions. The hybrids presented all came from the area of the transplant experiment. Pure BC and pure CA have genetic index scores of 0 and 1, respectively.

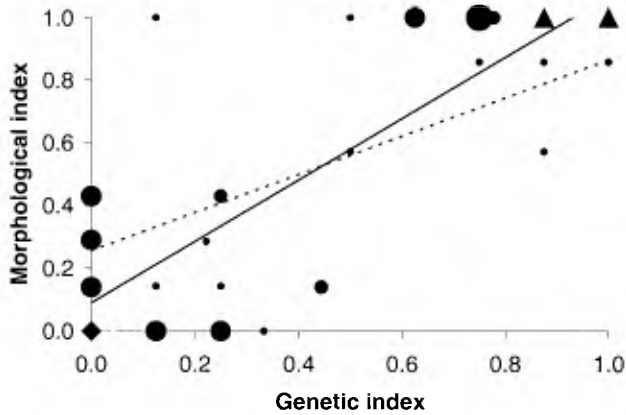


FIG. 2. Relationship between morphological and genetic indices of Ohio chickadees, calculated regression line (solid) based on the same 70 individuals from Fig. 1 ($r^2 = 0.778$, $df = 69$, $P < 0.001$), and calculated regression line (dashed) based on only the 18 individuals from the hybrid zone in Fig. 1 ($r^2 = 0.346$, $df = 17$, $P = 0.010$). For both indices, 0 and 1, respectively, indicate pure BC and pure CA. The four sizes of circles from smallest to largest denote sample sizes of 1, 2, 3, and 4, respectively. The triangles represent sample sizes of 10. The diamond represents a sample size of 12.

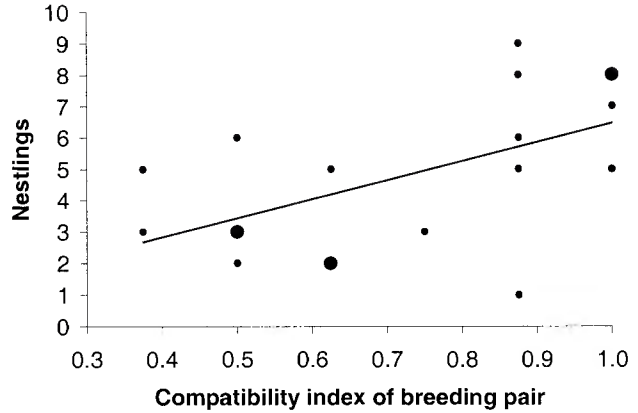


FIG. 3. Relationship of number of nestlings with compatibility index of a breeding pair of chickadees, and calculated regression line ($r^2 = 0.304$, $df = 18$, $P_{adj} = 0.03$). Compatibility indices of 0 and 1, respectively, indicate breeding pairs that would produce offspring heterozygous or homozygous for species-specific genetic markers. Each larger circle represents two datapoints.

cess (pairwise binomial comparisons: BC:CA, $z = 0.23$, $P = 0.82$; BC:HY, $z = 0.63$, $P = 0.53$; CA:HY, $z = 0.36$, $P = 0.72$).

Based on the three discrete morphological categories, the distributions of the number of fledglings per breeding pair differed across groups (see Table 1; Kruskal-Wallis $H = 6.84$, $df = 2$, $P = 0.033$) with hybrids producing fewest fledglings (BC [median:range] 6:1–8; CA 5:4–9; HY 3:2–5).

For the 19 pairs, CI ranged from 0.375 to 1.000 (see Table 1). CI was not significantly related to clutch size or fledging success (fledglings/nestling; see Table 2). However, CI was significantly positively related to both number of nestlings and number of fledglings (see Table 2, Figs. 3–4). Neither the female nor the male GI was significantly related to any of the reproductive measures (see Table 2).

