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THE EXTENT OF INTROGRESSION OUTSIDE THE CONTACT ZONE BETWEEN *NOTROPIS CORNUTUS* AND *NOTROPIS CHRYSOCEPHALUS* (TELEOSTEI: CYPRINIDAE)

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Abstract.—The cyprinid fishes, *Notropis cornutus* and *N. chrysocephalus*, hybridize in a long, narrow zone in the midwestern United States. To quantify the extent of introgression of genetic markers outside of this zone, samples were collected along transects starting near the region of contact (as defined by morphological characters), followed by samples progressively more distant. Diagnostic allozymic and mitochondrial DNA (mtDNA) restriction site markers were used to estimate the extent of introgression outside of the zone, while polymorphic allozyme and mtDNA markers were used to evaluate the potential for gene flow among populations within transects. Analysis of populations from the northern transect provided evidence for differentiation of populations for some of the markers; however, on average, enough gene flow has occurred to overcome substantial differentiation. Introgressed mtDNA and allozyme haplotypes were rare and found only in the population closest to the contact zone. The rarity of introgressed alleles in the more northern populations is consistent with the recent origin of these populations after the Wisconsin glaciation (less than 12,000 years bp) and/or selection maintaining the northern boundary of the contact zone. Analysis of populations from the southern transect revealed evidence for population subdivision but no evidence for introgression at the diagnostic allozyme loci; however, nearly all individuals from this transect possessed introgressed mtDNA haplotypes, with samples furthest from the contact zone exhibiting the highest frequencies of introgression. Patterns of variation for one of the polymorphic allozyme markers (*Est-A*) and introgressed mtDNAs were highly correlated, suggesting that allozymic heterogeneity at this locus is also the result of introgression. The most likely explanation for these data is that these introgressed haplotypes are indicators of a more southern position of the contact zone during the Pleistocene, with the contact zone shifting northward with the recession of the glacial front. Such movement implicates selection in the maintenance of distributional limits of these species, and hence, the width and position of the contact zone.

Key words.—Allozymes, introgression, mtDNA.

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Reproductive isolation has been of central importance in the development of speciation theory; however, the role and evolution of reproductive isolation in speciation has been controversial. The mechanistic nature of reproductive isolation is currently the focus of intensive debate (i.e., Dobzhansky, 1940; Muller, 1942; Paterson, 1978; Butlin, 1989), as is the role of reproductive isolation in defining species (i.e., Lambert et al., 1987; Coyne et al., 1988; McKitrick and Zink, 1988; Templeton, 1989). In spite of this controversy, forces maintaining the distinctness of species do exist. Without such forces, distinct taxa would fuse. Therefore, understanding the origin and maintenance of organismic diversity requires an understanding of the forces responsible for the observed discontinuities.

Reproductive isolation is most easily analyzed where taxa hybridize in natural situations. Such studies are most informative when several different sets of characters are utilized (i.e., Avise et al., 1984; Baker et al., 1989). The discovery and application of molecular characters have been particularly beneficial for the study of hybridization, providing multiple, independent data sets. Application of these characters as markers has allowed quantification of the extent and direction of hybridization within populations (i.e., Avise and Saunders, 1984; Lamb and Avise, 1986; Szymura and Barton, 1986; Bert and Harrison, 1988; Nelson et al., 1987; Baker et al., 1989; Dowling et al., 1989;

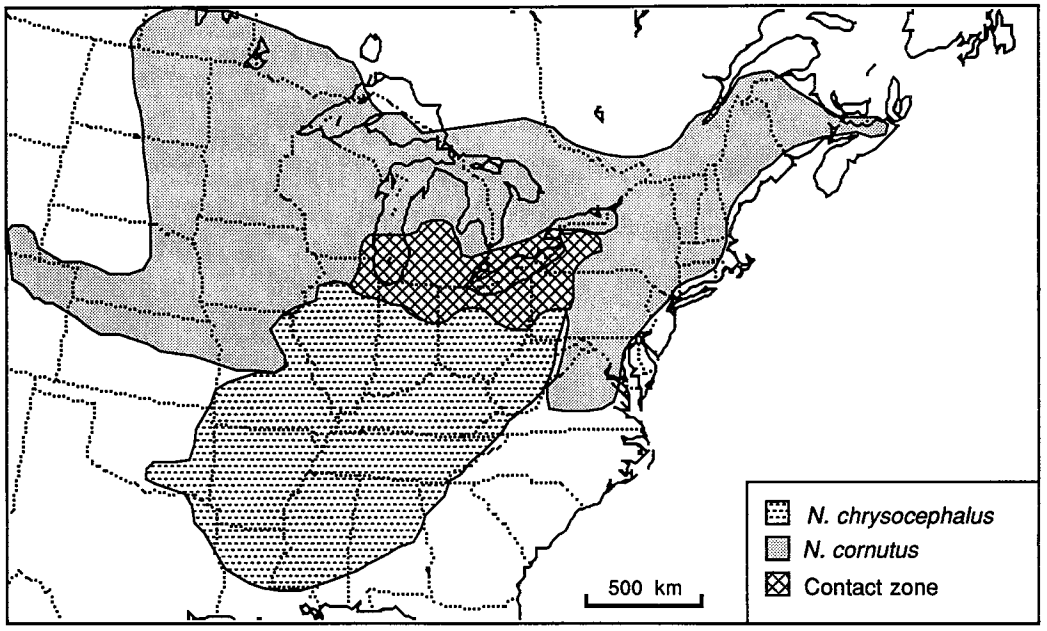

FIG. 1. Approximate position of contact zone between *N. cornutus* and *N. chrysocephalus* (modified from Gilbert, 1961, 1980*a*, 1980*b*).

