

PATTERNS AND PROCESSES WITHIN A NON-ECOTONE HYBRID ZONE:

THE CHICKADEES OF OHIO

DISSERTATION

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ABSTRACT

This dissertation uses an avian hybrid zone as a study system in which to explore potential causes for the configuration and movement of such zones. Stable hybrid zones are commonly considered to reflect a balance between selection and dispersal. Both exogenous and endogenous selection factors have been advanced to explain barriers to gene flow across narrow hybrid zones. Exogenous selection involves adaptation to local environments. In contrast, endogenous selection involves adaptation or coadaptation on the genomic level regardless of the external environment.

The focal avian species of this dissertation, black-capped (*Poecile atricapilla*) and Carolina (*Poecile carolinensis*) chickadees, are permanent residents in Ohio. Currently, their distributions abut in an east-west hybrid zone in the northern aspect of the state. The black-capped chickadee resides north of the hybrid zone and the Carolina chickadee south. Based on historical data, the hybrid zone has moved northward about 100 km in the past 70 years.

The first phase of this dissertation characterized, in a limited manner, a transect of the hybrid zone and identified patterns in reproductive success across the zone. A distinctive trough in reproductive success in the hybrid zone was observed and correlated with both location and parental genetics.

The second phase consists of a controlled experiment. The genetics of the chickadees within the hybrid zone (endogenous selection) and the location of the hybrid zone (exogenous selection) were tested against each other to determine which was more influential on the observed pattern of decreased reproductive success within the hybrid zone. In support of endogenous selection, transplanted hybrid pairs produced fewer nestlings and fledglings than did transplanted pairs of either parental species.

The final phase consists of a controlled, aviary experiment to determine if female mate preference might be a causal mechanism for the northward movement of the hybrid zone. The relative importance of morphological appearance (possibly denoting species identity) and social dominance of males were assessed. Without apparent knowledge of dominance, females preferred black-capped chickadee males. With knowledge of dominance, females preferred the dominant male which, when the dyad was of comparable size, was the Carolina male.

For Tim and AJ

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FIELDS OF STUDY

Major Field: Evolution, Ecology, and Organismal Biology

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CHAPTER 1

INTRODUCTION

This dissertation uses an avian hybrid zone as a study system in which to explore potential causes for the configuration and movement of such zones. Stable hybrid zones have been observed in a variety of taxa (see reviews in Barton & 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 always the same across all zones. Both exogenous and endogenous factors (Moore & Price 1993) have been advanced to explain barriers to gene flow across narrow hybrid zones (Barton & Hewitt 1985). Exogenous selection involves adaptation to local environments. For example, in a fern hybrid zone, the genetic composition across the hybrid population could be attributed to the amount of soil moisture and light levels (Kentner & 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 & Wood 1998; Pearson 2000; Pearson & Manuwal 2000; Pearson & Rohwer 2000; Rohwer et al. 2001). In contrast, endogenous selection involves adaptation or coadaptation on the genomic level regardless of the external environment. Individuals of mixed ancestry are selected against because alleles are favored either within a complementary genetic background or at high frequency (Barton & Gale 1993). For example, in a study controlling for vegetational type, reduced density in the zone between chromosomal races of a grasshopper *Podisma pedestris* was attributed to hybrid inviability (Barton 1980; Barton & Hewitt 1981b).

The focal avian species of this dissertation, black-capped (*Poecile atricapilla*, formerly *Parus atricapillus*; American Ornithologists' Union 1998, 2000) and Carolina (*Poecile carolinensis*, formerly *Parus carolinensis*; American Ornithologists' Union 1998) chickadees, are permanent residents in Ohio (Peterjohn 2001). Currently, their distributions abut in an east-west hybrid zone in the northern aspect of the state, approximately along US Highway 30 (Grubb et al. 1994, Peterjohn 2001). The black-capped chickadee resides north and the Carolina chickadee south of the hybrid zone. Before 1900, the Carolina chickadee was described as a permanent resident only within the southern portion of the state (Wheaton 1882). In the 1930s, the hybrid zone was located across the midline of the state (along approximately the current US Interstate 70; Trautman 1940). Based on these historical data, the hybrid zone has moved northward about 100 km in the intervening 70 years. The black-capped chickadee range has been receding and the Carolina range expanding at a

commensurate pace. This dissertation characterizes several measures of reproductive success across the hybrid zone, identifies a general mechanism causing variation in reproductive success across the hybrid zone, and evaluates a possible causal mechanism for the northward movement of the zone.

The hybrid zone has previously been studied at numerous locations in the eastern United States (e. g., Kansas – Rising 1968, Missouri – Braun & Robbins 1986, Sawaya 1990, Illinois – Brewer 1963, Ohio – Grubb et al. 1994, Virginia – Johnston 1971, Sattler 1996, Sattler & Braun 2000, West Virginia – Sattler 1996, Sattler & Braun 2000, Pennsylvania – Ward & Ward 1974). However, only Brewer (1963) studied reproduction (seven nests in western Illinois), noting a significant decrease in hatching success in the hybrid zone (<65%) compared to the parental species (~95%). Brewer (1963) attributed this reproductive deficit to infertility and retarded development of the eggs. In Ashland County, Ohio, after determining the location of the hybrid zone based on song type (Grubb et al. 1994), T. C. Grubb, Jr. (pers. comm.) followed a putative hybrid nest in the spring of 1993 that hatched only one of eight eggs. These limited empirical results and the evidence that these species are not sister taxa (Gill et al. 1989, Gill et al. 1993) suggest that only limited successful hybridization might be expected.

This dissertation approached the Ohio portion of the chickadee hybrid zone in three phases. The first phase (Chapter 2) includes two main components. First, a limited characterization of a transect of the hybrid zone in Ashland County, Ohio was developed based on five species-specific genetic markers (Sawaya 1990, Sattler 1996,

Sattler & Braun 2000). Second, reproductive measures were gathered for breeding pairs across that study transect. These measures were then compared to genetic and location information in a search for relevant patterns.

According to the frequency distributions of the five-enzyme/probe combinations, the hybrid zone was approximately 15 km wide. However, based on reduced values of reproductive measures, the hybrid zone was only approximately 6 km wide. The great contrast in widths indicates that conclusions about the widths of other hybrid zones based solely on frequency distributions of diagnostic genetic markers might be overestimations.

The reduced reproductive output appeared linked to the heterozygosity of males and to the genetic compatibility of a breeding pair. While number of nestlings and reproductive success were related to genetic characteristics of the breeding pairs, both of these two reproductive measures and the parents' genetic characteristics were also related to position within the zone. Thus, parental genotypes may not have been an exclusive cause for the reduced productivity in the middle of the study transect. For example, environmental attributes could also have been involved. While no gradients or other inconsistencies in environmental characteristics were detected across the sample transect, such exogenous factors could have existed and been causal.

Both the observed trough in reproduction across the zone and the recent northward movement of the zone are indications that this portion of the chickadee hybrid zone could be categorized as a "tension zone" (Key 1968, Barton & Hewitt

1985). In such cases, the location of a hybrid 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). The nature of such selection is not readily apparent based on observational data. Both exogenous and endogenous factors (Moore & Price 1993) have been advanced to explain barriers to gene flow across narrow hybrid zones (Barton & Hewitt 1985). The most convincing method for determining if such a balance is operating is to transplant parental taxa and their hybrids into the hybrid zone and measure the three groups' relative fitness (Moore & 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.

The second phase of the dissertation (Chapter 3) consists of a controlled experiment. The genetics of the chickadees within the hybrid zone (endogenous selection) and the location of the hybrid zone (exogenous selection) were tested against each other to determine which was more influential on the observed pattern of decreased reproductive success within the hybrid zone. The reproductive success of mated pairs of black-capped and Carolina chickadees and their hybrids transplanted into the hybrid zone was recorded. The 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 hybrids 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.

Although clutch sizes were similar, transplanted hybrid pairs produced fewer nestlings and fledglings than did transplanted pairs of either parental species. These results support the endogenous selection hypothesis for maintenance of this hybrid zone, and disprove the exogenous selection hypothesis.

The third and last phase of the dissertation (Chapter 4) consists of a controlled experiment to determine if female mate preference might be a causal mechanism for the northward movement of the hybrid zone. The relative importance of morphological appearance (possibly denoting species identity) and social dominance of males were addressed as part of the protocol.

Given a choice within an aviary setting, females of both species that had not observed the direct social interactions between a heterospecific dyad of males preferred to associate with the black-capped male. In nature, both species form consensual dominance hierarchies. In the aviary, Carolina males dominated black-capped males unless a Carolina male was substantially smaller. Once females had observed the physical interaction of a particular heterospecific dyad of males, they associated preferentially with the social dominant, regardless of species type. Thus,

this experiment suggests that the effect of Carolina male intrasexual dominance on female mate preference may be contributing to the northward movement of the hybrid zone.

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CHAPTER 2

REPRODUCTIVE SUCCESS ACROSS AN AVIAN HYBRID ZONE: IMPLICATIONS FOR CHARACTERIZATION OF HYBRID ZONES BASED ON GENETIC INDICES

ABSTRACT

Black-capped (*Poecile atricapilla*, BC) and Carolina (*P. carolinensis*, CA) chickadees hybridize in an east-west band from New Jersey to Kansas. Within the past century, the Ohio portion of this hybrid zone and the CA range to the south have been moving northward while the BC range has retracted. We characterized the genetic composition of an Ohio segment of the hybrid zone using five enzyme-probe combinations. Based on several reproductive variables (clutch size, number of nestlings, and reproductive success), we found a decrease in reproduction in the hybrid zone, a decrease significantly related to the genetic composition of the male. While, the decrease in reproduction was also significantly related to the compatibility index of the breeding pair (i. e., the probability of producing homozygous offspring) it was not significantly related to the genetic composition of the female. As the zone

of reduced reproductive success was considerably narrower than the zone of introgression, our results signal caution about solely using the distributions of genetic markers to characterize the functional width of a hybrid zone.

INTRODUCTION

Hybrid zone dynamics are 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 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 (*Poecile atricapilla*; BC) and Carolina (*P. carolinensis*; CA) 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). Because these species are not sister taxa (Gill *et al.* 1989, Gill *et al.* 1993), only limited successful hybridization might be expected.

Nationally, except for a peninsular distribution in the Appalachian Mountains, the BC distribution abuts the north edge of the CA distribution. In the southern Appalachians, BC is often found at high elevations with CA at lower elevations. In the early 1880s, CAs were described as permanent residents only within the southern

portion of Ohio (Wheaton 1882). By the late 1930s, the east-west oriented hybrid zone was probably located across the middle of the state approximately in the location of US Interstate 70 (Trautman 1940). Currently, while still in an east-west orientation, the zone is located about 100 km farther north, approximately along US Highway 30 (Grubb *et al.* 1994, Peterjohn 2001). In other words, the BC distribution has been receding northward.

Within the hybrid zone in Illinois, reproductive success is substantially reduced with respect to the parental populations on either side (Brewer 1963). Brewer (1963) found the hatching success in the hybrid zone was at most 65% (based on 7 nests) and significantly lower than for either parental species (~95%). Brewer (1963) attributed the reduction to infertility and retarded development of eggs.

The objectives of this study 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

The area of the hybrid zoned 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. 2.1). The study area was 23 km from north to south and 6 km from west to east. The landscape was rather equally divided among pasture, row crops and woodlands.

In November of 1993 and 1994, we placed remote-controlled feeder traps (Pierce and Grubb 1979) filled with sunflower seeds within privately owned woodlands along a north-south line within the zone. From December through 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 US Fish and Wildlife Service 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 determined based on behavioral observation subsequent to capture (i.e., males dominant to females) and the relative size of the members of a pair (Desrochers 1990, Smith 1991). Sex was later verified for many individuals based on vocalizations (e. g., singing males, begging females) and physiology (e. g., male cloacal protuberance, female brood patch).

For genetic analysis, a 50-75- μ l blood sample from each bird was taken in a heparinized capillary tube from a brachial vein punctured using a 25-gauge needle. We placed each blood sample 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 it at 4°C.

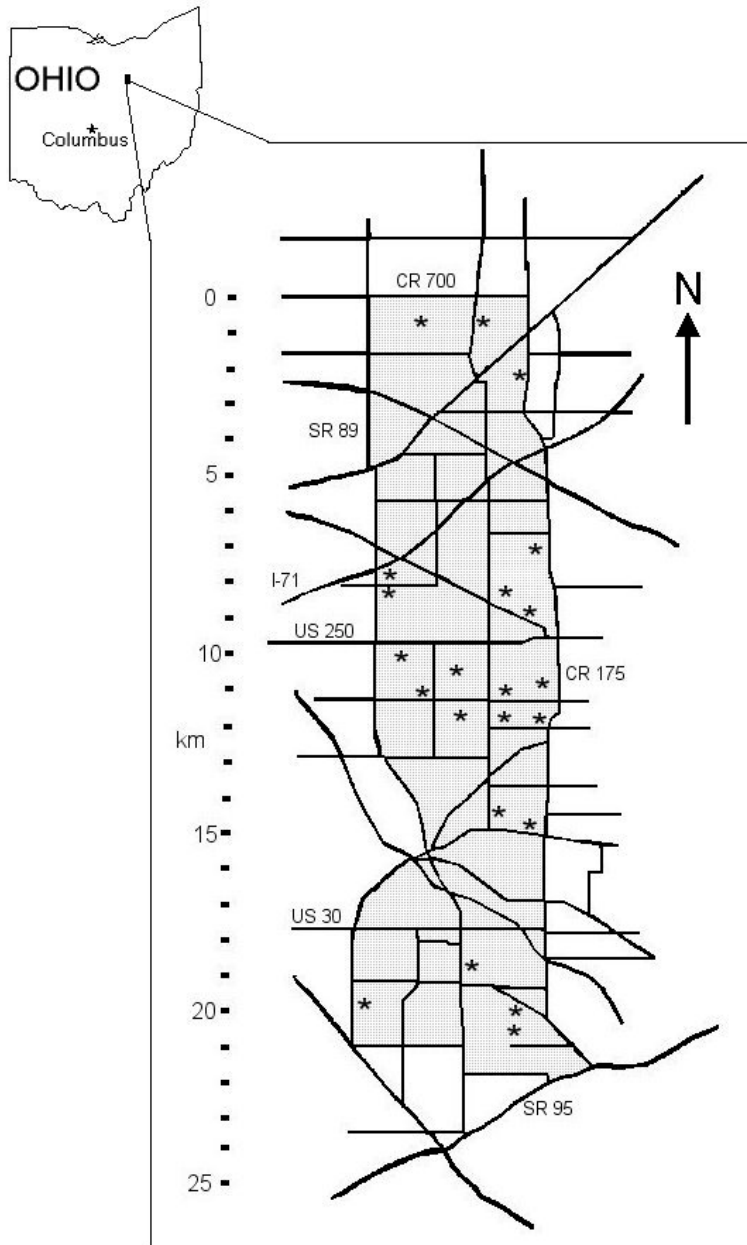


Figure 2.1: The study transect (stippled area) in Ashland County, Ohio with sampling points (stars) and major roadways bounding or traversing the study area indicated.

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

We assessed the concentration and purity of each extracted DNA sample using spectrophotometric measures of optical densities at 260 nm and 280 nm wavelengths. 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 ultra-violet light box. All the samples were determined to be unsheared.

Genetic analyses were performed following the methods of Sattler and Braun (2000). We used five probes to identify restriction fragment length patterns diagnostic for CA and BC on Southern blots (Sambrook *et al.* 1989). First, *Eco* RI digests were probed with a 1200-bp fragment of the domestic chicken (*Gallus g. domesticus*) oncogene *ski* (Li *et al.* 1986, Sawaya 1990, Sattler and Braun 2000). Second, *Bgl* II digests were probed with a randomly cloned fragment of CA DNA designated RP104 (Sattler 1996). Third, *Ava* II digests were probed with a randomly cloned fragment of CA DNA designated RP7 (Sattler 1996). Fourth, *Pst* I 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, *Pst* I 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), only four or five markers might be adequate to categorize coarsely individuals in a hybrid zone. However, the models assume that no F₁ x backcross or backcross x 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) calculated as the proportion of CA alleles present in an individual

$$GI = [(\sum_{i=1}^3 \alpha_i) + \beta + \gamma] / (8 + \sigma)$$

where σ is sex ($\sigma = 1$ if male and 0 if female), α_i are the autosomal loci ($\alpha_1 = Eco$ RI/*ski*, $\alpha_2 = Bgl$ II/RP104, $\alpha_3 = Ava$ II/RP7; $\alpha = 2$ if homozygous CA, 1 if heterozygous, and 0 if homozygous BC), β is the sex-linked locus *Pst* I/C7 ($\beta = 1 + \sigma$ if homozygous CA, 1 if heterozygous, and 0 if homozygous BC), and γ is the mtDNA locus *Pst* I/mtDNA ($\gamma = 1$ if CA and 0 if BC). For males, GI was based on nine alleles – one for the combination of *Pst* I/mtDNA and two for each of the other enzyme/probe combinations. For females, GI was based on eight alleles – one each

for *Pst* I/mtDNA and *Pst* I/C7 and two for each of the other enzyme/probe combinations. The combination of *Pst* I/mtDNA has only one variant because mtDNA is haploid. The combination of *Pst* I/C7 has only one allele in females because it is a sex-linked marker and females are the heterogametic sex in birds. For linear regressions, GI was transformed

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

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

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

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

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, 0.5 if at least one is heterozygous, and 0 if they are opposite homozygotes) and ϵ is the sex-linked locus *Pst* I/C7 ($\epsilon = 0.5$ if the parents are identical homozygotes, 0.25 if the male is heterozygous, and 0 if they are opposite homozygotes). At each locus, a higher proportion indicates a more complementary genetic background. For example, for the *Eco* RI/*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 *Pst* I/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).

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

For parental analysis, 5 µg of DNA from each chickadee were digested with 5x *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 membrane by Southern blot in 10 x SSC buffer and UV cross-linked to the membrane. Jeffreys' multilocus minisatellite probe 33.6 (Jeffreys *et al.* 1985a, b) was radiolabelled by primer extension. The hybridizations were run overnight at 62°C in 1.5 x SSC, 0.1% SDS, 5 x Denhardt's solution, and 6% w/v dextran sulfate. After hybridization, the membranes were washed four times for 30 min at 62°C in 1.5 x SSC, 0.1% SDS. The membranes were then exposed to X-ray film at -20°C for approximately one week.