No evidence of extrapair parentage was found. Of 85 offspring in 18 families (one family of five was not analyzed), only nine individuals had one or more unattributable bands. Based on band-sharing scores (Wetton et al. 1987), none of those nine individuals could be excluded as the offspring of the putative parents.

DISCUSSION

Our original experimental design called for three discrete categories of breeding pairs (CA, BC, and HY), but the molecular markers revealed genetic introgression (Fig. 1) that allowed us to discern a gradient of parental pairings. The wide range of GIs within the HY category furnished additional relevant information for statistical analysis and biological interpretation. While F_1 hybrids are often viable and fertile, the F_2 generation and a variety of backcrosses exhibit increased mortality and sterility (i.e., Wu and Palopoli 1994; Hutter 1997). A possible cause is that the negative epistatic interaction between loci of the parental species tends to be recessive (Muller 1942; Orr 1993). True F_1 hybrids would be heterozygous at all differentiated loci, possibly masking negative epistasis, but backcrosses would begin exposing homozygous recessives.

Analysis under both the categorical (CA, BC, HY) or regression (CI) models supported the conclusion that hybrids had lower relative fitness than either parental species. The results support the endogenous selection hypothesis, and fail to support the exogenous selection hypothesis.

The genetically pure pairs (BC or CA) had on average 7.0 fledglings (Fig. 4). Comparable data (fledglings per successful clutch) are not available for either parental species in areas immediately adjacent to the study area or for CA in

TABLE 2. Regressions of reproductive measures of chickadees on female- and male-transformed genetic indices and compatibility index of the breeding pair. Fledging success is the proportion of nestlings that fledge. The sample size for all regressions is 19. Significant relationships based on the sequential Bonferroni (Hochberg 1988) technique ($P_{adj} = P \times [5 - Rank]$) are shown in boldface.

Reproductive variables	Transformed genetic index								Compatibility index of breeding pair			
	Female				Male				r^2	P	Rank	P_{adj}
	r^2	P	Rank	P_{adj}	r^2	P	Rank	P_{adj}				
Clutch size	0.071	0.27	2	0.81	0.001	0.89	4	0.89	0.154	0.10	3	0.20
Nestlings	0.005	0.77	4	0.77	0.258	0.03	2	0.09	0.304	0.01	2	0.03
Fledglings	0.013	0.65	3	1.30	0.287	0.02	1	0.08	0.314	0.01	1	0.04
Fledging success	0.190	0.06	1	0.24	0.096	0.20	3	0.40	0.012	0.66	4	0.66

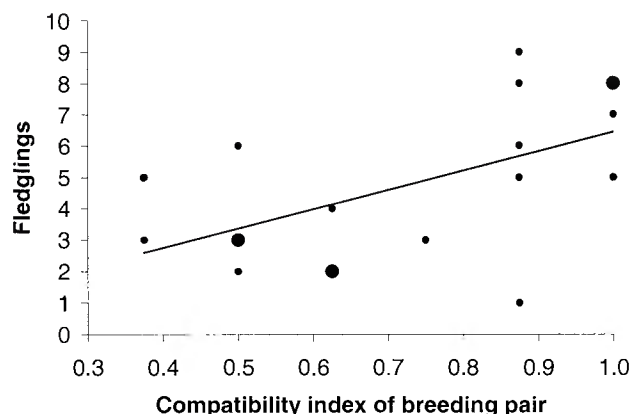


FIG. 4. Relationship of number of fledglings with compatibility index of a breeding pair of chickadees, and calculated regression line ($r^2 = 0.314$, $df = 18$, $P_{adj} = 0.04$). Compatibility indices of 0 and 1, respectively, indicate breeding pairs that would produce offspring heterozygous or homozygous for species-specific genetic markers. Each larger circle represents two datapoints.

general. Nickell (1956) found a similar number of fledglings (6.6) for BC in the southern peninsula of Michigan. Since, in general, CA tend to have a smaller clutch than BC (5–6 versus 6–8; Harrap 1996), neither parental species appeared to experience a reduction in the number of fledglings produced when relocated into the hybrid zone.