Rand and Harrison, 1989). Biochemical markers have also allowed tests for concordant variation within hybrid zones (reviewed in Hewitt, 1989), and the extent of introgression outside of hybrid zones (i.e., Ferris et al., 1983; Powell, 1983; Spolsky and Uzzell, 1984; Tegelstrom, 1987; Marchant et al., 1988). In addition, knowledge of population structure and levels of gene flow obtained from biochemical studies can provide information critical for interpreting patterns of variation in hybrid zones (i.e., Szymura and Barton, 1986; Hewitt, 1989). Combination of these population genetic features with ecological and historical attributes can provide a powerful synergism necessary for a better understanding of evolution and reproductive isolation (Avise, 1989; Rand and Harrison, 1989).

The hybridizing cyprinid fishes, *Notropis cornutus* and *Notropis chrysocephalus,* are ideally suited for the study of reproductive isolation. They are widely distributed and abundant in many streams and rivers of eastern North America. *Notropis cornutus* is more northern in its distribution, ranging from Ontario and Quebec southward to northern Illinois, Indiana, and Ohio in the midwest, and as far south as Virginia on the Atlantic Coast (Fig. 1). *Notropis chrysocephalus* is more southern in its distribution, ranging from central Michigan, southern Ontario, and New York, south to near the Gulf of Mexico (Fig. 1). Based on morphological characteristics (Gilbert, 1961), the contact zone between these species is relatively narrow, including northern Illinois, Indiana, and Ohio, southern Michigan and Ontario, and western New York and Pennsylvania (Fig. 1). The hybrid zone between these species differs from classical hybrid zones and more closely resembles the mosaic hybrid zone models described by Harrison and Rand (1989). Hybridization does not follow a distinct clinal pattern, but is determined by local environments.

Hybridization between these species has been intensively scrutinized. Early morphological studies documented considerable introgression (Gilbert, 1961; Miller, 1968; Coburn, 1975; Smith et al., 1981). Application of allozyme markers verified information obtained from previous studies, but also provided evidence for some reproductive isolation in the form of heterozygote deficiency for diagnostic allozyme markers (Dowling and Moore, 1984). Analysis of individuals from the same year class and lo-

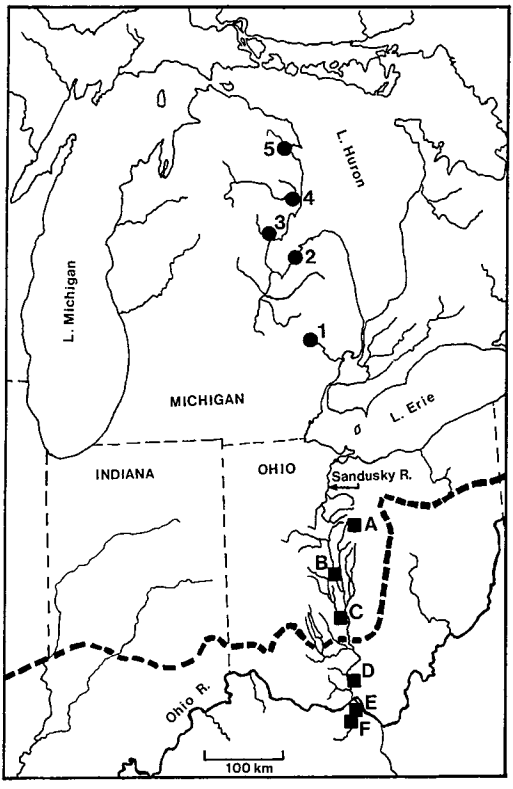

FIG. 2. Localities collected along the northern and southern transects. Names and sample sizes are provided in Table 1. The heavy dashed line marks the approximate position of the furthest advance of the Wisconsin glaciation (Flint, 1971; Trautman, 1981).

cality provided evidence for reduced fitness of adult hybrids relative to both parental species (Dowling and Moore, 1985*a*). Application of restriction endonuclease analysis of mitochondrial DNA (mtDNA) to the study of reproductive isolation provided evidence for asymmetrical introgression at some localities, with directionality the result of differences in assortative mating and/or survivorship of hybrids from different reciprocal crosses (Dowling et al., 1989).