Minitab version 13.1 (Anonymous 2000) was used for the regression analyses. 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 breeding pair) would have reduced values in the middle of the hybrid zone. Consequently, for the relevant comparisons, a second order polynomial trend line was included instead of a linear regression line.

This project was performed under The Ohio State University ILACUC protocol 93A0206, federal banding permit 20653 and Ohio banding and collecting permit 509. None of the birds was held captive for longer than an hour.

RESULTS

The frequencies of various alleles at marker loci changed in consistent fashion across the hybrid zone from a low proportion of CA alleles in the north to a high proportion in the south (Figs. 2.2-2.6). The samples for this analysis were taken from only adults, not nestlings, to limit non-independence of data points due to relatedness. The unusually small sample size for *Pst* I/C7 stemmed from a need to know the sex of each individual to determine the number of alleles to be considered. Consequently, only individuals observed to breed were included to insure the correct attribution of sex.

Across the hybrid zone, we obtained records of reproductive output for 29 pairs of chickadees. Two nests could not be analyzed because of a laboratory accident causing the loss of the DNA. We analyzed DNA fingerprints for the remaining 27 nests, finding no evidence of extra-pair fertilizations. Of the 100 offspring tested, 14

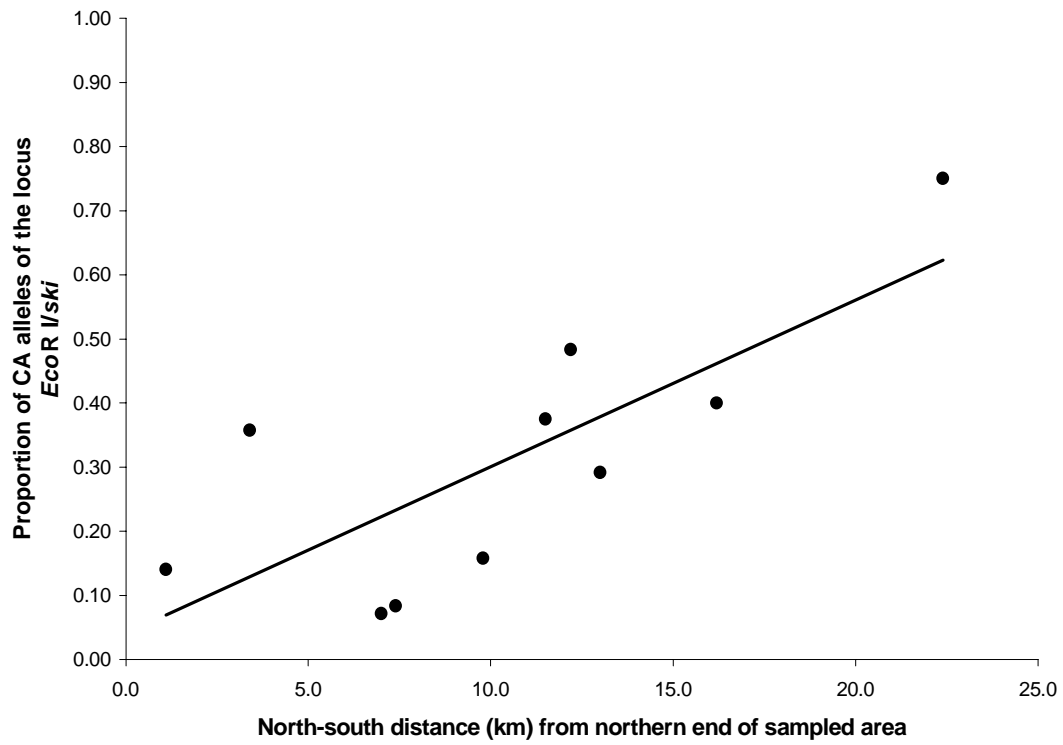


Figure 2.2: Distribution and trend line for CA alleles at the locus *EcoR I/ski* across the study site in north central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The sample size is 143.

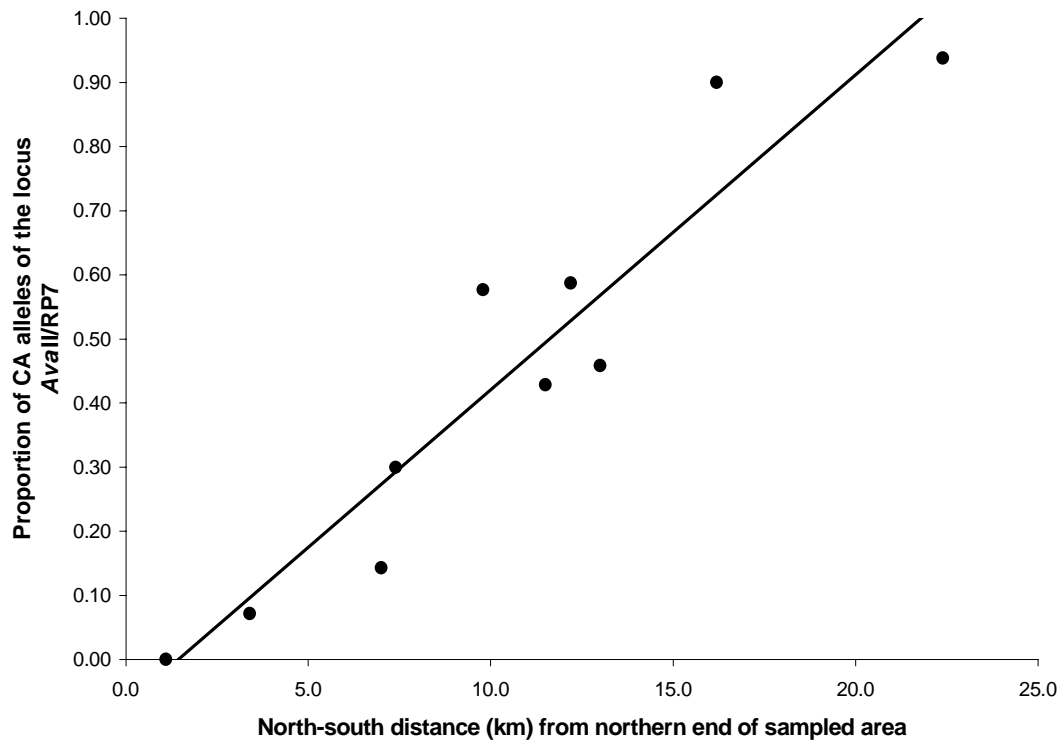


Figure 2.3: Distribution and trend line for CA alleles at the locus *Ava II/RP7* across the study site in north central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The sample size is 121.

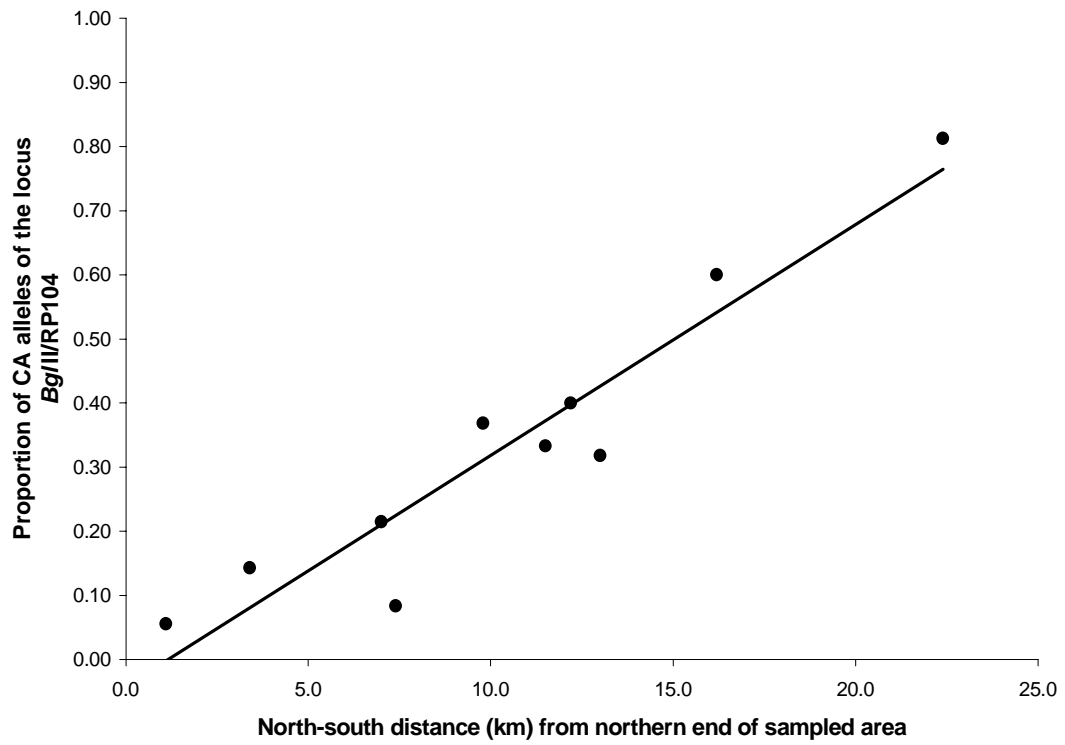


Figure 2.4: Distribution and trend line for CA alleles at the locus *Bgl* II/RP104 across the study site in north central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The sample size is 142.

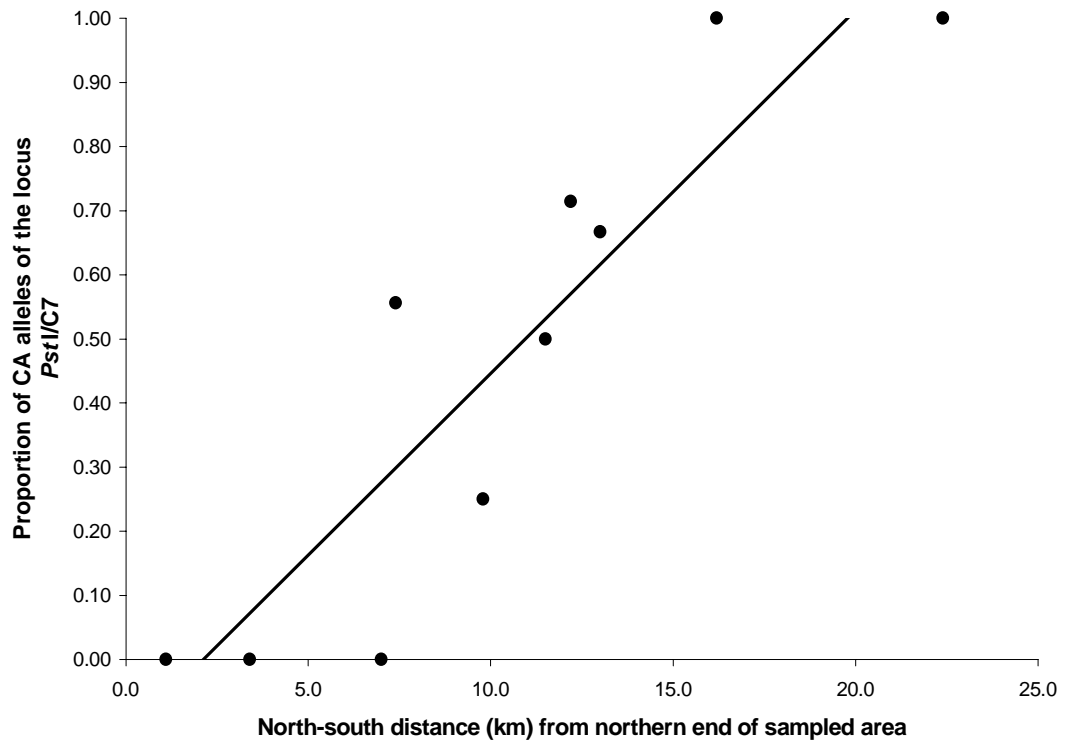


Figure 2.5: Distribution and trend line for CA alleles at the locus *Pst I/C7* across the study site in north central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The sample size is 60.

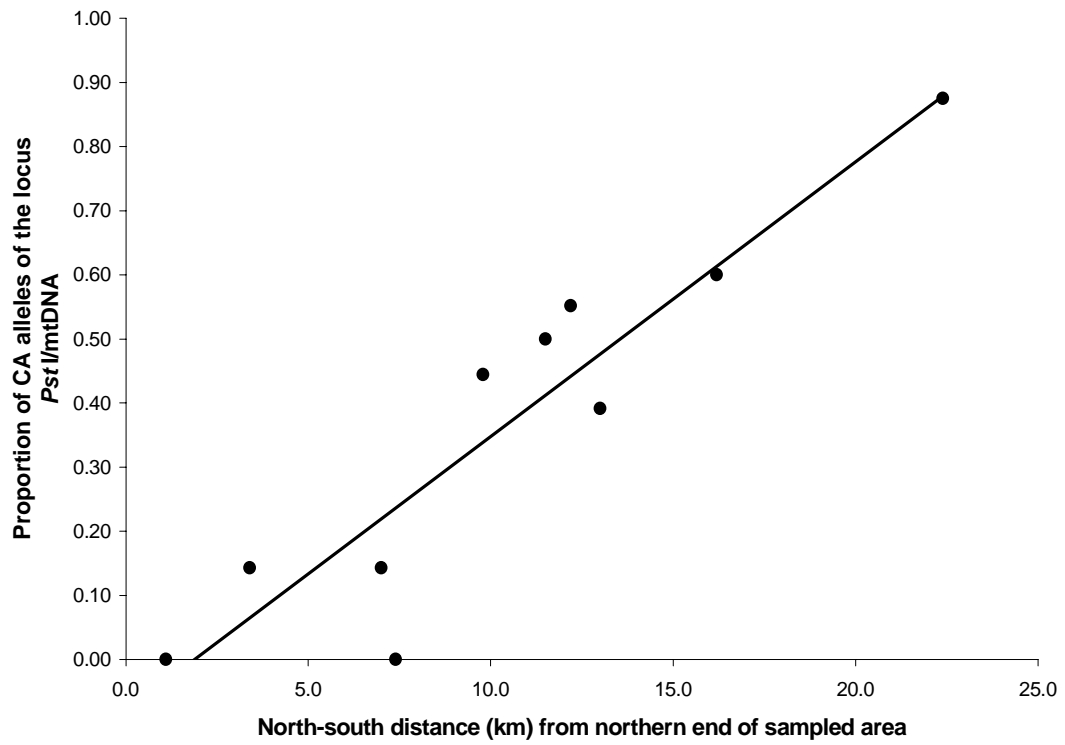


Figure 2.6: Distribution and trend line for CA alleles at the locus *Pst I*/mtDNA across the study site in north central Ohio. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The sample size is 142.

had one or more unattributable bands. None of these 14 individuals could be excluded as the offspring of the putative parents based on band-sharing scores (Wetton *et al.* 1987).

For all 29 pairs, there was no loss of offspring between hatching and fledging. Therefore, for these 29 pairs of chickadees, number of nestlings equaled number of fledglings, fledging success (fledglings/nestling) was 100% and reproductive success (fledglings/egg) equaled hatching success (nestlings/egg).

Figures 2.7-2.9 place various reproductive measures within the study landscape with a second order polynomial trend line included. No trend was apparent in clutch size across the zone, but both number of nestlings/fledglings and reproductive success displayed apparent troughs near the midway point of the zone.

GI of each sex had a positive and significant relationship with location in the study landscape (female GI, $R^2 = 0.354$, $n = 29$, $p = 0.001$, Fig. 2.10; male GI, $R^2 = 0.528$, $n = 29$, $p < 0.001$, Fig. 2.11). The CI of breeding pairs was lowest in the middle of the transect (Figure 2.12).

Transformed GI of the female ranged from 0.12 to 0.50 (Fig. 2.13 and 2.14) and was not significantly related to any reproductive measure (Table 2.1). While transformed GI of the male, ranging from 0.05 to 0.50 (Fig. 2.15 and 2.16), was not significantly related to clutch size (Table 2.1), it was positively and significantly related to both number of nestlings (Table 2.1, Fig. 2.15) and reproductive success (Table 2.1, Fig. 2.16).

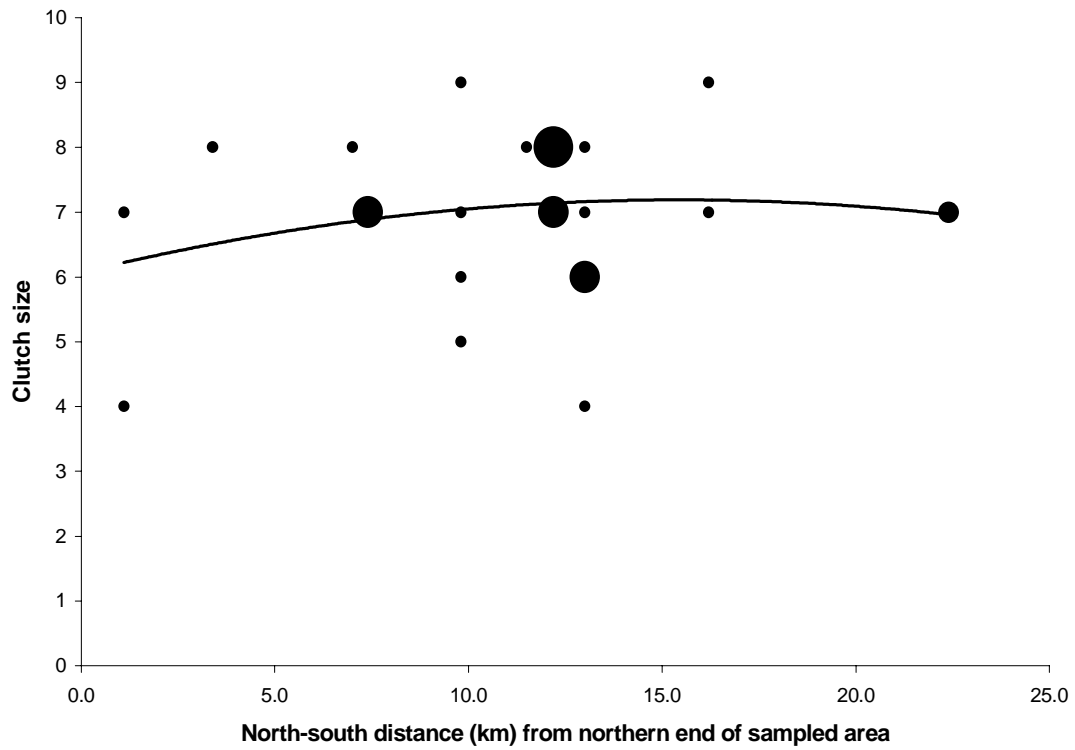


Figure 2.7: Relationship of clutch size with location in the hybrid zone, and the second order polynomial trend line. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The four sizes of circles from smallest to largest denote sample sizes of 1, 2, 3 and 4, respectively.