Our protocol did not formally address one type of exogenous selection, frequency-dependent selection (Mallet 1986; Mallet and Barton 1989), most typically selection favoring the most common form. Combining the results of this experiment with additional transplanting of the three groups into the two parental distributions would provide the necessary data to support or eliminate exogenous frequency-dependent selection. If the relative reproductive success of the three groups remained the same in all three areas (i.e., hybrids doing more poorly than either parental species), frequency-dependent selection could then be eliminated.

Our protocol did provide data that could address this issue incidentally. Frequency-dependent selection might favor rare or common forms. Selection favoring rare forms causes global polymorphism and an unstable cline (Mallet and Barton 1989). Therefore, frequency-dependent selection is unlikely in these chickadees because they show a series of stable clines (even though the zone as a whole is moving). Thus, frequency-dependent selection favoring common forms is the type to be addressed. If such were the case and the three groups were placed within the hybrid zone, the two parental types should be at a disadvantage relative to the more common hybrids. In this experiment, disappearance of introduced birds did not differ among the three genetic categories. Since the introductions were made during the winter, which is the time of most mortality in chickadees (Smith 1991), we believe it is unlikely that there is a difference in annual survivorship within the zone. Thus, exogenous selection based on differential survival (e.g., frequency-dependent selection) does not appear likely in this zone.

According to the regression model depicted in Figure 4, perfectly compatible chickadees ($CI = 1.00$) would produce 1.91 or 2.48 times more fledglings per nesting attempt, re-

spectively, than the average ($CI = 0.50$) or least compatible ($CI = 0.375$) hybrid pair in the experiment. As chickadees seldom produce more than one brood per year (Smith 1991), our experiment demonstrated an average 191% or a maximal 248% reduction in annual reproduction of hybrids. Such a marked decrease in reproductive success of hybrids relative to parental taxa provides strong support for the endogenous selection hypothesis. While it may be argued that relative fitness should not be calculated until offspring at least survive to the next breeding season, it is quite unlikely that the required increase in survivorship of hybrids relative to the parental taxa (1.91 to 2.48 times) could exist to offset the reduced reproduction. For example, just south of the hybrid zone and study area, the annual survivorship of CA juveniles without supplemental food is 31% (Doherty and Grubb 2002). Consequently, the offspring of either the average or the lowest CI pair in this study would need an annual survivorship of 59% ($1.91 \times 31\%$) or 77% ($2.48 \times 31\%$), respectively, to offset the reduced number of fledglings. Given that adult CA annual survivorship without supplemental food just south of the hybrid zone is only 43% (Doherty and Grubb 2002) and adult BC annual survivorship with supplemental food in Connecticut is 62% (Loery et al. 1997), an annual survivorship of either 59% or 77% for juvenile HY without supplemental food is not likely.

This experiment attempted to control for any exogenous factors that might be contributing to the reduction in reproductive success observed across the Ohio portion of this chickadee hybrid zone. However, unlike the case in our isolated woodlots, most chickadees in the hybrid zone live and breed amongst other chickadees, so exogenous social factors could be in operation more generally (Rohwer et al. 2001). For example, while the endogenous selection observed in this experiment apparently limits the width of the zone, social interactions might be functioning in the northward movement of the zone.

While transplant experiments in nature have addressed hybrid zones between species or subspecies of less mobile taxa (Levin and Schmidt 1985; Emms and Arnold 1997; Wang et al. 1997; Fritsche and Klatz 2000), none has demonstrated endogenous selection. More importantly, only Wang et al. (1997) have actually examined production of successful sexual offspring, but a key step (germination rate) of that experiment was conducted in the laboratory rather than within the hybrid zone. We have presented the first simultaneous field test of the exogenous and endogenous selection hypotheses using reproductive success as the test criterion. Our results provide some of the most conclusive evidence to date for endogenous selection in a hybrid zone.

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