While much is known about the dynamics of hybridization between *N. cornutus* and *N. chrysocephalus,* most of this knowledge is derived from analyses of populations within the contact zone. We collected samples at regular intervals along transects north and south of the contact zone in order to determine: 1) the extent of introgression north and south of the zone, and 2) the extent of population subdivision along these transects. Data obtained from these transects will allow us to compare the potential and extent of introgression outside the zone and consider the relative importance of other factors (i.e., physiology, ecology, climatic history) that determine the position and width of this hybrid zone.

MATERIALS AND METHODS

Sampling Strategy.—Samples of *N. cornutus* and *N. chrysocephalus* were collected along transects north and south of the contact zone (as defined by Gilbert, 1961). The transects extended into the ranges of morphologically "pure" *N. cornutus* (northern transect) and *N. chrysocephalus* (southern transect). Transects started at the approximate northern and southern boundaries of the zone, with each following collection progressively more distant (see Fig. 2 and Table 1 for localities and sample sizes). Collections from the northern transect were taken from Saginaw Bay (Lake Huron) and four rivers draining into the Great Lakes, whereas collections from the southern transect were primarily from the Scioto River (a tributary of the Ohio River), with additional samples collected from Tygart's Creek, whose mouth is almost directly across the Ohio River from the Scioto River. Specimens were collected with a 4.6 m × 1.5 m seine in August 1986 (northern transect) and August 1987 (southern transect). For most specimens, heart, liver, and gonad were removed and stored in MSB buffer (0.003 M $CaCl_2$, 0.01 M EDTA, 0.21 M mannitol, 0.07 M sucrose, 0.05 Tris-HCl, pH 7.5; Ball et al., 1988) for one to four days prior to isolation of mtDNA. Specimens were then frozen on dry ice, transported back to the laboratory, and stored at −80°C until use in protein electrophoresis. For small specimens, the caudal musculature was removed and frozen for use in protein electrophoresis, while the carcass was stored in MSB buffer for isolation of mtDNA.

Protein Electrophoresis.—The following allozymic loci found to be polymorphic or diagnostic for *N. cornutus* and *N. chrysocephalus* were resolved using procedures described previously (Dowling and Moore, 1984, 1985*b*; Dowling and Brown, 1989): esterase (*Est-A,* nonspecific), glucose phosphate isomerase (*Gpi-A,* EC 5.3.1.9), pep-

TABLE 1. Locality data and genotypes for polymorphic genetic markers for the collections from the northern and southern transects (see Fig. 2).

Locality	N	Genotypes[a]				
		Est-A	*Gpi-A*	*Pep-B*	*AvaI*	*HindIII*
Northern transect						
1) Coon Creek, Clinton River drainage, Macomb Co., MI	20	bb (7) bc (7) cc (4) ? (2)	ab (1) ac (4) ad (1) bc (1) cc (2) cd (6) dd (5)	aa (9) ab (10) bb (1)	A (2) 1 (14) 2 (4)	A (2) 1 (5) 2 (12) 3 (1)
2) Saginaw Bay, Huron Co., MI	15	bb (1) bc (8) cc (6)	cc (4) cd (3) dd (8)	aa (6) ab (8) bb (1)	1 (4) 2 (11)	1 (15)
3) Rifle River, Arenac Co., MI	20	ac (1) bb (5) bc (10) cc (4)	ac (5) cc (7) cd (8)	aa (9) ab (7) bb (4)	1 (1) 2 (19)	1 (17) 2 (1) 3 (2)
4) AuSable River, Iosco Co., MI	29	ac (1) bb (11) bc (9) cc (8)	cc (14) cd (7) dd (4) ce (3) ee (1)	aa (8) ab (15) bb (6)	2 (29)	1 (26) 3 (3)
5) Devil's Creek, Alpena Co., MI	20	bb (8) bc (7) cc (5)	ac (2) ad (1) cc (12) cd (5)	aa (7) ab (12) bb (1)	1 (1) 2 (19)	1 (20)
Southern transect						
A) Olentangy River, Marion Co., OH	21	bb (14) bc (6) cc (1)	cc (21)	bb (21)	A (5) 1 (13) 4 (2) 6 (1)	A (5) 1 (13) 2 (3)
B) Mill Creek, Scioto River drainage, Delaware Co., OH	20	bb (13) bc (6) cc (1)	cc (19) ce (1)	bb (20)	A (5) 1 (12) 3 (1) 5 (2)	A (5) 1 (15)
C) Big Darby Creek, Scioto River drainage, Pickaway Co., OH	24	bb (18) bc (3) cc (3)	cc (23) ce (1)	bb (24)	A (6) 1 (12) 4 (3) 6 (1) 7 (2)	A (6) 1 (16) 2 (1) 4 (1)
D) Miller Run Creek, Scioto River drainage, Scioto Co., OH	20	bb (9) bc (9) cc (2)	cc (19) ce (1)	bb (20)	A (1) 1 (10) 3 (2) 4 (4) 5 (2) 6 (1)	A (1) 1 (19)
E) Tygart's Creek 1, Greenup Co., KY	20	bb (1) bc (3) cc (16)	cc (19) cd (1)	bb (20)	1 (12) 3 (1) 4 (2) ? (5)	1 (17) ? (3)
F) Tygart's Creek 2, Greenup Co., KY	20	bb (1) bc (2) cc (17)	cc (17) ce (3)	bb (20)	1 (12) 3 (3) 4 (4) 5 (1)	1 (20)

[a] Allelic and haplotype designations as described in the Materials and Methods section. A question mark identifies the number of individuals unscorable for that particular locus/restriction enzyme.