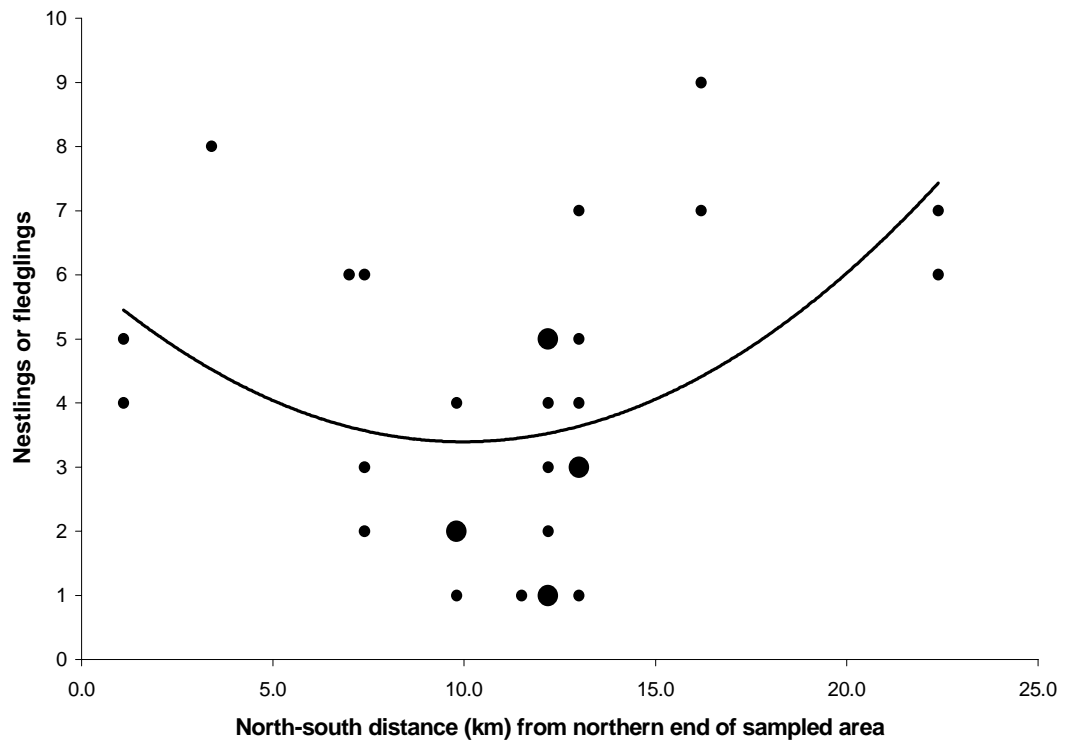


Figure 2.8: Relationship of number of nestlings or fledglings with location in the hybrid zone, and the second order polynomial trend line. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. Small and large circles denote sample sizes of 1 and 2, respectively.

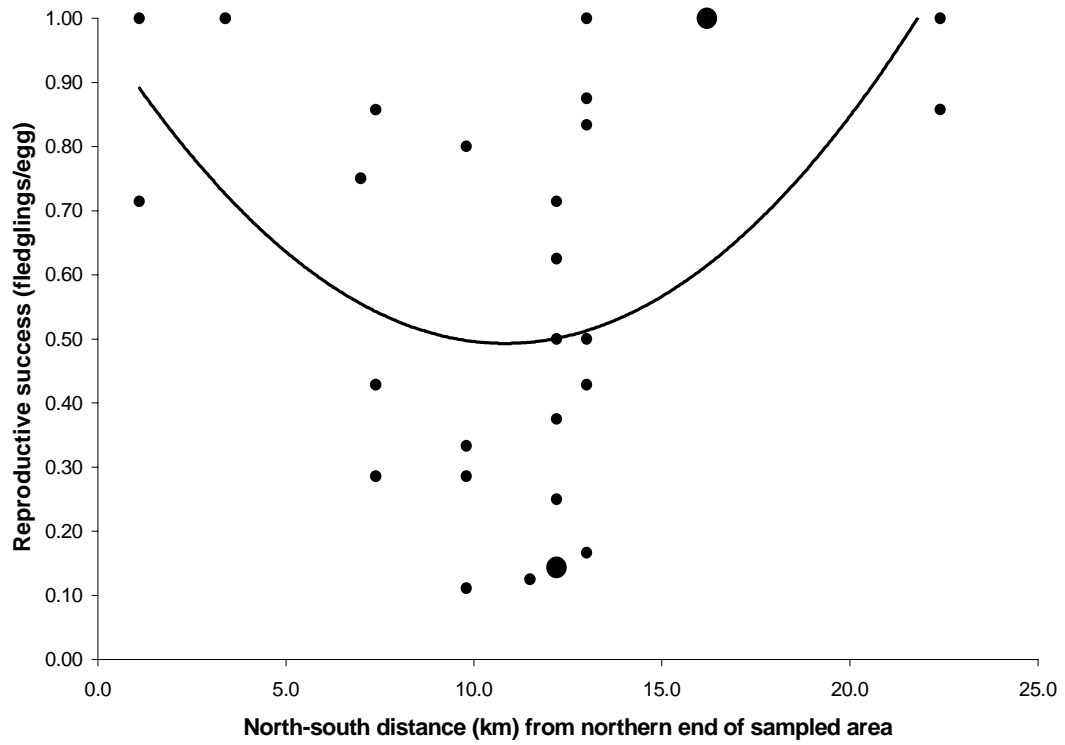


Figure 2.9: Relationship of reproductive success (fledglings/egg) with location in the hybrid zone, and the second order polynomial trend line. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. Small and large circles denote sample sizes of 1 and 2, respectively.

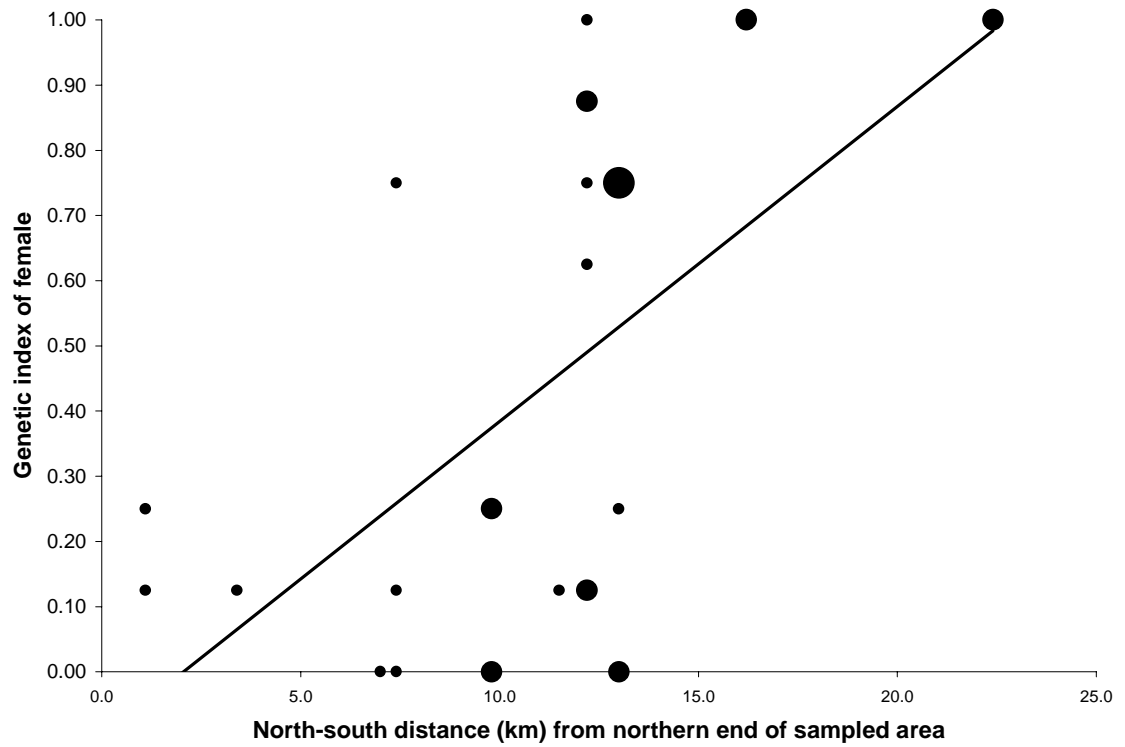


Figure 2.10: Relationship of female genetic index with location in the hybrid zone, and calculated regression line ($R^2 = 0.354$). Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

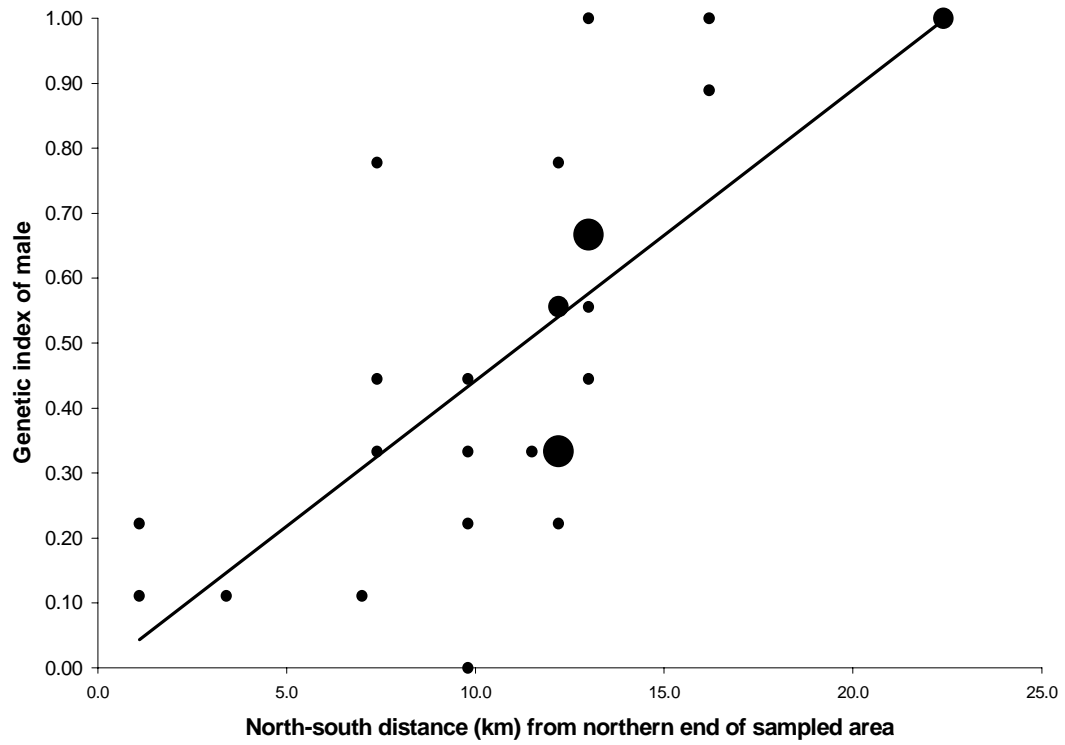


Figure 2.11: Relationship of male genetic index with location in the hybrid zone, and calculated regression line ($R^2 = 0.528$). Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

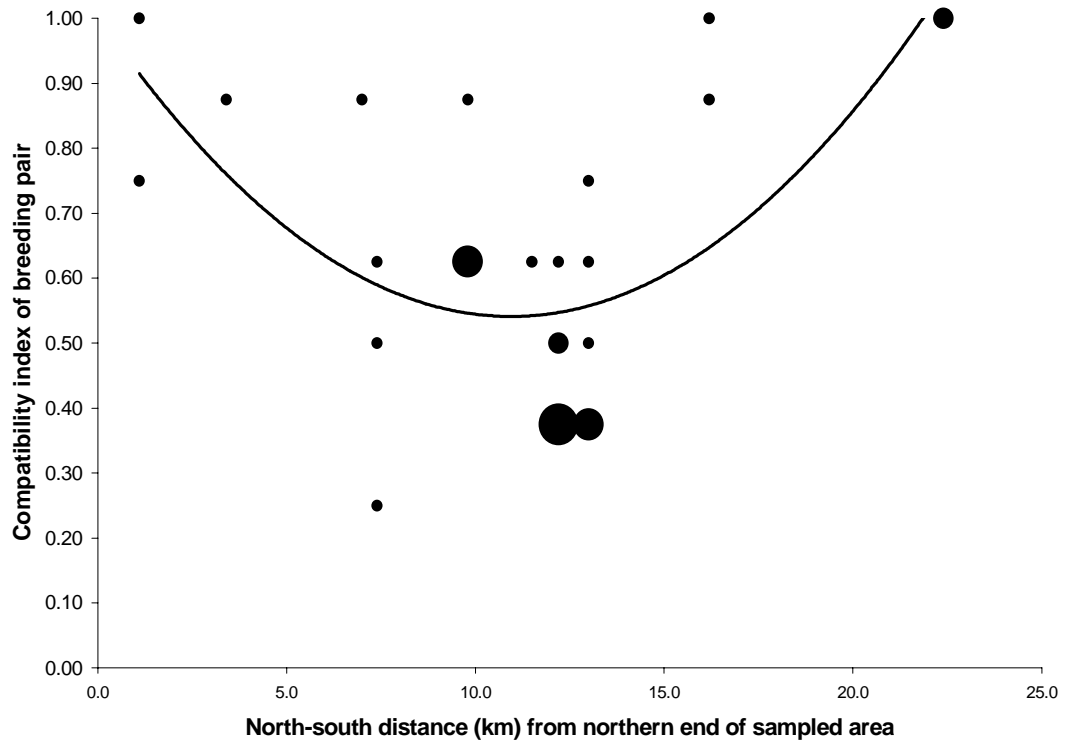


Figure 2.12: Relationship of compatibility index of breeding pair with location in the hybrid zone, and the second order polynomial trend line. Kilometer 0 is Ashland County Road 700, the northern boundary of the study area. The four sizes of circles from smallest to largest denote sample sizes of 1, 2, 3 and 4, respectively.

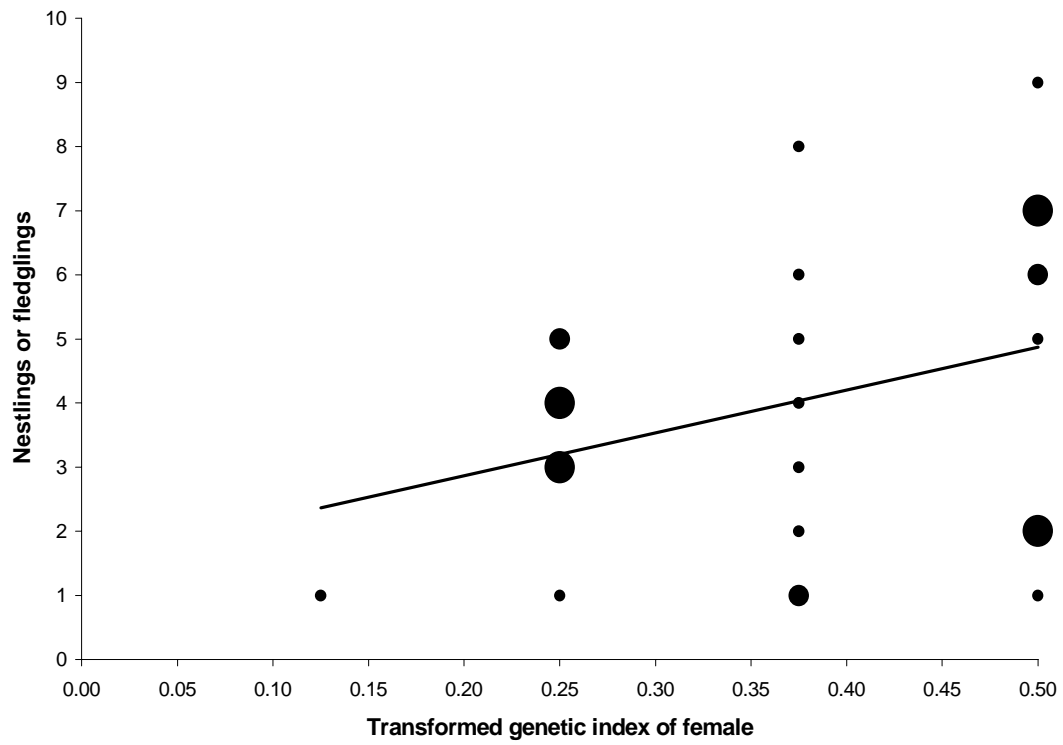


Figure 2.13: Relationship of number of nestlings or fledglings with transformed genetic index ($GI' = |GI - 0.5|$) of the female, and calculated regression line ($R^2 = 0.112$). Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