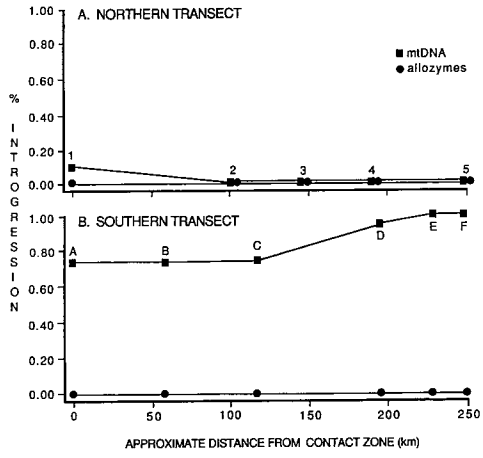

FIG. 3. Extent of introgression (measured in percentage) north (A) and south (B) of the contact zone. Distances from the contact zone are approximate. The symbols represent localities (identified by numbers and letters—see Table 1), with squares and circles depicting data for mtDNA and allozymes, respectively.

tidases (*Pep-B, Pep-S,* EC 3.4.13.11), and phosphoglucomutase (*Pgm-A,* EC 2.7.5.1). Electrophoretic mobility variants are assumed to represent genetic differences and are hereafter considered alleles. *Pgm-A* and *Pep-S* were previously identified as fixed mobility differences, as allopatric populations of *N. cornutus* and *N. chrysocephalus* are fixed for fast and slow alleles, respectively (Dowling and Moore, 1984). *Pep-B* is partially diagnostic, with *N. cornutus* expressing two alleles, while *N. chrysocephalus* exhibits only the fast allele found in *N. cornutus. Est-A* and *Gpi-A* are polymorphic, with each species sharing multiple alleles. Locus nomenclature follows Buth (1984); however, alleles are designated by lower case letters, with "a" representing the least anodal allele, "e" representing the most anodal allele.

Restriction Endonuclease Analysis of Mitochondrial DNA.—A detailed description of the methods used for restriction endonuclease analysis of mtDNA is presented in Dowling et al. (1989, 1990). MtDNAs were isolated by equilibrium density ultracentrifugation, and digested with restriction endonucleases. The resulting fragments were separated on 1.0% agarose and 4.0% polyacrylamide gels, and visualized by end-labeling and autoradiography. Surveys of individuals from several populations distant from the region of hybridization indicate that mtDNAs of *N. cornutus* and *N. chrysocephalus* are distinct (approximately 7–8% sequence divergence, Dowling and Brown, 1989; Dowling, unpubl. data), and easily differentiated using a variety of restriction enzymes. In this instance, mtDNAs were digested with the restriction endonucleases *Ava*I (CPyCGPuG), *Hind*III (AAGCTT), and *Mbo*I (GATC). These enzymes were chosen because each produces fragment patterns diagnostic for *N. cornutus* and *N. chrysocephalus* mtDNAs and showed variation within species. Comparison of variants with cleavage maps for *Ava*I and *Hind*III (Dowling and Brown, 1989; Dowling, unpubl. data) indicated that all haplotypes resulted from the gain or loss of restriction sites. Each individual was assigned a letter or number according to haplotype (*N. cornutus* mtDNAs were identified with numbers; those of *N. chrysocephalus* with letters).

Statistical Analysis.—The extent of gene flow outside the hybrid zone was quantified by direct count of introgressed alleles for the diagnostic markers (i.e., mtDNA species haplotype, *Pgm-A, Pep-S*). *Pep-B* is only diagnostic for *N. chrysocephalus* since *N. cornutus* possesses both alleles in populations from well outside the area of hybridization (Dowling and Moore, 1984). Therefore, this locus was used to quantify the extent of introgression only for the southern transect. Heterogeneity among populations was estimated by calculating Wright's F_{ST} for the polymorphic markers (i.e., *Ava*I and *Hind*III haplotypes; *Est-A, Gpi-A,* and, for *N. cornutus, Pep-B* alleles), using programs written by Swofford and Selander (1989) and Weir (1990). Data for different restriction enzymes were considered separately because this reduced the number of rare haplotypes and simplified analysis and interpretation. Statistical significance of F_{ST} was determined using the V-statistic described in DeSalle et al. (1987). Where more than two alleles or haplotypes were found per allozyme locus/restriction enzyme, the test was performed for each allele separately, pooling the other alleles. Estimates of gene flow (*Nm*) were determined from the allozyme data by back calculation from F_{ST} val-

TABLE 2. Measures of genetic heterogeneity (F_{ST}) and their significance levels from the northern and southern transects (Table 1, Fig. 2). Calculations were made only for polymorphic loci. NA indicates alleles absent or characters that were monomorphic from a particular transect. Negative F_{ST} values are effectively equal to zero.