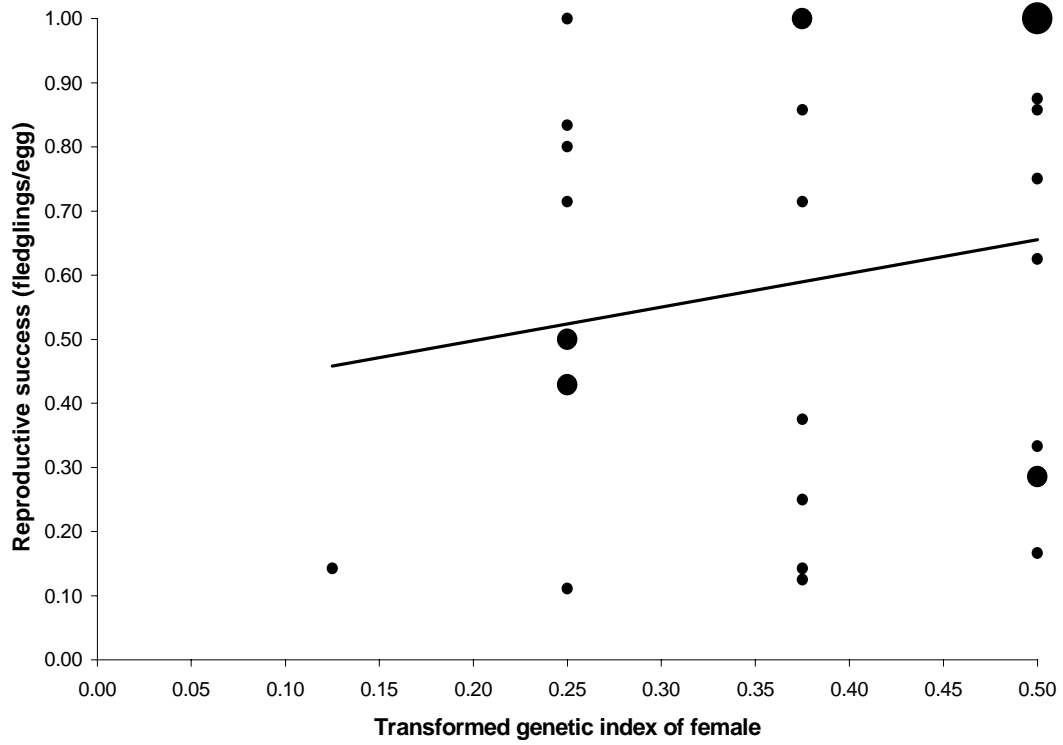


Figure 2.14: Relationship of reproductive success (fledglings/egg) with transformed genetic index ($GI' = |GI - 0.5|$) of the female, and calculated regression line ($R^2 = 0.036$). Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

Sex	Reproductive Variable	Regression Equation	R ²	p	Rank	p _{adj}
Female	Clutch size	Y = 6.13 + 2.33 X	0.049	0.250	2	0.500
	Nestlings or fledglings	Y = 1.53 + 6.67 X	0.112	0.079	1	0.237
	Reproductive success	Y = 0.393 + 0.526 X	0.036	0.326	3	0.326
Male	Clutch size	Y = 6.75 + 0.98 X	0.015	0.520	3	0.520
	Nestlings or fledglings	Y = 1.82 + 8.83 X	0.357	0.001	1	0.003
	Reproductive success	Y = 0.298 + 1.20 X	0.336	0.001	2	0.002

Table 2.1: Regressions of chickadee reproduction on the transformed genetic indices (GI' = |GI - 0.5|) of the female and male of a pair. Sample size for all regressions is 29. Significant relationships based on the sequential Bonferroni technique (p_{adj} = p * [4 - Rank]; Hochberg 1988) are shown in boldface.

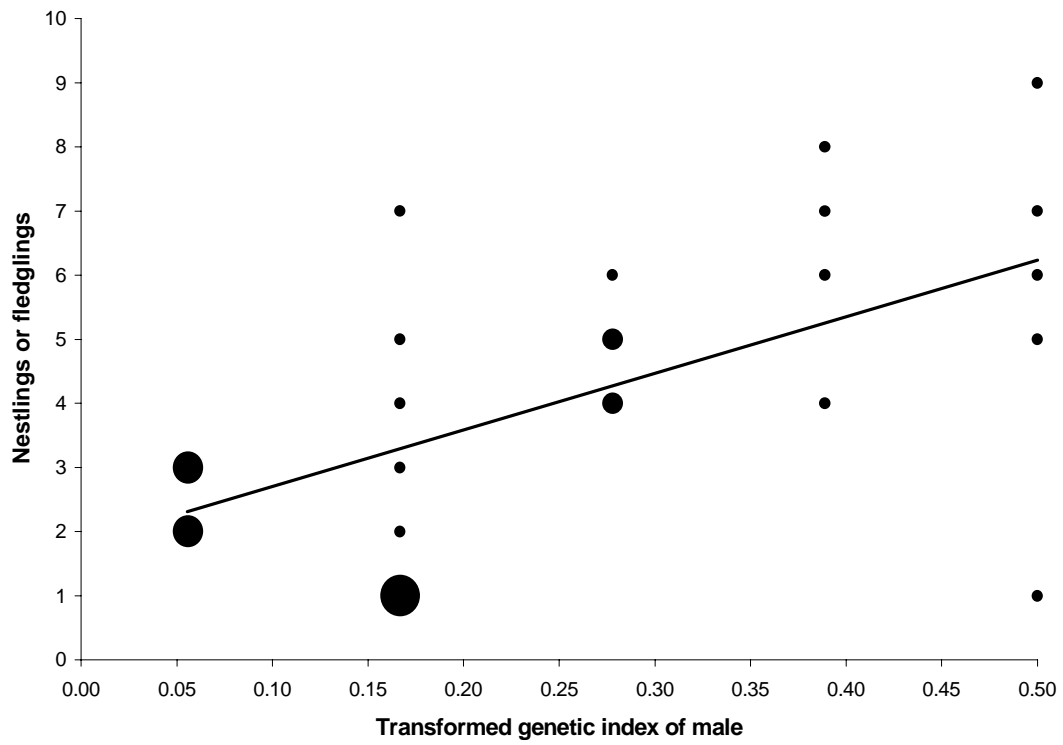


Figure 2.15: Relationship of number of nestlings or fledglings with transformed genetic index ($GI' = |GI - 0.5|$) of the male, and calculated regression line ($R^2 = 0.357$). The four sizes of circles from smallest to largest denote sample sizes of 1, 2, 3 and 4, respectively.

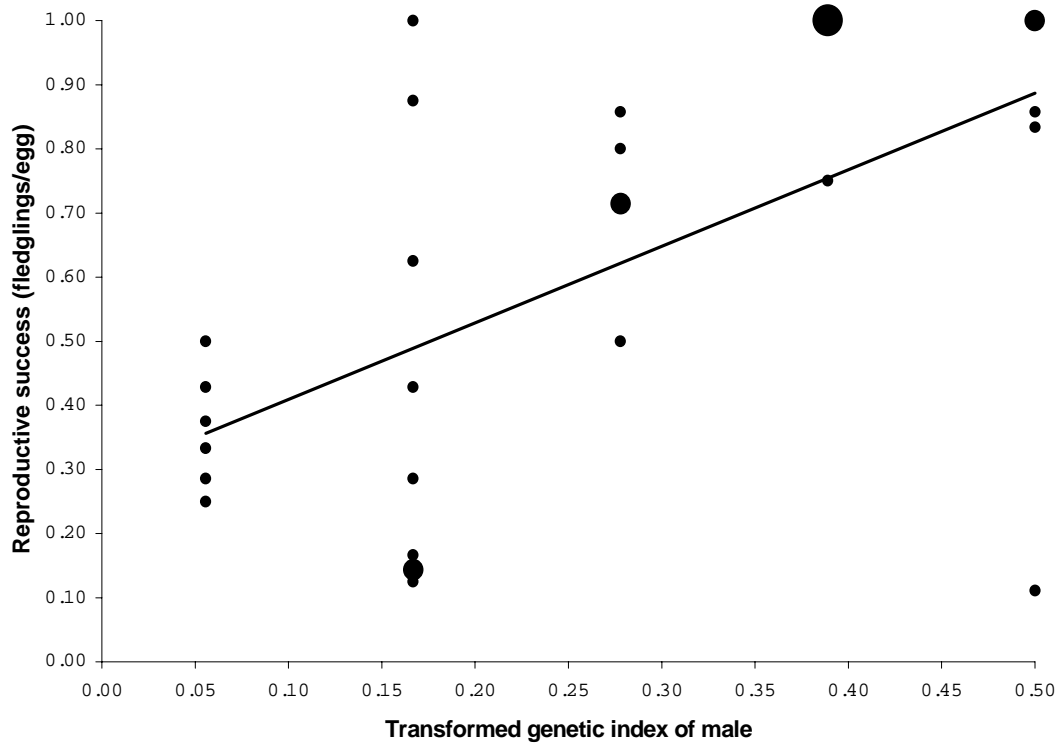


Figure 2.16: Relationship of reproductive success (fledglings/egg) with transformed genetic index ($GI' = |GI - 0.5|$) of the male, and calculated regression line ($R^2 = 0.336$). Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

The CI between the male and female of a breeding pair ranged from 0.25 to 1.0 (Fig. 2.17-2.18). While CI was not significantly related to clutch size (Table 2.2), it was positively and significantly related to both number of nestlings (Table 2.2, Fig. 2.17) and reproductive success (Table 2.2, Fig. 2.18).

DISCUSSION

Based on the frequency distributions of the five enzyme/probe combinations (Fig. 2.2-2.6), our data on reproduction were taken within the chickadee hybrid zone. Located in the middle of the transect we monitored was an area about 6 km in width where reduced reproductive output appeared linked to the heterozygosity of males and to the genetic compatibility of a breeding pair. While number of nestlings and reproductive success were related to genetic characteristics of the breeding pairs, these reproductive measures (approximately 6 km wide) and genetic characteristics (approximately 15 km wide for both female and male GI) were both also related to position within the zone (Fig. 2.7-2.12). 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 could also have been involved. While we could not detect any gradients or other inconsistencies in environmental characteristics across our sample transect, such exogenous factors (Harrison 1990; Arnold 1997)

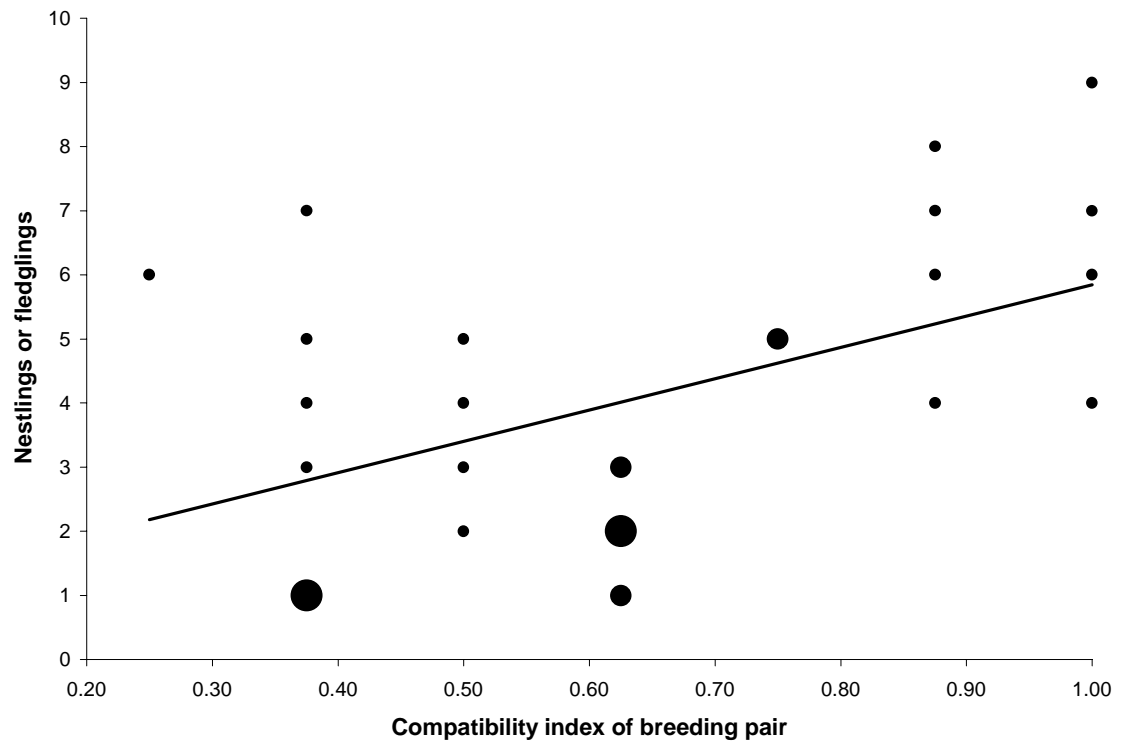


Figure 2.17: Relationship of number of nestlings or fledglings with compatibility index of breeding pair, and calculated regression line ($R^2 = 0.238$). Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

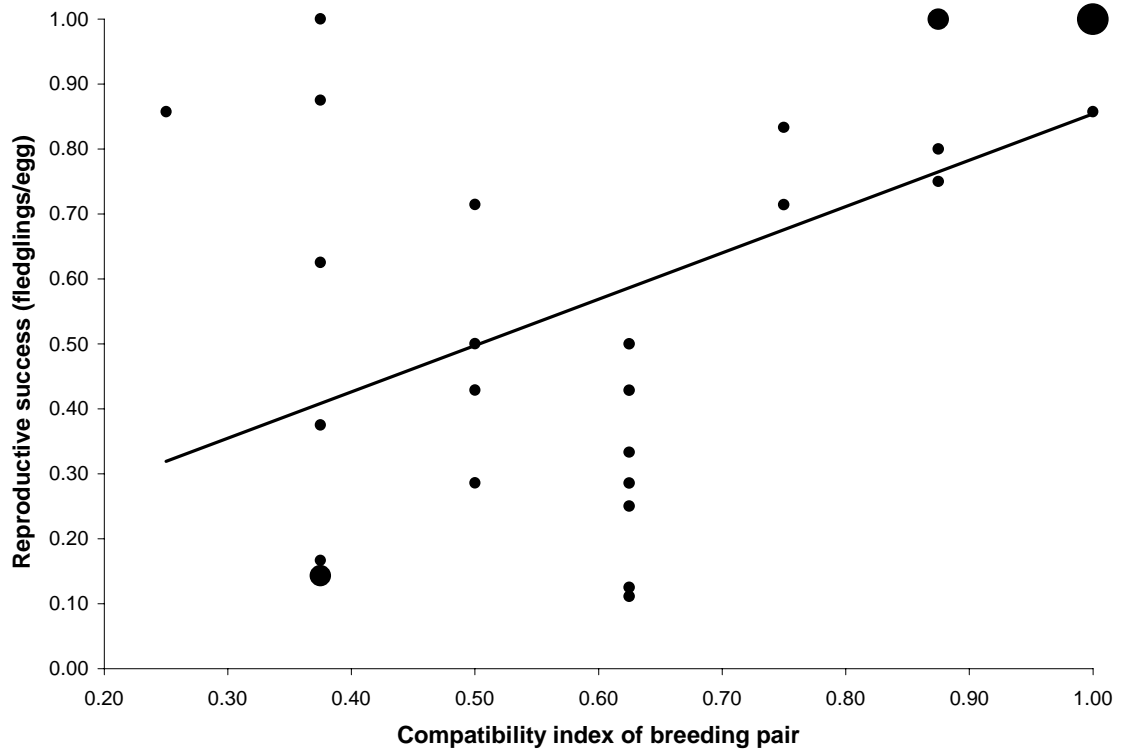


Figure 2.18: Relationship of reproductive success (fledglings/egg) with compatibility index of breeding pair, and calculated regression line ($R^2 = 0.261$). Small, medium and large circles denote sample sizes of 1, 2 and 3, respectively.

Reproductive Variable	Regression Equation	R ²	p	Rank	p _{adj}
Clutch size	Y = 7.16 - 0.25 X	0.002	0.807	3	0.807
Nestlings or fledglings	Y = 0.96 + 4.88 X	0.238	0.007	2	0.014
Reproductive success	Y = 0.141 + 0.714 X	0.261	0.005	1	0.015

Table 2.2. Regressions of chickadee reproduction on the compatibility index of the breeding pair. Sample size for all regressions is 29. Significant relationships based on the sequential Bonferroni technique ($p_{adj} = p * [4 - Rank]$; Hochberg 1988) are shown in boldface.

could have existed and been causal. However, we have recently obtained experimental evidence indicating that exogenous factors may not be at work in this part of the chickadee hybrid zone (Chapter 3).

Reduced productivity was apparently related to the genetic composition of males, but not females. Haldane's Rule (Haldane 1922) 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). Based on the lack of a significant relation between female GI and any reproductive variable, Haldane's Rule does not appear to be operating in this part of the chickadee hybrid zone. On the other hand, the data set does provide some evidence for Haldane's Rule. For example, there is a distinct gap between 0.3 and 0.6 in the distribution of female GI (Fig. 2.10). In males (Fig 2.10), 12 individuals are within that same GI range. Apparently, highly heterozygous females are removed from the population before reproduction (i. e., sampling point of this study). As an additional consideration, the small number of genetic markers employed could be contributing to the difference in relationships of female GI and male GI with reproductive measures. Male GI had a larger number of possible values due to inclusion of a sex-linked marker.

Haldane's Rule might not directly apply to comparisons of parental genetics with reproductive measures. Reproductive variables such as hatching success should be compared to the sex of the offspring themselves and, unfortunately, we do not

know the sex of nestlings or unhatched eggs. Under Haldane's Rule, we would expect an increased ratio of females to males in unhatched eggs with a compensatory decrease in the ratio for viable offspring.

Both the observed trough in reproduction across the zone and the recent northward movement of the zone are indications that this portion of the chickadee hybrid zone could be categorized 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). The most convincing method for determining if such a balance is operating is to transplant parental taxa and their hybrids into the hybrid zone and measure the three groups' relative fitness (Moore and Price 1993).

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 since we know that two components of GI (*Eco RI/ ski* and *Bgl II/RP104*) show significant introgression of BC alleles in southern Ohio (Sattler 1996). Since the introgression we measured could be a residual result of the northward movement of the Ohio portion of the zone (e. g. Shaw 1993), our results signal caution about using solely the distributions of genetic markers to characterize the functional width of a hybrid zone. Without adequate knowledge about historical

movements of a zone, relying solely on genetic distributions might cause an overestimate of the amount of hybridization occurring between a pair of species. Conversely, the ratio of reduced-fitness width to introgression width might indicate the degree of past movement of a hybrid zone.

The pattern of reproductive success across a hybrid zone provides information about the first stage of hybridization and its impact, 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. In comparison, the patterns in the genetic markers across a zone also provide information about the strength of the barrier because they represent the long-term impact of the hybridization. When hybridization is occurring (i. e., back-crosses are present), a hybrid zone is less analogous to a wall and more like a semi-permeable membrane that allows alleles to pass through at various rates depending on allele-specific selection factors.