Marker	Allele/ haplotype	Northern transect		Southern transect	
		F_{ST}	SIG	F_{ST}	SIG
Allozymes					
Est-A	a	0.000	NS	NA	NA
	b	0.008	NS	0.335	$P < 0.001$
	c	0.010	NS	0.335	$P < 0.001$
Gpi-A	a	0.036	$P < 0.005$	NA	NA
	b	0.016	NS	NA	NA
	c	0.092	$P < 0.001$	0.000	NS
	d	0.118	$P < 0.001$	0.000	NS
	e	0.054	NS	0.011	NS
Pep-B	a	0.000	NS	NA	NA
	b	0.000	NS	NA	NA
mtDNA					
Species	CO	NA	NA	0.085	$P < 0.005$
	CH	NA	NA	0.085	$P < 0.005$
*Ava*I	1	0.585	$P < 0.001$	0.012	NS
	2	0.585	$P < 0.001$	NA	NA
	3	NA	NA	0.000	NS
	4	NA	NA	−0.015	NS
	5	NA	NA	0.014	NS
	6	NA	NA	−0.026	NS
	7	NA	NA	0.055	NS
*Hind*III	1	0.459	$P < 0.001$	0.082	NS
	2	0.601	$P < 0.001$	0.090	NS
	3	−0.003	NS	NA	NA
	4	NA	NA	−0.002	NS

ues (Slatkin and Barton, 1989). Nucleon diversity was estimated from the *Mbo*I haplotype data, using the formula described by Nei and Tajima (1981).

RESULTS

This study of hybridization has two distinct components: 1) analysis of population subdivision along transects, and 2) estimation of the extent of introgression outside the contact zone. Analysis of population subdivision provides information concerning the potential for introgression, where significant subdivision would indicate a reduction in the ability of genes to introgress along the transect. Without this information, interpretation of the extent of introgression would be difficult.

Northern Transect.—Electrophoretic analysis of specimens collected along a transect north of the hybrid zone into the range of *N. cornutus* revealed no evidence of allozymic introgression, as none of the 104 specimens from the five populations analyzed exhibited alleles typical of *N. chrysocephalus* at the two diagnostic loci (Fig. 3). Analysis of mtDNA from these same individuals uncovered limited introgression, with only two individuals collected at the northern border of the contact zone possessing the mtDNA typical of *N. chrysocephalus* (locality 1—Fig. 3).

The three polymorphic allozyme loci (*Est-A, Gpi-A, Pep-B*) and mtDNA restriction fragment haplotypes produced by two enzymes (*Ava*I, *Hind*III) were used to test for structuring of populations along the transect. Estimates of population subdivision (Table 2) revealed significant genetic heterogeneity among the five populations for only one of the three allozyme loci (*Gpi-A*) and the *Ava*I and *Hind*III mtDNA haplotypes typical of *N. cornutus* (the mtDNA data from the two individuals with haplotypes typical of *N. chrysocephalus* were excluded). At the *Gpi-A* locus, three of the five alleles (a, c, d) exhibited significant F_{ST} values. Although values for the remaining two alleles (b, e) were not significant, their distribution (Slatkin, 1985) also indicates some

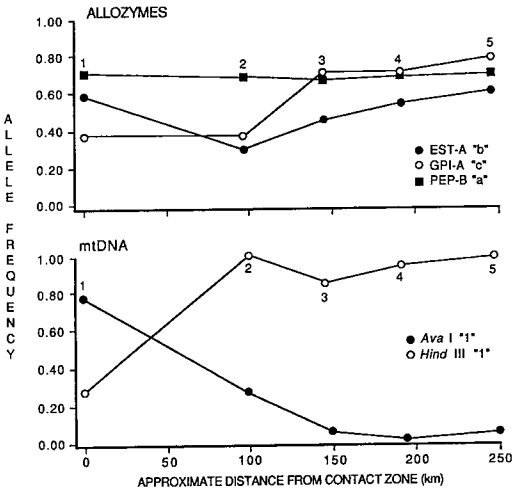

FIG. 4. Graphical representation of allele frequency variation across populations from the northern transect. Distances from the contact zone are approximate. Locality of collection is identified by number (Table 1). A single common allele/haplotype was chosen for each character.

heterogeneity since the two "b" and five "e" alleles were found at only single localities (1 and 4, respectively). For *Hind*III, statistical analysis of the two common haplotypes (1 and 2) provided evidence for heterogeneity; however, the pattern for the rare haplotype (3) suggests more gene flow between populations (Slatkin, 1985), because it was found in six individuals from three localities. Only two haplotypes were identified for *Ava*I, both indicating significant heterogeneity across populations. The patterns of frequency variation for the common alleles of these three markers (*Gpi-A, Ava*I, and *Hind*III) are roughly correlated (Fig. 4). Each shows differentiation of the collection closest to the edge of the contact zone (Clinton River, locality 1), and to a lesser extent the collection from Saginaw Bay (locality 2), from the more northern populations.