We anticipate that future work of a similar nature in the same area will furnish useful information on the stability of the hybrid zone's width and the rate of its northward movement.

ACKNOWLEDGMENTS

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CHAPTER 3

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

ABSTRACT

The contributions of genetic and environmental factors to differential reproductive success across hybrid zones have rarely been tested (Fritsche & Klatz 2000). 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, hybrid pairs produced fewer nestlings and fledglings than did pairs of either parental species. Our results support the endogenous selection hypothesis for maintenance of this hybrid zone, and disprove the exogenous selection hypothesis.

INTRODUCTION

Stable hybrid zones have been observed in a variety of taxa (see reviews in Barton & 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 always the same across all zones. Both exogenous and endogenous factors (Moore & Price 1993) have been advanced to explain barriers to gene flow across narrow hybrid zones (Barton & Hewitt 1985). Exogenous selection involves adaptation to local environments. 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 & 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 & Wood 1998; Pearson 2000; Pearson & Manuwal 2000; Pearson & Rohwer 2000; Rohwer et al. 2001). In contrast, endogenous selection involves adaptation or coadaptation on the genomic level regardless of the external environment. Individuals of mixed ancestry are selected against because alleles are favored either within a complementary genetic background or at high frequency (Barton & 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 & Hewitt 1981b).

Barton & 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 where extensive life-history data have been collected for over 20 years (Veen et al. 2001). This long-term data set shows no recruitment of offspring from female F₁ hybrids and slightly reduced fertility of male F₁ hybrids (Veen et al. 2001).

The most convincing method for distinguishing the type of selection operating is to transplant parental taxa and their hybrids into the hybrid zone and measure the

three groups' relative fitness (Moore & 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.

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.

METHODS

The subjects and the zone

BC and CA chickadees are known to hybridize in many areas along their common border (e. g., Kansas – Rising 1968, Missouri – Braun & Robbins 1986,

Sawaya 1990, Illinois – Brewer 1963, Ohio – Grubb et al. 1994, Virginia – Johnston 1971, Sattler 1996, Sattler & Braun 2000, West Virginia – Sattler 1996, Sattler & Braun 2000, Pennsylvania – Ward & Ward 1974). While 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, per. obs.), 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 US Interstate 70 (Trautman 1940). Since then, the BC distribution has been receding northward. Currently, while still in an east-west orientation, the zone is located about 100 km farther north in the state, approximately along US Highway 30 (Grubb et al. 1994; Peterjohn 2001).

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 & 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 from 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. At the time of capture, each bird was banded with a US 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 & 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 – 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 a 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 μ g 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 ultra-violet light box. All samples were determined to be unsheared.

Genetic index

Genetic analyses were performed following the methods of Sattler & 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, *Eco* RI digests were probed with a 1200-bp fragment of the domestic chicken (*Gallus g. domesticus*) oncogene *ski* (Li et al. 1986; Sawaya 1990; Sattler & Braun 2000). Second, *Bgl* II digests were probed with a randomly cloned fragment of CA DNA designated RP104 (Sattler 1996). Third, *Ava* II digests were probed with a randomly cloned fragment of CA DNA designated RP7 (Sattler 1996). Fourth, *Pst* I digests were probed with a randomly cloned 4000-bp fragment of tufted titmouse (*Baeolophus bicolor*) DNA designated C7 (Sawaya 1990; Sattler & Braun 2000). Fifth, *Pst* I digests were also probed with house finch (*Carpodacus mexicanus*)

mitochondrial DNA (mtDNA) (Mack et al. 1986; Sawaya 1990; Sattler & Braun 2000) purified by subcellular fractionation and CsCl equilibrium-gradient centrifugation (Dowling et al.1990).

Based on the statistical models of Boecklen & 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 x backcross or backcross x 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 & Howard (1997).

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) 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 *Pst* I/mtDNA has only one variant because mtDNA is haploid. The combination of *Pst* I/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 *Pst* I/mtDNA and two for each of the

other enzyme/probe combinations. For females, GI was based on eight alleles – one each for *Pst* I/mtDNA and *Pst* I/C7 and two for each of the other enzyme/probe combinations. Hence the index was calculated as:

$$GI = [(\sum_{i=1}^3 \alpha_i) + \beta + \gamma] / (8 + \sigma)$$

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

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

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

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

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, 0.5 if at least one is heterozygous, and 0 if they are opposite homozygotes) and ϵ is the sex-linked locus *Pst I/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 *Eco RI/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 *Pst I/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 & Orr 1989).

Parental analysis

While only one pair of chickadees occupied each woodlot, extra-pair 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 basis (Grubb & Doherty 1999). To confirm that extra-pair parentage did not confound results, we conducted standard paternity and maternity analyses (Parker & Burley 1998).

For parental analysis, 5 µg of DNA from each chickadee were digested with 5 x *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 x SSC buffer and UV cross-linked to the nylon membrane. Jeffreys' multilocus minisatellite probe 33.6 (Jeffreys et al. 1985a, b) was radiolabelled by primer extension. The hybridizations were run overnight at 62°C in 1.5 x SSC, 0.1% SDS, 5 x 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 x SSC, 0.1% SDS, and then exposed to X-ray film at -20°C for approximately one week.

Statistical Analyses

Minitab version 13.1 (Anonymous 2000) was used for regression, Kruskal-Wallis, and pairwise binomial comparison analyses. As 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.

Ethical Statement

This experiment was performed under The Ohio State University's ILACUC protocol 97A016, federal banding permit 20653 and Ohio banding and collecting permit 509. None of the birds was held captive for longer than a day.

RESULTS

The genetic markers confirmed the result from plumage scores that all experimental woodlots were within the hybrid zone (Table 3.1, Fig. 3.1). Additionally, the MI and GI were significantly and positively related (Fig. 3.2; $r^2 = 0.778$, $df = 69$, $p < 0.001$) 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 (Table 3.1; Kruskal-Wallis $H = 45.76$, $df = 2$, $p < 0.0001$). However, the molecular markers revealed greater introgression of foreign alleles into parental populations (Fig. 3.1) than was evident from the morphological hybrid index, a result which has been obtained in independent studies of this hybrid zone at other locations (Sattler & 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

Species	Morphology and genetics of experimental pair				Reproductive variables of experimental pair				
	Morphological index of:		Genetic index of:		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

Table 3.1: The morphological, genetic and reproductive data for each experimental pair of chickadees. Fledging success is the proportion of nestlings that fledge.

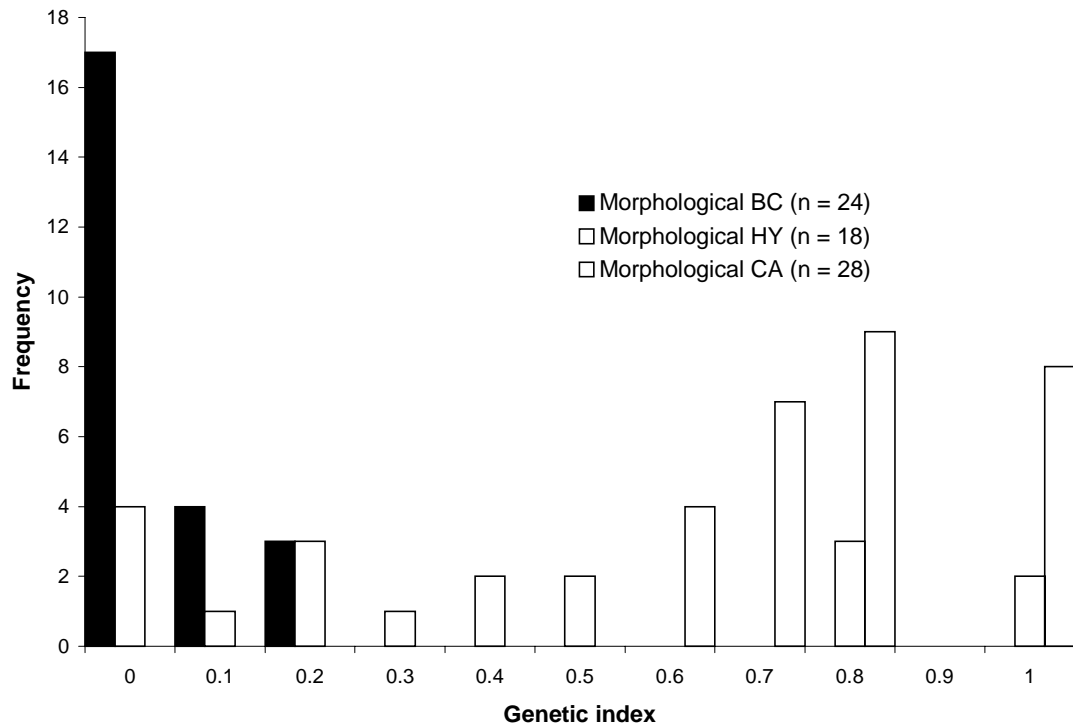


Figure 3.1: Genetic indices of Ohio chickadees taken from within the morphological black-capped, morphological hybrid and morphological Carolina distributions. The hybrids presented all came from the area of the transplant experiment. Pure BC and pure CA have genetic index scores of 0 and 1, respectively.

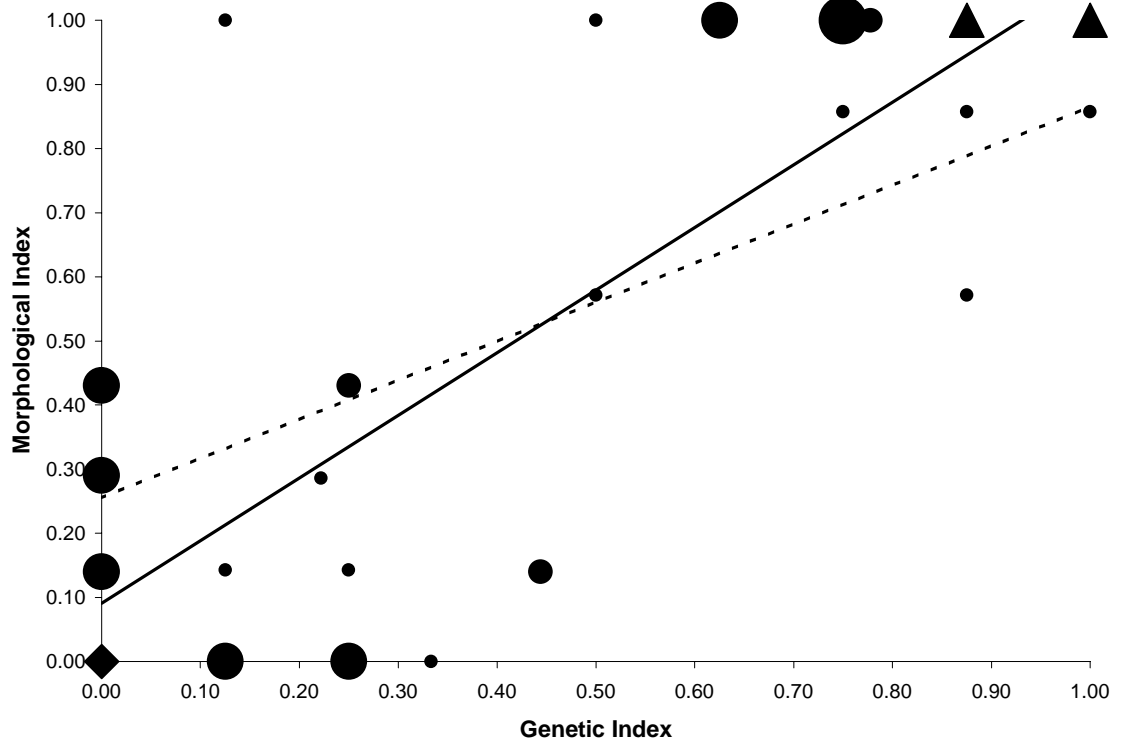


Figure 3.2: Relationship between morphological and genetic indices of Ohio chickadees, calculated regression line (solid) based on the same 70 individuals from Fig. 3.1 ($r^2 = 0.778$, $df = 69$, $p < 0.001$), and calculated regression line (dashed) based on only the 18 individuals from the hybrid zone in Fig. 3.1 ($r^2 = 0.346$, $df = 17$, $p = 0.010$). 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.

proportion of introductions lost prior to determining reproductive success (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 (Table 3.1; 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 (Table 3.1). CI was not significantly related to clutch size or fledging success (fledglings/nestling) (Table 3.2). However, CI was significantly positively related to both number of nestlings and number of fledglings (Table 3.2, Fig. 3.3 and 3.4). Neither the female nor the male GI was significantly related to any of the reproductive measures (Table 3.2).

No evidence of extra-pair 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. 3.1) that allowed us to discern a gradient of parental pairings. The

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

Table 3.2: 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 technique ($p_{adj} = p * [5 - Rank]$; Hochberg 1988) are shown in boldface.

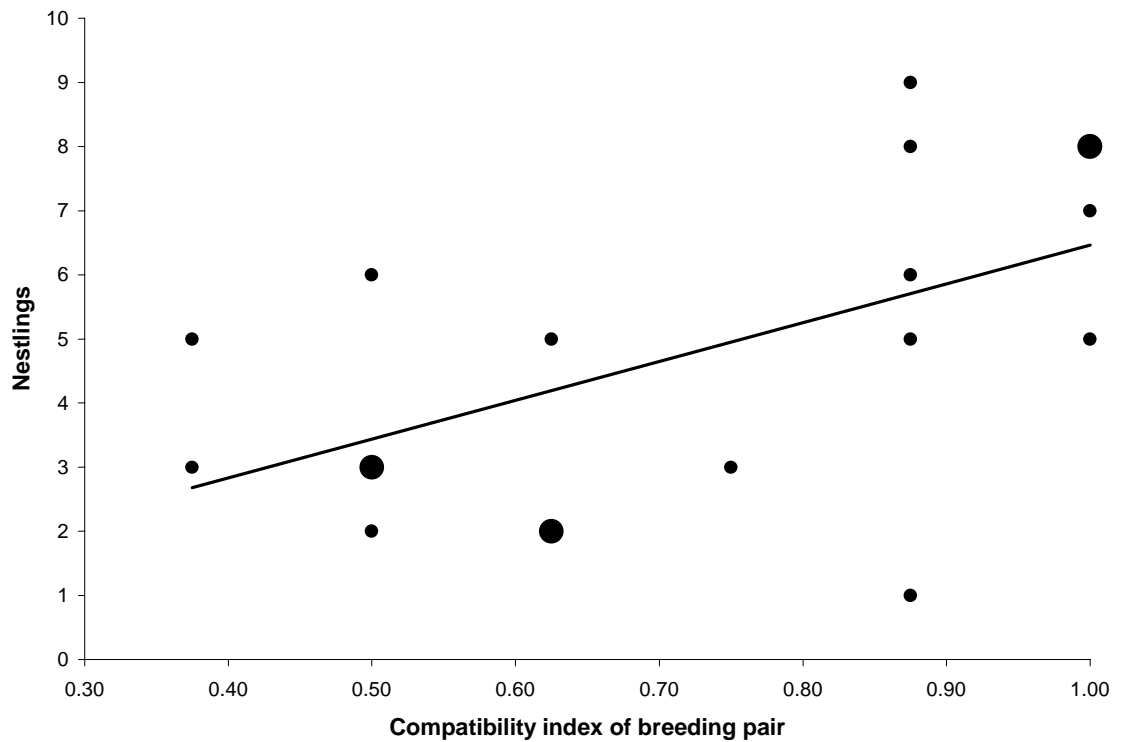


Figure 3.3: Relationship of number of nestlings with compatibility index of a breeding pair of chickadees, and calculated regression line ($r^2 = 0.304$, $df = 18$, $p_{adj} = 0.03$). Compatibility indices of 0 and 1, respectively, indicate breeding pairs that would produce offspring heterozygous or homozygous for species-specific genetic markers. Each larger circle represents two data points.

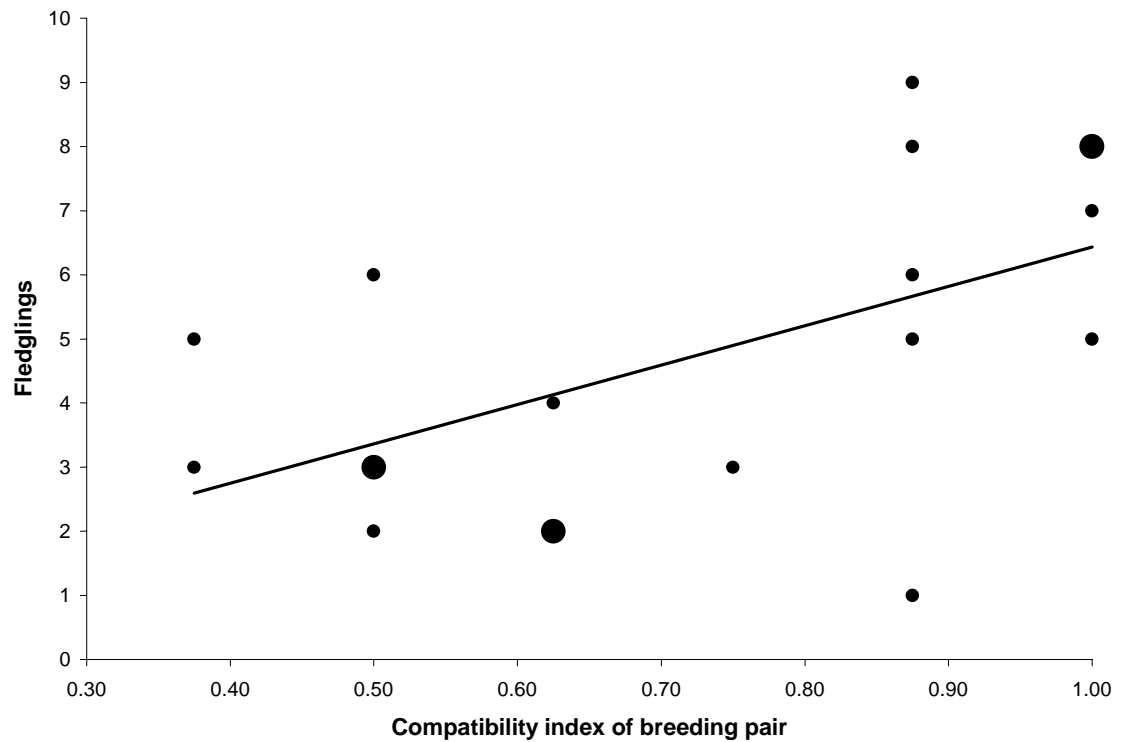


Figure 3.4: Relationship of number of fledglings with compatibility index of a breeding pair of chickadees, and calculated regression line ($r^2 = 0.314$, $df = 18$, $p_{adj} = 0.04$). Compatibility indices of 0 and 1, respectively, indicate breeding pairs that would produce offspring heterozygous or homozygous for species-specific genetic markers. Each larger circle represents two data points.

wide range of GIs within the HY category furnished additional relevant information for statistical analysis and biological interpretation. While F₁ hybrids are often viable and fertile, the F₂ generation and a variety of backcrosses exhibit increased mortality and sterility (i.e., Wu & 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 F₁ 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 disprove the exogenous selection hypothesis.

The endogenous mechanism(s) reducing hybrid fitness operated early in development as shown by the significant reduction in nestling production. Haldane's Rule (Haldane 1922) 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). However, based on the lack of a significant relation between the female's GI and any reproductive variable, Haldane's Rule did not appear to operate in the endogenous selection observed. On the other hand, the nearly significant relationships between male GI and the number of nestlings ($p_{\text{adj}} = 0.081$)

and fledglings ($p_{\text{adj}} = 0.072$) might be an indication that Haldane's Rule was operating in the females but not observable given the power of the analysis (small sample size and small number of diagnostic genetic markers).

The genetically pure pairs (BC or CA) had on average 7.0 fledglings (Fig. 3.4). 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 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 & 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 & 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 3.4, perfectly compatible chickadees ($CI = 1.00$) would produce 1.91 or 2.48 times more fledglings per nesting attempt, respectively, 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 & 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 & 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 controlled 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 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 & Schmidt 1985; Emms & Arnold 1997; Wang et al. 1997; Fritsche & 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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CHAPTER 4

MATE PREFERENCE: A POSSIBLE CAUSAL MECHANISM FOR A MOVING HYBRID ZONE

Revision submitted to the journal *Animal Behaviour* by C. L. Bronson, Thomas C. Grubb, Jr., Gene D. Sattler & Michael J. Braun

ABSTRACT

The study of patterns and underlying mechanisms within hybrid zones may provide insight into speciation. Black-capped (*Poecile atricapilla*; BC) and Carolina (*P. carolinensis*; CA) chickadees hybridize in an east-west band from New Jersey to Kansas. Within the past century, the Ohio portion of this hybrid zone and the CA range to the south have been moving northward while the BC range has retracted. We examined mate preference in females of both species as one possible causal mechanism for this shift. To be conservative about the nomenclature and results, the samples are referred to as either “BC-like” or “CA-like” due to the observed genetic introgression in the study individuals. Given a choice within an aviary setting, in the aggregate, BC-like and CA-like females that had not observed the direct social interactions between a dyad of a BC-like male and a CA-like male preferred to

associate with the BC-like male. In nature, both species form consensual dominance hierarchies. In the aviary, CA-like males dominated BC-like males unless a CA-like male was substantially smaller. Once females of both types had observed the physical interaction of a particular heterospecific dyad, they associated preferentially with the dominant male, regardless of species type. Thus, the effect of CA male intrasexual dominance on female mate preference may be contributing to the northward movement of the hybrid zone.

INTRODUCTION

A focal area in the study of organisms is the nature of genetic cohesion within groups. Hybrid zone dynamics are a fertile area for such research because of the exchange of genes between such distinct groups (Hewitt 1988, Harrison 1990, 1993). In a variety of taxa, all or portions of hybrid zones have been observed to move over time (e. g., Arntzen & Wallis 1991, Hairston et al. 1992, Shaw et al. 1993, Rohwer & Wood 1998, Shapiro 1998). For many hybrid zones, each subsection of a zone has individualized ecological constraints and evolutionary history that greatly impact the observed dynamics in that area (e.g., Bert & Harrison 1988, Hairston et al. 1992, Kallioinen et al. 1995). Correlational evidence indicates that movement in a warbler hybrid zone might result from male aggression and mate choice (Pearson 2000, Pearson & Rohwer 2000), rather than from either naturally occurring habitat transition or anthropogenic modification of the habitat (Pearson & Manuwal 2000).

Similarly, experimental evidence of male territorial behaviour linked with anthropogenic modification of the habitat might explain movement of a magpie hybrid zone (Kallioinen et al. 1995).

Black-capped (*Poecile atricapilla*; BC) and Carolina (*P. carolinensis*; CA) chickadees are known to hybridize in many areas along the common distribution border (e. g., Missouri – Braun & Robbins 1986, Sawaya 1990, Ohio – Grubb et al. 1994, Virginia and West Virginia – Sattler 1996, Sattler & Braun 2000, Pennsylvania – R. L. Curry, pers. com.). While quite similar in appearance, the two species do have distinguishable phenotypes (Pyle 1997).

In Ohio, the BC distribution abuts the north edge of the CA distribution. In the 1880s, CAs were described as permanent residents within the southern portion of the state (Wheaton 1882). Within Ohio in the early 1940s, the east-west oriented hybrid zone was probably located across the middle of the state approximately along the current location of US Interstate 70 (Trautman 1940). Currently, while still in an east-west orientation, the zone is located about 100 km farther north in the state, approximately along US Highway 30 (Grubb et al. 1994, Peterjohn 2001). In other words, the BC distribution has been receding northward.

Two potential factors that may influence the movement of this portion of the chickadee hybrid zone are mate choice and social dominance. Mate choice in a mixed-species context has not been addressed previously in BC and CA. However, studies of mate choice in individual chickadee species have yielded results germane to interspecific mate choice. Both species form linear, consensual dominance

hierarchies in the winter (Hartzler 1970). Within such winter flocks, females have been shown to desert their mate in favor of a mate with a higher rank (Smith 1991, Otter & Ratcliffe 1996). Potential benefits a female could gain from making such a switch are increased winter survival (Ekman 1990, Hogstad 1992, Lemmon et al. 1997) and a larger breeding territory (Smith 1976). Because male dominance appears to be an important causal factor in female mate choice in chickadees, we investigated: 1) whether BC and CA females prefer a conspecific or heterospecific male, 2) whether such females prefer a dominant or subordinate male, and 3) the relative strengths of male species identity and male dominance status as determinants of female preference for a male.

METHODS

Subjects

From January to March of 1998 and 1999, chickadees were captured as pairs using either mist nets or remote-controlled feeder traps (Pierce and Grubb 1979). All birds were subsequently genotyped for five diagnostic marker loci (see below), and the large majority of subjects possessed either mostly BC or CA alleles. However, because of the presence of apparent hybrids in the study (see “Results”), the terms BC-like and CA-like are used for the samples in order to be conservative about species nomenclature. The 10 BC-like pairs were taken from Seneca County, Ohio (41° 4’ N, 82° 53’ W, approximately 25 km north of the center of the hybrid zone).

The 10 CA-like pairs came from Morrow (40° 24' N, 82° 49' W) and Union (40° 14' N, 83° 22' W) Counties, Ohio; both areas are approximately 45 km south of the center of the hybrid zone.

At the time of capture, each bird was banded with a US Fish and Wildlife Service aluminum band for identification. The use of color-bands for individual identification has been shown to influence female mate choice in several avian species (Burley 1986, 1988, Metz & Weatherhead 1991, Johnson et al. 1993). Fortunately, such a potential complication from using color bands was avoidable in this experiment. First, each heterospecific dyad of males employed in the experiment was always in physical isolation from the experimental female in the aviary. Second, within each male dyad, the BC-like male had readily apparent whiter wings (secondary coverts and feather margins) and longer tail; therefore the males were individually identifiable when housed together.

Each bird was weighed at capture 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 tip of bent “elbow” to tip of bent “wrist”) to the nearest 0.1 mm. Sex was determined based on behavioral observation prior to capture (i.e., males dominant to females) and the relative size of the pair (Desrochers 1990, Smith 1991). We could not accurately determine previous reproductive activity, previous mating status, previous mating partner, or age for the experimental individuals.

Each bird was scored on four morphological characters, whiteness of secondary wing coverts, whiteness of secondary feather margins, whiteness of tail

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 a 0 if jagged (BC-like) and 1 if straight (CA-like). As a percentage of the possible total of seven, pure BC and CA phenotypes would yield 0% and 100%, respectively.

For genetic analysis, a 50-75- μ l blood sample from each bird was taken in a heparinized capillary tube from a brachial vein punctured with a 25-gauge needle. 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 for later DNA extraction.

All birds were maintained on ad libitum black oil sunflower seeds, a daily mealworm supplementation and ad libitum water/snow in individual welded-wire cages at the aviary site (40° 24' N, 82° 49' W). To limit the females' experience with stimulus males, the two sexes were housed in different buildings. Within each building, the birds were visually but not acoustically isolated from each other. Both buildings were maintained at ambient temperature and photoperiod.

Genetic Analysis

DNA was extracted from the blood samples at The Ohio State University in the laboratory of Patricia G. Parker. Each blood sample had 200 μ g 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. Then, the remaining aqueous phase of each sample

was 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 using spectrophotometric measures of optical densities at 260 nm and 280 nm. The concentration estimate and integrity (sheared vs. unsheared) 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 ultra-violet light box. All the samples were determined to be unsheared.

Genetic analyses were performed at the Smithsonian Institution's Laboratory of Molecular Systematics following the methods of Sattler & Braun (2000). Five probes were used to identify restriction fragment length patterns diagnostic for CA and BC on Southern blots (Sambrook et al. 1989). First, *EcoR* I digests were probed with a 1200-bp fragment of the domestic chicken (*Gallus g. domesticus*) oncogene *ski* (Li et al. 1986, Sawaya 1990, Sattler & Braun 2000). Second, *Bgl* II digests were probed with a randomly cloned fragment of CA DNA designated RP104 (Sattler 1996). Third, *Ava* II digests were probed with a randomly cloned fragment of CA DNA designated RP7 (Sattler 1996). Fourth, *Pst* I digests were probed with a randomly cloned 4000-bp fragment of tufted titmouse (*Baeolophus bicolor*) DNA designated C7 (Sawaya 1990, Sattler & Braun 2000). Fifth, *Pst* I digests were also probed with house finch (*Carpodacus mexicanus*) mitochondrial DNA (mtDNA) (Mack et al. 1986, Sawaya 1990, Sattler & Braun 2000) purified by subcellular fractionation and CsCl equilibrium-gradient centrifugation (Dowling et al. 1990). The

five resulting genotypes were combined into an index calculated as the percentage of CA restriction fragment length alleles present in an individual. The combination of *Pst* I/mtDNA has only one variant because mtDNA is haploid. The combination of *Pst* I/C7 has only one allele in the females because it is a sex-linked marker and females are the heterogametic sex in birds. Thus for males, the genetic index was based on nine marker alleles – one for the combination of *Pst* I/mtDNA and two for each of the other enzyme and nuclear probe combinations. For females, the genetic index was based on eight alleles – one each for *Pst* I/mtDNA and *Pst* I/C7 and two for each of the other enzyme and probe combinations.

Aviary

The aviary building was located in a meadow and more than 20 m from wooded areas. The animal use portion of the aviary building (response and stimulus sections in Fig. 4.1) had a sand floor, welded-wire and translucent corrugated fiberglass walls, and a welded-wire ceiling open to the sky. The observation chamber had a wooden floor, opaque corrugated fiberglass walls, an insulated ceiling, and was separated from the response section by a “one-way” Plexiglas wall. The animal use portion of the aviary was permanently divided by welded wire into two main areas – the response section and the stimulus section. Within the response section, a floor-to-ceiling wall was present at the mid-line of the aviary extending 1.47 m from the section divider. This wall limited the number of perching locations in the response section from which both stimulus subsections were visible. A permanent wall 1.04 m

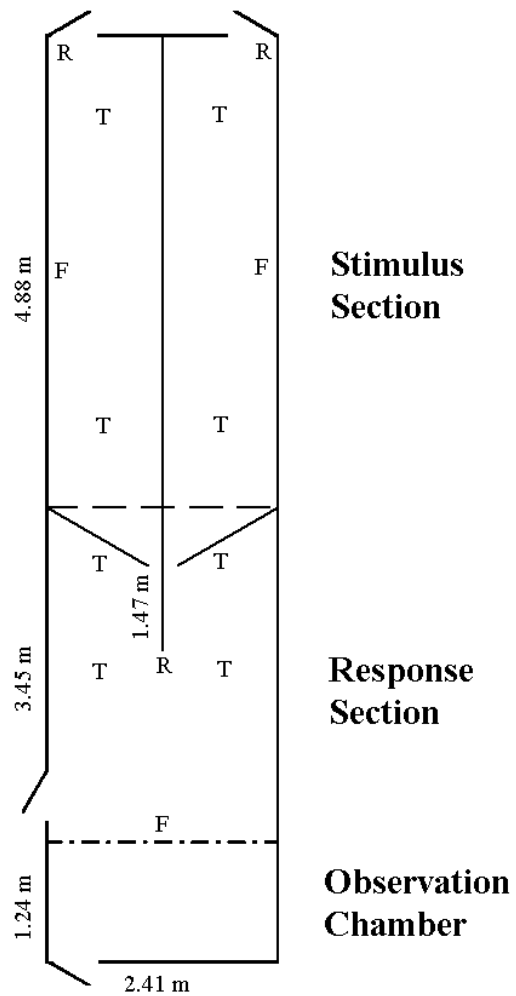


Figure 4.1: An overhead diagram of the aviary building. Ceiling height = 2.05 m, height of the permanent portion of stimulus section divider = 1.04 m, T = artificial tree, F = remote-controlled feeder trap, and R = roosting chamber. Solid lines are corrugated fiberglass, dashed line is welded-wire, and dash-dot line is one-way Plexiglas.

high extended along the full length of the stimulus section midline, while a series of translucent corrugated fiberglass panels were suspended from the ceiling above the permanent wall. The combination of this permanent wall and the removable fiberglass panels above it divided the stimulus section into two visually isolated subsections of equal size. The series of corrugated fiberglass panels could be dropped down into the permanent wall to allow a bird to fly throughout the entire stimulus section.

Translucent corrugated fiberglass doors were used to temporarily allow either visual access (doors open) or no visual access (doors closed) between the response section and stimulus subsections.

The response section and two stimulus subsections each contained artificial trees, a roosting chamber and a remote-controlled feeder trap (Fig. 4.1). Two of the response section's four artificial trees had perches allowing visual access to only the left stimulus subsection. Likewise, two artificial trees in this section had perches with potential visual access to only the right stimulus subsection. Each stimulus subsection had two artificial trees. Each artificial tree consisted of a 3x3x185-cm piece of wood suspended from the welded-wire ceiling by a hook screwed into one end. Each artificial tree contained 12 22x1-cm dowel-rod perches, three on each face.

Each of the three roosting chambers had a sawdust floor and was fashioned from an artificial nesting snag (Grubb & Bronson 1990). In the response section, the roosting chamber was attached to the mid-line wall near the ceiling. The opening of the roosting chamber was oriented towards the observation chamber. In each stimulus subsection, the roosting chamber was positioned farthest from the response section in

the corner of the two outside walls. The openings of these roosting chambers were oriented towards the response section and observation chamber. The remote-controlled feeder trap in the response section was attached midway along the wall between the response section and the observation chamber with its opening oriented towards the response section. The remote-controlled feeder trap in each of the two stimulus subsections was attached midway along the outside wall with its opening oriented towards the wall subdividing the stimulus section.

While in the aviary, birds received ad libitum black oil sunflower seeds, daily mealworm supplementation, and ad libitum water/snow. The aviary's sections were not acoustically isolated from either each other or the surrounding habitat.

Experimental Procedure

The ideal design for a mate-preference experiment would expose each female to an unique heterospecific dyad of males. However, such a design would require twice as many males as females. The availability of birds and the use of these birds in a subsequent experiment requiring male-female pairs constrained this study to use equal numbers of males and females. Therefore, each dyad of males consisting of one BC-like male and one CA-like male was used as the stimulus for one BC-like female and one CA-like female. During the course of the presentation of a dyad of males to the first female, the males were allowed to physically interact and establish a dominance hierarchy. Therefore, when the dyad of males was presented to the second female, they could potentially have conveyed their dominance status to her (e.g. via vocalizations, pheromones) even when the dyad of males were both physically and

visually isolated from each other. To limit any bias from this potential information transfer, each dyad of males was presented in random order to the two females. No female was presented with a dyad of males containing a conspecific male captured at the same location where she was captured.

Five days (plus an initial day of acclimation) were required to collect data for each female (Table 4.1). Ten females of each species were used. A data point consisted of the female's presence on the side of the response section near one side of the stimulus section. The mid-line location of the wall in the response section was used for determining a female's position. The location of the artificial trees for perching and the Plexiglas wall (no perching opportunity) separating the observation chamber and response section minimized the number of entries into a "neither" category. The "neither" category consisted of midline activities (perching on the feeder or roosting chamber, or feeding at the feeder). A data collection session consisted of 30 data points collected 30 s apart (instantaneous sampling; Lehner 1979) beginning approximately 90 min after sunrise and at least 30 min after the observer had entered the observation chamber.

The female was not provided with a refuge area where she could go and not participate in the experiment. As a result, it is possible that some of her time spent in association with a male should not be attributed to her attempting to associate with that male. The area of the aviary is not much larger than the recommended distance for calculating association indices in nature (Smith 1991). Unless the female systematically changed the area she preferred in accordance with the treatments, both

Component	Day	Dyad of males present in stimulus subsections	Female could view the stimulus subsections	Female had observed the dominance relationship of the dyad of males
Control 1	1	No	No	No
Control 2	2	No	Yes	No
Control 3	3	Yes	No	No
Treatment 1	4	Yes	Yes	No
Treatment 2	5	Yes	Yes	Yes

Table 4.1: Daily components of the mate-preference trials of female chickadees.