The remaining allozyme loci (*Est-A* and *Pep-B*) failed to show significant heterogeneity across populations (Fig. 4). For *Est-A*, the Saginaw Bay population (locality 2) appeared to be slightly different from the others. *Pep-B* showed no differentiation, with all populations having approximately the same frequencies for each of the two alleles.

Indirect estimates of gene flow (*Nm*; Table 3) averaged across the three polymorphic allozyme loci indicated considerable gene exchange among populations (6.5 individuals per generation). The F_{ST} and *Nm* values for *Est-A* from this study (F_{ST} = 0.009; *Nm* = 27.5) are similar to those reported for *N. cornutus* from a single river drainage in northern Michigan (F_{ST} = 0.017; *Nm* = 14.6; Dowling and Moore, 1986).

Southern Transect.—Electrophoretic analysis of specimens collected along the transect south of the hybrid zone into the range of *N. chrysocephalus* also failed to detect introgression of allozymic characters, as none of the 125 individuals from the six populations analyzed exhibited *N. cornutus* alleles at the three diagnostic marker loci (Fig. 3). However, unlike the northern transect, there was considerable evidence for introgression of mtDNA south of the hybrid zone, with approximately 75–100% of the individuals in each population exhibiting *N. cornutus* mtDNA haplotypes (Fig. 3, Table 1). The pattern of introgression is opposite to that expected, since the populations farthest away from the contact zone have the highest frequencies of introgressed mtDNA haplotypes.

As for the northern transect, the polymorphic allozyme (*Est-A, Gpi-A*) and mtDNA (*Ava*I, *Hind*III) markers were used to test for structuring of populations along the transect; however, unlike the northern transect, most individuals exhibited mtDNAs obtained by introgression from *N. cornutus*. No variation was found in any of the mtDNAs typical of *N. chrysocephalus* for these enzymes (as well as all others surveyed, Dowling and Brown, 1989), while the mtDNAs typical of *N. cornutus* exhibited considerable variability. Therefore, only variation exhibited by *N. cornutus* mtDNAs was used in the analysis of population structure. It is important to keep in mind that these *N. cornutus* mtDNA haplotypes from the southern transect were found in individuals indistinguishable from "pure" *N. chrysocephalus* for all morphological and allozymic features, and estimates of subdivision obtained from mtDNA variation of these *N. cornutus* haplotypes address heterogeneity of introgressed characters.

The large numbers of introgressed (i.e., *N. cornutus*) mtDNA haplotypes permitted

TABLE 3. Measures of genetic heterogeneity (F_{ST}) and levels of gene flow (Nm) for each of the characters. Nm was calculated from F_{ST} using the relationship provided in Slatkin and Barton (1989). NA indicates values that were not calculated.

Marker	Northern transect		Southern transect	
	F_{ST}	Nm	F_{ST}	Nm
Allozymes				
Est-A	0.009	27.5	0.335	0.5
Gpi-A	0.093	2.4	0.004	62.3
Pep-B	0.000	NA	NA	NA
Average for allozyme loci	0.037	6.5	0.301	0.6
mtDNA				
*Ava*I	0.585	NA	0.003	NA
*Hind*III	0.438	NA	0.077	NA
Species haplotype	NA	NA	0.085	NA

use of species haplotype as an additional character for the analysis of population structure. Unlike the other characters that provide an indication of the distribution of variation between populations, the results from the analysis based on species haplotype reflects the extent of introgression.

Estimates of genetic heterogeneity among populations revealed evidence for subdivision at only one of the two allozymic loci (*Est-A*; Fig. 5). Variability at this locus takes the form of a cline, with the "b" allele more frequent in northern populations (localities A–D), and the "c" allele prevalent in more southern populations (localities E and F). Variability at the *Gpi-A* locus was restricted to one major allele and two minor alleles (each occurring at frequencies less than 0.05). The widespread distribution of the rare "e" allele (6 individuals from four populations) suggests that considerable gene flow exists among these populations (Slatkin, 1985).

Neither of the two mtDNA markers revealed significant levels of genetic heterogeneity. The *Hind*III and *Ava*I haplotypes, while showing considerable variability, failed to provide evidence of significant heterogeneity (Fig. 5, Table 2). The distribution of species haplotype varies clinally, with *N. chrysocephalus* mtDNAs most common in northern populations (localities A–C), but absent in the more southern populations (localities E–F). The patterns of heterogeneity for *Est-A* and mtDNA species haplotype are significantly correlated (r=0.88, P=0.01, Spearman rank correlation of arcsine square root transformed frequencies), with the transition occurring in the area of population D, just north of the Ohio River. This population is intermediate in terms of its mtDNA and *Est-A* characteristics, with its mtDNA haplotypes more like the southern populations (E, F) and the *Est-A* allele frequencies more similar to the northern localities (A, B, C).