Translucent fiberglass doors were opened or closed to control visual access between the response section and the stimulus subsections.

the categorization of a location preference based on statistical significance (vs. a majority) and the use of numerous controls should have limited likelihood that females were not associating with particular males. Additionally, due to their identical nature with the data presented here, the results of two later 15-min sessions (separated by 45 min) each day per female were not included. Thus, it is unlikely that a female might have systematically changed her area preference with treatment (even across three observational periods) based on a pattern of “loafing” rather than associating with a male.

The afternoon before the first day of data collection, a female was released into the response section with the doors to the stimulus subsections closed. This procedure allowed the female to acclimate to the response section of the aviary without visual access to the stimulus subsections and without a dyad of males present. The first day of data collection, Control 1, served two purposes. First, it was used to determine if a female had a positional bias in the response section when she was without visual access to the stimulus subsections and without a dyad of males present. Second, it was used as a within-female control (see “Data Analysis”) for a female’s responses to a dyad of males in the stimulus subsections when she could not see them. After Control 1 had been completed, the doors to the stimulus sections were opened so the female would acclimate to the response section of the aviary with visual access to the stimulus subsections without a dyad of males present. The second day of data collection, Control 2, served three purposes. First, it was used to determine if a female had a positional bias in the response section when she had visual access to the

stimulus subsections without a dyad of males present. Second, it was used as a within-female control (see “Data Analysis”) for when she could see a dyad of males in the stimulus subsections but had not yet observed the dyad physically interact (i.e., dominance unobserved). Third, it was used as a within-female control for when she could see a dyad of males after having observed them physically interact (i.e., dominance observed).

After Control 2 had been completed, the doors to the stimulus subsections were closed and each member of a dyad of males was released within seconds into a different, randomly determined, stimulus subsection. The female was allowed to acclimate to the response section of the aviary without visual access to the stimulus subsections and with a dyad of males present. Also, each male of the dyad was allowed to acclimate to its stimulus subsection without visual access to the response section or to the other stimulus subsection. The third day of data collection, Control 3, determined if a female had a preference for one of the two males of a dyad based on non-visual characteristics. This control was included because a variety of factors (i. e., vocalizations, pheromones) could not be eliminated from the experimental design without greatly altering the aviary (i.e., anechoic sections, independent air systems for each section) and/or the males (i.e., surgically preventing vocalizations).

After Control 3 had been completed, the doors to the stimulus subsections were opened, allowing the female to acclimate to the response section of the aviary with visual access to the stimulus subsections and with a dyad of males present. Also, each male of the dyad could acclimate to its stimulus subsection with visual access to

the response section and the female, but without visual access to the adjoining stimulus subsection or the male within it. The fourth day of data collection, Treatment 1, served two purposes. First, it was used to determine if a female had a preference for one of the two males of a dyad without having observed the males physically interact. Second, it was used in determining if a female switched her preference after observing the dominance relationship between the two males of a dyad.

After Treatment 1 had been completed, the fiberglass panels suspended between the two stimulus subsections were lowered into the permanent, dividing wall. Because the two males had precedence on one half of the aviary and were similarly new to the respective other half, the seniority effect (Glase 1973, Hogstad 1987) should have been minimized. From the observation chamber, the dyad of males was continuously monitored until their dominance relationship had been determined. One male was scored as dominant each time he chased the other one, supplanted it from a perch, or caused it to wait before entering a feeder. When 10 consecutive interactions favored one male, he was considered the dominant of the dyad, a decision typically reached within 30 min. However, the dyad of males was still allowed to interact for two hours, all the while in view of the female in the response section. Since each dyad of males was presented to two females, dominance records were collected twice for each dyad. Male dominance was used as a factor in several analyses (see “Data Analysis”).

At the completion of their interaction, the two males, without being handled, were confined into their original subsections and the dividing fiberglass panels were suspended from the ceiling. The female was allowed to acclimate to the response section of the aviary with visual access to the stimulus subsections and with a dyad of males present whose dominance relationship she had had a chance to observe. Also, each male of the dyad was allowed to acclimate to its stimulus subsection with visual access to the response section and the female, but without visual access to the other stimulus subsection. The fifth day of data collection, Treatment 2, served two purposes. First, it was used to determine if a female had a preference for one of the two males of a dyad based on dominance criteria. Second, it was used to determine if a female switched her preference after observing the dominance relationship within a dyad of males.

There were two irregularities in the data collection. First, for one CA-like female, the data for the Control 3 could not be collected. A storm during the prior night had blown open the doors to the stimulus subsections, so the female had been exposed prematurely to visual contact with the two stimulus subsections. Second, for another female, on the morning the Control 2 data were to be taken, flooding made the aviary inaccessible. For that female, Control 2 records were taken the following day, as the extra time allowed to acclimate was thought not pivotal.

General data analysis

Data analysis occurred in a series of nested stages. In stage one, a within-bird analysis tested each female for her location preference. This location testing was done in two ways depending on the question of interest; both ways employed the large sample approximation (i.e. normal theory approximation) of the two-tailed binomial distribution ($n = \text{observations}$; Hollander and Wolfe 1973) with a 5% criterion level. For the hypothesis testing concerning whether a female had a location bias in the absence of a dyad of males, the comparison frequency for the binomial was 0.5. For the hypothesis testing involving whether a female had a location bias with a dyad of males present, the comparison frequency was the location frequency of the female from the appropriate control. The changed comparison frequency for the latter set of hypothesis testing most conservatively standardized for any within-bird bias for a side of the response chamber. If a female's location preference differed at all from the random expectation of 15 observations on each side (i. e., a location frequency of 0.5), that deviation, regardless of size (i. e., a statistically significant deviation not required), would be employed in the subsequent determinations.

In stage two analysis, the within-bird results (e. g., preference or no preference) were combined for each of the hypothesis testing situations (i. e., treatments) mentioned above and detailed below. Because the data for this analysis stage are only "preference" or "no preference" for each of the females, these across-treatment analyses consider all "preferences" equally so females with underlying opposite preferences (addressed in stage three analysis) do not "cancel each other

out.” The one-tailed binomial distribution ($n = \text{females}$) was employed for the across-treatment (i. e., all of the females under a given treatment) analyses. Three separate analyses were then performed – for the CA-like females, for the BC-like females, and for all females combined.

In stage three analysis, subsets of the stage two analyses were used to address further hypothesis testing. The two-tailed binomial distribution ($n = \text{females}$) was employed for these additional across-treatment analyses, except one hypothesis test that had an a priori directionality. Because all of these stage three analyses focused only on females that had previously demonstrated a preference for one male or the other, they all had sample sizes less than 10 and 20, respectively, for each species separately and for the two species combined.

Finally, two tests assessed male dominance interactions. First, the two-tailed binomial distribution ($n = \text{dyads}$) was employed to determine if dominance within a dyad was related to species. Second, the Mann-Whitney U test ($N_1 = \text{number of dyads of males with BC-like male dominant}$, $N_2 = \text{number of dyads of males with CA-like male dominant}$) was employed to determine if dominance within a dyad was related to the ratio of male weights.

Hypothesis testing

Based on Control 1, individual females (stage one analysis) were tested to determine if they had a left-right position preference without visual access to the stimulus subsections when no dyad of males was present. The null hypothesis was

that a female would not prefer either the left or right side (i. e., would not differ from 15 observations per side of the aviary). The alternative hypotheses were that a female would prefer either the left or right side (i. e., would differ from 15 observations per side of the aviary). The results from all the females (no preference or preference) were combined first into the two species categories (10 BC or 10 CA) and then as one inclusive group (all 20). These pooled data (stage two analysis) were tested to determine if females (either BC, CA or all) had a left-right position preference without visual access to the stimulus subsections when no dyad of males was present. The null hypothesis was that females would not prefer either the left or right side (i. e., would not differ from five or 10 females preferring a side of the aviary and five or 10 females not preferring a side of the aviary). The alternative hypothesis was that females would prefer a side of the aviary (i. e., would have significantly more than five or 10 females preferring a side of the aviary).

Based on Control 2, individual females (stage one analysis) were tested to determine if they had a left-right position preference with visual access to the stimulus subsections when no dyad of males was present. The null hypothesis was that a female would not prefer either the left or right side (i. e., would not differ from 15 observations per side of the aviary). The alternative hypotheses were that a female would prefer either the left or right side (i. e., would differ from 15 observations per side of the aviary). The results from all the females (no preference or preference) were combined first into the two species categories (10 BC or 10 CA) and then as one inclusive group (all 20). These pooled data (stage two analysis) were tested to

determine if females (either BC, CA or all) had a left-right position preference with visual access to the stimulus subsections when no dyad of males was present. The null hypothesis was that females would not prefer either the left or right side (i. e., would not differ from five or 10 females preferring a side of the aviary and five or 10 females not preferring a side of the aviary). The alternative hypothesis was that females would prefer a side of the aviary (i. e., would have significantly more than five or 10 females preferring a side of the aviary).

Based on Control 3 against Control 1, individual females (stage one analysis) were tested to determine if they had a left-right position preference without visual access to the stimulus subsections when a dyad of males was present. The null hypothesis was that a female would not prefer either the left or right side (i. e., would not differ from 15 observations per side of the aviary after adjusting against her position in Control 1). The alternative hypotheses were that a female would prefer either the left or right side (i. e., would differ from 15 observations per side of the aviary after adjusting against her position in Control 1). The results from all the females (no preference or preference) were combined first into the two species categories (10 BC or 9 CA) and then as one inclusive group (all 19). These pooled data (stage two analysis) were tested to determine if females (either BC, CA or all) had a left-right position preference without visual access to the stimulus subsections when a dyad of males was present. The null hypothesis was that females would not prefer either the left or right side (i. e., would not differ from five or 10 females preferring a side of the aviary and five or 10 females not preferring a side of the

aviary). The alternative hypotheses were that females would prefer a side of the aviary (i. e., would have significantly more than five or 10 females preferring a side of the aviary).

From the pooled data, the females that showed a preference were separated into a group (stage three analysis). First, this group was tested to determine if females (either BC, CA or all) had a species-based preference without visual access to the stimulus subsections when a dyad of males was present. The null hypothesis was that females would not prefer either species category (i. e., would not differ from fifty percent of the females preferring either species). The alternative hypotheses were that females would prefer one of the species categories (i. e., would have significantly more than 50 percent of the females preferring either the BC-like or CA-like male). Second, this group was tested to determine if females (either BC, CA or all) had a dominance-based preference without visual access to the stimulus subsections when a dyad of males was present. The null hypothesis was that females would not prefer either dominance type (i. e., would not differ from 50 percent of the females preferring either dominance type). The alternative hypotheses were that females would prefer one of the dominance types (i. e., would have significantly more than 50 percent of the females preferring either the dominant or subordinate male).

Based on Treatment 1 against Control 2, individual females (stage one analysis) were tested to determine if they had a preference for one male of the dyad without having observed them physically interact. The null hypothesis was that a female would not prefer either male of the dyad (i. e., would not differ from 15

observations per side of the aviary after adjusting against her position in Control 2). The alternative hypotheses were that a female would prefer either one of the males (i. e., would differ from 15 observations per side of the aviary after adjusting against her position in Control 2). The results from all the females (no preference or preference) were combined first into the two species categories (10 BC or 10 CA) and then as one inclusive group (all 20). These pooled data (stage two analysis) were tested to determine if females (either BC, CA or all) had a preference for one male of the dyad without having observed them physically interact. The null hypothesis was that females would not prefer either male of the dyad (i. e., would not differ from 50 percent of the females having and not having a preference for a male). The alternative hypotheses were that a female would prefer either one of the males (i. e., would have significantly more than 50 percent of the females having a preference for one males in the dyad). From the pooled data, the females that showed a preference were separated into a group (stage three analysis) and tested to determine if females (either BC, CA or all) had a species-based preference without having observed the dyad of males physically interact. The null hypothesis was that females would not prefer either species category (i. e., would not differ from 50 percent of the females preferring either species). The alternative hypotheses were that females would prefer one of the species categories (i. e., would have significantly more than 50 percent of the females preferring either the BC-like or CA-like male).

Based on Treatment 2 against Control 2, individual females (stage one analysis) were tested to determine if they had a preference for one male of the dyad

after having observed them physically interact. The null hypothesis was that a female would not prefer either male of the dyad (i. e., would not differ from 15 observations per side of the aviary after adjusting against her position in Control 2). The alternative hypotheses were that a female would prefer either one of the males (i. e., would differ from 15 observations per side of the aviary after adjusting against her position in Control 2). The results from all the females with a preference (preferring dominant or preferring subordinate) were combined first into the two species categories (BC or CA) and then as one inclusive group (all). These pooled data (stage two analysis) were used to determine if females (either BC, CA or all) had a preference for the socially dominant male of the dyad. The null hypothesis was that females would not prefer the socially dominant male of the dyad (i. e., would not differ from 50 percent of the females preferring the socially dominant male and preferring the socially subordinate male). The alternative hypothesis was that females would prefer the socially dominant male of the dyad (i. e., would have significantly more than 50 percent of the females having a preference for the socially dominant male of the dyad).

Based on Treatment 2 against Treatment 1, individual females (stage one analysis) were tested to determine if they switched their preference for one male of the dyad after having observed them physically interact. The null hypothesis was that a female would not switch her preference for a male of the dyad (i. e., would not differ from 15 observations per side of the aviary after adjusting against her position in Treatment 1). The alternative hypotheses were that a female would switch her

preference for one of the males (i. e., would differ from 15 observations per side of the aviary after adjusting against her position in Treatment 1). The results from all the females with a preference (switching or not switching) were combined first into the two species categories (BC or CA) and then as one inclusive group (all). These pooled data (stage two analysis) were used to determine if females (either BC, CA or all) switched their preference after observing the males physically interact. The null hypothesis was that females would not switch their preference (i. e., would not differ from 50 percent of the females switching and not switching). The alternative hypothesis was that females would switch their preference after observing the dyad of males interact (i. e., would have significantly more than 50 percent of the females switching their preference). From the pooled data, the females that switched their preference were separated into a group (stage three analysis) and tested to determine if females (either BC, CA or all) switched to the socially dominant male after observing the dyad of males physically interact. The null hypothesis was that females would not switch their preference to the socially dominant male (i. e., would not differ from 50 percent of the females switching to the socially dominant male). The alternative hypothesis was that females would switch their preference to the socially dominant male (i. e., would have significantly more than 50 percent of the females switching their preference to the socially dominant male).

Additionally, the pooled female data (maintained/switched preference for socially dominant or maintained/switched preference for socially subordinate) from Treatment 2 against Treatment 1, were used to determine if females maintained or

switched their preference to the dominant male. The null hypothesis was that females would not preferentially maintain or switch their preference based on social dominance (i. e., would not differ from 50 percent of the females maintaining or switching their preference based on social dominance). The alternative hypothesis was that females would maintain or switch their preference based on social dominance (i. e., would have significantly more than 50 percent of the females switching their preference based on social dominance).

Ethical statement

This project was performed under The Ohio State University's ILACUC protocol 97A016. The birds were banded and bled under federal banding permit 20653 and Ohio banding and collecting permit 509, and were held for longer than 24 h under federal collecting permit MB673317-0.

None of the birds was held captive for longer than a month. The physical interactions within each dyad of males never resulted in physical contact. The most extreme interaction was one sustained chase of approximately 1 min. All birds were released into woodlots at the end of their participation in the experiment, and many were resighted or recaptured during a subsequent experiment.

RESULTS

We attempted to obtain genetically pure BC and CA individuals. Based on their plumage scores, the large majority (36 of 40) of the birds employed were pure BC or CA (Fig. 4.2). Only one BC-like individual did not exhibit a pure morphological phenotype, its secondary wing coverts being tan instead of white. Only three of the CA-like individuals did not exhibit a pure morphological phenotype, all had tan secondary feather margins instead of gray.

Contrary to the morphological index, post-experiment genetic analysis indicated that the majority of individuals (26 of 40) exhibited some level of introgression (Fig. 4.2). Of the BC-like individuals, four females and five males had all the BC alleles at the five marker loci (0%), two females and four males had all but one BC variant, three females and one male had all but two BC alleles, and one female had all but four BC alleles. Of the CA-like individuals, three females and two males had all the CA alleles (100%), three females and four males had all but one CA variant, two females and three males had all but two CA alleles, two females had all but three CA alleles, and one male had all but four CA alleles. Nonetheless, the distribution of the genetic species index was clearly bimodal (Fig. 4.2).

Since the observation session occurred approximately 20 hours after the birds were initially exposed to each treatment, only typical flock behaviour was seen in all three individuals (i. e., contact calls). We employed the extended period of acclimation both to eliminate time of day from confounding the differences between

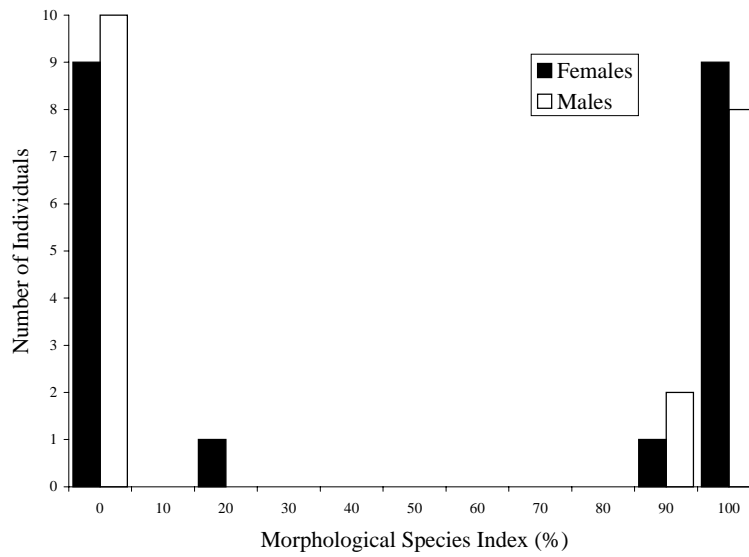
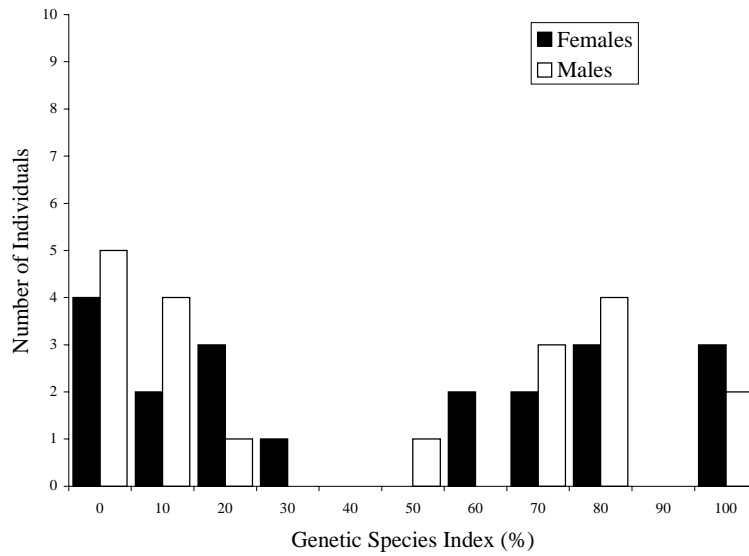


Figure 4.2: Morphological (top) and genetic indices (bottom) of male and female chickadees used in a female mate-preference experiment. For both indices, pure BC and pure CA would have index values of 0% and 100%, respectively.

components (controls, treatments) and to lower the likelihood of overt interactions still occurring during the observation time. In stage two analysis, neither BC-like (binomial test: $P = 0.62$), CA-like (binomial test: $P = 0.17$), nor the aggregate (binomial test: $P = 0.25$) of females exhibited a left-right position preference when they had no visual access to the stimulus subsections and when a dyad of males was absent. Individually (stage one analysis), three BC-like females significantly preferred the right side of the response section, two significantly preferred the left side, and five had no significant preference. Individually (stage one analysis), four CA-like females significantly preferred the right side of the response section, three significantly preferred the left side, and three had no significant preference.