Indirect estimates of Nm calculated from southern transect populations are variable (Table 2). The mean value of Nm calculated from the two allozyme loci was 0.6 individuals per generation; however, estimates from *Gpi-A* and *Est-A* were quite different, 62.3 and 0.5, respectively.

DISCUSSION

The patterns of introgression and genetic heterogeneity vary among the northern and southern transects. Specimens collected north of the hybrid zone exhibit limited introgression of genetic characters, but significant levels of geographically structured genetic variation for some characters, whereas populations collected south of the hybrid zone exhibit considerable genetic introgression, with observed population structure apparently linked to the extent of introgression. The contrasting patterns between transects could be due to sampling design or historical differences between these regions. While both transects were collected from single drainage systems, samples from the southern transect were mostly taken from a single river, whereas those from the northern transect were taken from tributaries of a large lacustrine system. Therefore, one might expect to see more differentiation and

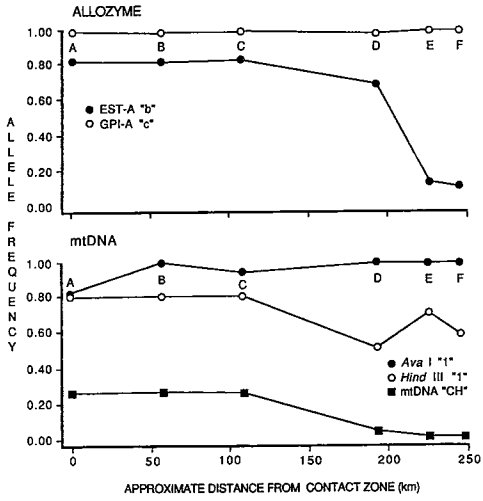

FIG. 5. Graphical representation of allele frequency variation across populations from the southern transect. Distances from the contact zone are approximate. Locality of collection is identified by letter (Table 1). A single common allele/haplotype was chosen for each character.

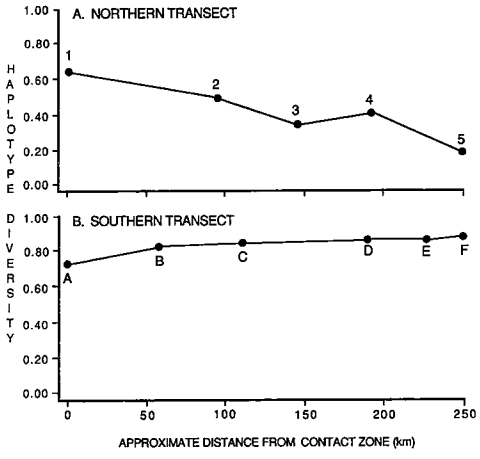

FIG. 6. Nucleon diversity north (A) and south (B) of the contact zone. Distances from the contact zone are approximate. The symbols represent localities (identified by numbers and letters—see Table 1).

less introgression along the northern transect if lakes act as barriers to dispersal.

The different results for the northern and southern transect are not likely due to sampling design for several reasons. These species are not restricted to the tributary rivers, reducing the likelihood of their isolation (locality 2 and Scott and Crossman, 1973). F_{ST} values were, on average, relatively small, indicating the existence of enough gene exchange to prevent population subdivision among these tributary populations (Hartl and Clark, 1989). Based on this evidence, the northern transect, like the southern, represents a single drainage with considerable interconnection of populations. Therefore, historical factors have likely had the most significant impact on observed differences between the northern and southern transect populations. Since regions north and south of the hybrid zone have distinct histories, data from these transects need to be considered separately.

Patterns of Introgression and Genetic Heterogeneity

Northern Transect.—The Great Lakes region was severely impacted by the Pleistocene glaciation, with the ice sheet extending into southern Ohio (Fig. 2) at the peak of the most recent glacial age (the Wisconsin, approximately 24,000 years ago; Flint, 1971). The five localities from the northern transect were covered by the ice sheet until approximately 12,000 years ago (Bailey and Smith, 1981).

Patterns of genetic heterogeneity are consistent with a recent origin of these populations. The more northern localities (3–5, Fig. 4) are genetically homogeneous, while the more southern populations (1, 2) show some differentiation from the others. This pattern could be produced by differences in the founding stocks for these sets of localities, or progressive dispersal from a single founding stock, with loss of diversity as *N. cornutus* dispersed northwards. All five localities exhibit, for the most part, genotypes that are found in other rivers in southeastern Michigan (Dowling, unpubl. data). Estimates of nucleon diversity based on *Mbo*I restriction digests reveal that genetic diversity gradually decreases from southern to northern populations (Fig. 6), indicating that dispersal northward occurred recently, with more northern populations possessing only a portion of the diversity found in southern populations from the transect. If this is the case, the observed heterogeneity is a result of the mode and timing of establishment of these populations and does not identify barriers to dispersal.