In stage two analysis, neither BC-like (binomial test: $P = 0.62$), CA-like (binomial test: $P = 0.17$), nor the aggregate (binomial test: $P = 0.41$) of females exhibited a left-right position preference when they had visual access to the stimulus subsections and when a dyad of males was absent. Individually (stage one analysis), four BC-like females significantly preferred the right side of the response section, one significantly preferred the left side, and five had no significant preference. Individually (stage one analysis), six CA-like females significantly preferred the right side of the response section, one significantly preferred the left side and three had no significant preference.

In stage two analysis, BC-like and CA-like females both tended to prefer one or the other male of a dyad based on non-visual characteristics of the males. BC-like females (nine of 10; binomial test: $P = 0.01$) significantly preferred one male of a

dyad based on non-visual characteristics. While having a similar trend, CA-like females (seven of nine; binomial test: $P = 0.09$) did not exhibit a significant preference for either male of a dyad. When both species were combined, females (16 of 19; binomial test: $P < 0.01$) significantly preferred one male of a dyad based on non-visual characteristics.

Because of the significant preference for one male of a dyad based on non-visual characteristics, preferences related to the males' species and relative dominance were further tested. In stage three analysis, neither BC-like (binomial test: $P = 0.51$), CA-like (binomial test: $P = 0.73$) nor the aggregate (binomial test: $P = 0.80$) of females that significantly preferred one male of a dyad based on non-visual characteristics exhibited a species preference. Individually (stage one analysis), three BC-like females significantly preferred the BC-like male and six significantly preferred the CA-like male. Individually (stage one analysis) four CA-like females significantly preferred the BC-like male and three significantly preferred the CA-like male.

Similarly and also in stage three analysis, neither BC-like (binomial test: $P = 0.75$), CA-like (binomial test: $P = 0.07$) nor the aggregate (binomial test: $P = 0.22$) of females that significantly preferred one male of a dyad based on non-visual characteristics exhibited a social dominance preference prior to observing the males physically interact. Individually (stage one analysis), five BC-like females significantly preferred the socially dominant male and four significantly preferred the

socially subordinate male. Individually (stage one analysis), six CA-like females significantly preferred the socially dominant male and one significantly preferred the socially subordinate male.

In stage two analysis, once in visual contact with the males, both (eight of 10 for each based on stage one analysis) BC-like (binomial test: $P = 0.05$) and CA-like (binomial test: $P = 0.05$) females exhibited a significant preference for one male of a dyad without having observed the males physically interact. Because of the significant preferences for one male or the other, preference related to the males' species was further tested. Neither the BC-like (binomial test: $P = 0.29$) females nor the CA-like (binomial test: $P = 0.07$) females that significantly preferred one male of a dyad significantly preferred one species, although six of eight and seven of eight, respectively, did prefer the BC-like male. But females of both species combined (13 of 16; binomial test: $P = 0.02$) significantly preferred the BC-like male without having observed the males physically interact.

In stage two analysis, both BC-like (binomial test: $P = 0.04$) and CA-like (binomial test: $P = 0.04$) females exhibited a significant preference for the socially dominant male of a dyad after observing the males interact. In stage one analysis, for both BC-like and CA-like females, eight significantly preferred the dominant male, one significantly preferred the subordinate male, and one had no significant preference.

In stage two analysis, neither BC-like (binomial test: $P = 0.50$), CA-like (binomial test: $P = 0.25$), nor the aggregate (binomial test: $P = 0.24$) of females

exhibited a significant tendency to switch their preference after observing the males of a dyad interact socially. Individually (stage one analysis), five BC-like females significantly changed their preference for a male, four significantly maintained their preference for a male, and one had no significant preference. Individually (stage one analysis), six CA-like females significantly changed their preference for a male, three significantly maintained their preference for a male, and one had no significant preference.

In stage three analysis, of the females that switched their preference after watching the dyad of males physically interact, both BC-like (binomial test: $P = 0.03$) and CA-like (binomial test: $P = 0.11$) females tended to switch from the subordinate to the dominant male. Individually (stage one analysis), all five BC-like females that switched their preference changed to the dominant male. Individually (stage one analysis), five of the six CA-like females that switched their preference for a male changed to the dominant male. In the aggregate (binomial test: $P = 0.01$), females of the two species significantly switched their preference to the dominant male after observing the males physically interact.

In stage two analysis, both BC-like (binomial test: $P = 0.02$) and CA-like (binomial test: $P = 0.10$) females tended to maintain or switch their preference to the dominant male of a dyad. The BC-like females and the aggregate (binomial test: $P = 0.001$) were significant. Individually (stage one analysis), nine of 10 BC-like females maintained or switched their preference to the dominant male, while one female maintained or switched her preference to the subordinate male. Individually (stage

one analysis), eight of 10 CA-like females maintained or switched their preference to the dominant male, while two females maintained or switched their preference to the subordinate male. In the aggregate, 17 of 20 females of the two species maintained or switched their preference to the dominant male while three females maintained or switched their preference to the subordinate male.

Across the two observational periods of physical interaction of the dyad of males, the dominance relationship never changed. The CA-like male was dominant in seven of the 10 dyads (binomial test: $P = 0.17$; Fig. 4.3). In the three dyads where the BC-like male was dominant, the CA-like male was noticeably smaller. For the seven pairs in which the CA-like male was socially dominant, the average within-dyad ratio of CA-like to BC-like body mass ($AVE \pm SE = 93.16 \pm 7.44\%$) was greater than that for the three dyads in which the BC-like male was socially dominant ($AVE \pm SE = 85.27 \pm 0.86\%$) (Fig. 4.3). The difference between the two ratios closely approached significance (Mann-Whitney U test: $U = 3$, $N_1 = 3$, $N_2 = 7$, $P = 0.11$).

DISCUSSION

The post-experiment genetic identification of some individuals as impure BC or CA was not unexpected. Sattler and Braun (2000) found that genetic introgression exceeds morphological introgression in Appalachian transects of this hybrid zone. Also, the northward progression of the Ohio portion of the hybrid zone into the BC range has potentially left BC alleles in the wake of the movement. The greater

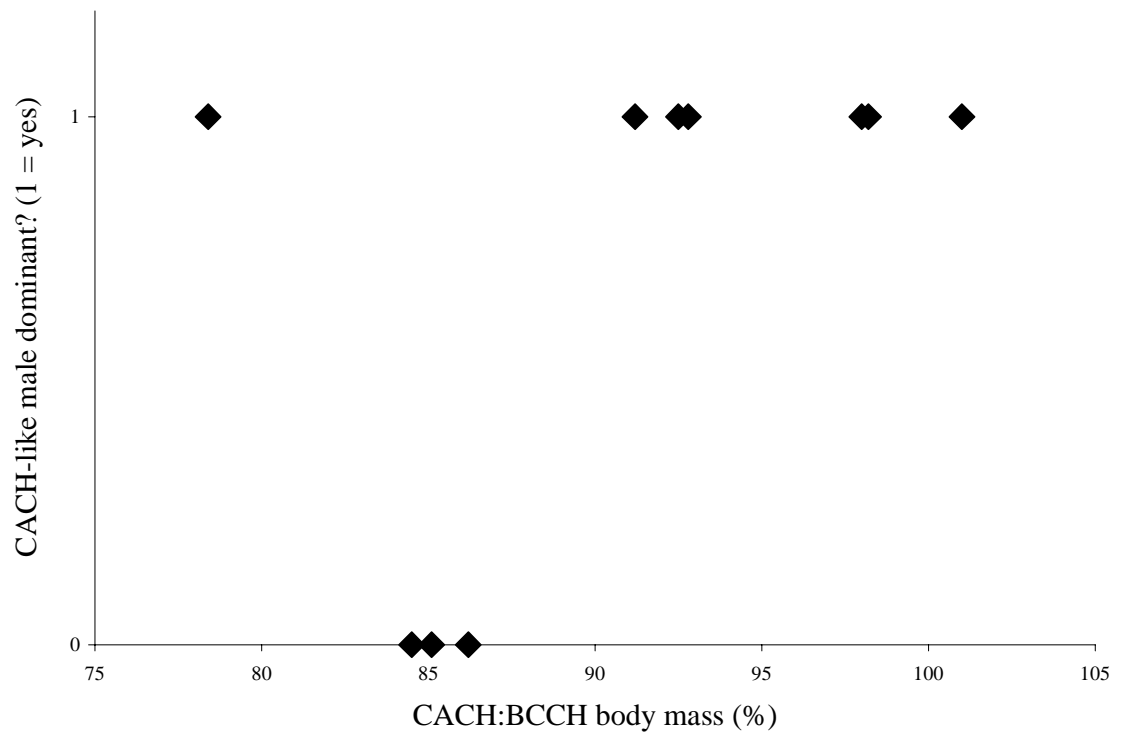


Figure 4.3: Social dominance of CA-like males over BC-like males in relation to the ratio of body masses.

proportion of pure BC individuals (nine of 20) than pure CA individuals (five of 20) could support the hypothesis that the BC alleles within the CA individuals are the residual remnants of a previously more southern BC distribution (e.g., Shaw et al. 1993). Additionally, a wide genetic range of individuals exists within the Ohio hybrid zone (see Chapter 2). This diversity of individuals could indicate widespread interbreeding between the two species and might increase the likelihood of foreign alleles spreading farther from the center of the hybrid zone. Regardless of the cause, the limited genetic impurity in these experimental individuals would, if anything, bias against observing species-driven significant results. The two species samples were not as distinctly different genetically as morphological identification indicated. For a behavioural difference between these two samples to be detected, the corresponding difference between two genetically pure samples would have to have been greater. Therefore, the results presented are conservative.

Sex, size, age and precedence are known to affect the dominance of a dyad of chickadee individuals (Smith 1991). In our protocol, either these factors were not relevant or we attempted to control for them. First, since it was the same within a dyad of males, sex likely did not influence the relationship. Second, our analysis shows that relative size is a contributing factor to the dominance relationship. We tried to use males of similar size. Finally, we contend that precedence links the age and seniority effects. Typically, old birds dominate younger birds, especially in males (Smith 1991); however, older birds have also typically been established longer in a flock, hence also have seniority. The studies of seniority effects across Paridae tend to

involve juveniles and show that in individuals of similar age, the first to arrive ranks higher (BC – Glase 1973; marsh tits (*Parus palustris*) – Nilsson & Smith 1985, 1988, Nilsson 1989a, 1989b; tufted titmice – Brawn & Samson 1983; willow tits – Hogstad 1987). Thus, in studies where age was controlled, seniority was the principal factor determining dominance status. This experiment carefully controlled for seniority and in doing so, we contend, controlled for any potential effects of differences in the ages of the males.

Reproductive history can influence a female's mate choice. For instance, infrequently an older female can remain with her subordinate mate, allowing a younger female to pair with the alpha male (Glase 1973, Ficken et al. 1990, Smith 1991). While we were not able to determine the reproductive history of the experimental females, we believe the protocol limited any potential influence of such history. Since a female had been removed from contact with all previously known males, her mate was effectively dead. She was introduced to a new "flock." Because chickadees form pairs in their winter flocks (Ficken et al. 1981, Smith 1991), she would presumably need to reacquire a mate. Thus, it seems reasonable to assume that, regardless of their previous reproductive history, all the females in our experiment should have reacted similarly.

The behaviour of females of both chickadee species supported biased mate choice as a potential mechanism for the movement of the Ohio portion of the hybrid zone. In the aggregate, before observing the dominance interaction of the males, females preferred BC-like males. This preference, if it were the sole factor, would

tend to cause the hybrid zone to move toward the CA range, opposite to the observed direction. Unfortunately, the design of this experiment did not allow for specifically attributing the preference to any BC-like morphological phenotype independent of body size.

In the aggregate, both BC-like and CA-like females preferred the dominant male to the subordinate. Females could gain several benefits from associating with a higher-dominance mate. First, a high-dominance pair has a better chance of obtaining a larger (Smith 1976) and/or higher-quality (Smith 1991, Otter et al. 1999) breeding territory. Second, the dominance status of a female's pair-bond male influences her winter survival more than does her own dominance level (Ekman 1990, Hogstad 1992, Lemmon et al. 1997).

Aside from obtaining a high-dominance mate, female preference for dominant males also influences choice of extra-pair mating partners (Otter et al. 1998). BC females actively seek higher ranked males than their own mate for extra-pair copulations (EPCs) (Smith 1988). They also choose nest-sites close to territories of high-ranking males, thus, increasing their chances of an EPC with a high-rank male (Ramsay et al. 1999).

Based on the relatively quick identification of dominance by a human observer (under 30 min for all dyads), we were not able to develop a measure for potential differences in the relative dominance between different dyads. For instance, one particular heterospecific dyad might have been between sequentially ranked males if placed within a larger group of males while another heterospecific dyad

might have had a couple of individuals between them in a larger group. Based on the similar and short time frame it took to categorize the dominant, we assume that all the dyads were of relatively equal asymmetry. However, if females do have a requisite threshold for dominance to be important, our results are conservative. For example, in closely “ranked” dyads, females may not change their preference to the dominant male from the subordinate. Within this experiment, such females would be categorized as “not switching to the dominant male” and the likelihood of detecting a significant relationship between dominance and switching would be decreased.

The dominance observed in the aviary is likely to also exist in a natural setting since aviary dominance interactions have been shown to be behaviourally similar to those at feeders in BC (Lempriere 1990). Additionally, dominance hierarchies observed at feeders (two present in the stimulus section of the aviary) and away from them in nature are identical (Smith 1976).

In nature, both species form consensual dominance hierarchies. While studies in BC have not shown a relationship between weight and rank (Glase 1973, Smith 1976), neither of the studies controlled for known variation in weight (e. g. season, time of day). In willow tits, when daily weight fluctuations were limited, weight explained 77% of variance in rank (Hogstad 1987). In the aviary, the tendency for CA-like males to dominate similar-size BC-like males could lead to a preference in females of both species for CA-like males. Across a north-south transect within the distribution of either parental species, both species display a positive relationship between body size and latitude (Lunk 1952, Brewer 1961). Within areas where their

distributions abut, these size clines tend to overlap and the species are generally of similar size (Lunk 1952, Brewer 1961). Assuming that any CA-like male likely to be encountered by a BC-like female in the hybrid zone would be about as large as a BC-like male, the observed preference for the dominant male would cause a movement of the hybrid zone in a northward direction, as observed.

Female preferences for BC-like males, on the one hand, and dominant males, on the other hand, could potentially negate each other. The design of the experiment allowed the relative strengths of these two preferences to be assessed. Based on existing knowledge of conspecific mate choice in chickadees, social dominance was assumed a priori as the more important factor and was presented second. The females did maintain or switch from their initial preference to the dominant male, indicating that conspecific dominance status was more influential than any species-specific male attributes.

Either of two mechanisms could have been responsible for females maintaining or switching their preference to the dominant male after observing the two males interact. Females of both species might have preferred the BC morphological phenotype in the absence of other cues. Alternatively, in the absence of directly observing males interact, the females might have used a surrogate cue (e. g. body size) as an indicator of dominance. In nine of 10 male dyads, the BC was larger than the CA.

Near and within the hybrid zone, the two species and their hybrids are of similar size (Lunk 1952, Brewer 1961) and the two species exhibit interspecific

territorial defense (Brewer 1961). The combination of female preference for dominant individuals (Smith 1991, Otter & Ratcliffe 1996, Ramsay et al. 2000) and dominant individuals commanding better territories (Smith 1991, Otter et al. 1999) would appear to promote movement of the zone in the observed direction, towards the BC range. A similar process of male aggression affecting the movement of an avian hybrid zone was indicated in an experimental study of white-collared manakins (*Manacus candei*) and golden-collared manakins (*M. vitellinus*) (McDonald et al. 2001). The greater aggression of the golden-collared birds has been suggested as the cause for introgression of golden-collared plumage traits into the range of the white-collared manakin (McDonald et al. 2001).

While potentially providing some insight into the northward movement of the hybrid zone in Ohio, this experiment suggests several avenues for further investigation. For example, what criteria are the females using in the absence of visual information to make a choice? Both species tended to show a preference when visually isolated from the males. The preference was not associated with the species or dominance of the males. Some other factor needs to be identified. Also, do females really prefer the BC morphological phenotype independent of body size? Possibly CA males altered to the BC morphological phenotype would be preferred over the CA morphological phenotype. Also, in hybrids, is the CA tendency for male dominance linked to the CA morphological phenotype, or could “super-hybrids” exist with the BC morphological phenotype, but the CA capacity to dominate social interactions?

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