Given that introgression north of the con-

tact zone is possible, two hypotheses can explain the lack of *N. chrysocephalus* alleles north of the contact zone. The recent origin of *N. cornutus* populations north of the contact zone may indicate that there has been too little time for dispersal of *N. chrysocephalus* into these populations. Alternatively, selection against the *N. chrysocephalus* phenotype could be responsible for limiting the introgression of *N. chrysocephalus* alleles north of the hybrid zone. The latter model does not require selection against specific mtDNA or allozyme genotypes, but linkage of *N. chrysocephalus* alleles to other characters that are inferior outside of their current range or in combination with *N. cornutus* genotypes. Within the area of sympatry, hybrids are less fit than either parental species (Dowling and Moore, 1985*a*), making this hypothesis plausible.

It is important to note that evidence for recent establishment of northern populations does not preclude the existence of selection in maintaining the integrity of the northern border of this contact zone. Given the probable age of these populations (4,000–6,000 generations) and the magnitude of gene flow estimated here, it seems likely that more introgressed alleles should have been detected north of the contact zone if selection was not involved in maintaining the northern boundary of this zone.

Southern Transect.—Unlike the northern transect, not all localities from the southern transect were directly affected by the most recent glacial advance (Fig. 2). During this time, it is likely that *N. cornutus* was distributed much farther south, with a concordant southward shift of the hybrid zone. As the glaciers receded, the range of *N. cornutus* shifted northward, presumably because changing conditions favored *N. chrysocephalus*. *Notropis cornutus* was actually distributed further southward in Ohio in historical times, with a recent recession in its range likely due to increased agricultural activity (Trautman, 1981). The pattern of mtDNA introgression is consistent with a considerably more southern distribution of *N. cornutus* in former times, with *N. cornutus* mtDNAs found in every individual at the two southern localities of the transect (E and F, Fig. 5). Under this scenario, the *N. cornutus* mtDNAs well south of the hybrid zone would be relicts, indicators of a more southerly placement of the area of hybridization in earlier times.

Alternatively, *N. cornutus* may have been isolated in different glacial refugia than *N. chrysocephalus,* and the contact zone may actually be a recent (post-Pleistocene) phenomenon. If this were the case, the position of the contact zone probably has not changed dramatically, and the *N. cornutus* mtDNAs must have introgressed southward into the range of *N. chrysocephalus.* Takahata and Slatkin (1984) have demonstrated mathematically that mtDNA can easily flow across special boundaries. Accordingly, gene flow of mtDNA across species boundaries has been frequently used to explain introgressed mtDNA haplotypes well outside of an area of hybridization. In this instance, however, gene flow through the contact zone does not seem as likely an explanation. The pattern of introgression (*N. chrysocephalus* from populations closer to the contact zone exhibit fewer *N. cornutus* mtDNAs than those farther removed—Fig. 3) is exactly opposite the pattern expected if continuing gene flow were responsible.

Given the high frequency of *N. cornutus* mtDNAs in samples analyzed here, it is possible *N. chrysocephalus* mtDNAs were completely absent from these populations until recently, when *N. chrysocephalus* may have reinvaded from the north through the adjacent Sandusky River (Fig. 2). The lack of heterogeneity among populations along the transect for *N. cornutus* mtDNA haplotypes and *Gpi-A* alleles suggests considerable interconnection of populations; yet, the strong correlation of the *Est-A* "b" allele with the occurrence of the *N. chrysocephalus* mtDNA haplotype suggests that these patterns are both the result of a reinvasion of a differentiated stock of *N. chrysocephalus.* Further studies of geographic variation in *N. chrysocephalus* are needed to verify the existence of such a stock.

This distribution of mtDNAs suggests a more southern position of the contact zone in the past, making the fixation of introgressed mtDNAs in more southern populations a point of interest. The high frequency of *N. cornutus* mtDNAs could be the result of selection favoring *N. cornutus* mtDNAs, fixation by genetic drift, long-term

undirectional introgression, or some combination of these factors.

The genetic interaction between nuclear and mitochondrial DNA provides a means of addressing the hypotheses that selection favors *N. cornutus* over *N. chrysocephalus* mtDNA in a *N. chrysocephalus* nuclear background. Mitochondrial DNA does not encode all of the enzymes necessary for replication, transcription, and translation, and encodes only some protein subunits (i.e., cytochrome oxidase, subunits I, II, and III), with the remaining subunits encoded in the nucleus. Because of this integration, mtDNA coevolves with the nuclear genome (reviewed by Avise, 1986; Moritz et al., 1987). Given this coevolution, it is difficult to envision selection favoring a highly divergent *N. cornutus* mtDNA in the nuclear background of *N. chrysocephalus.*

The observed pattern could also have resulted from a bottleneck in effective population size and chance fixation of *N. cornutus* mtDNAs. If this were the case, one might expect a reduction in levels of variation from the reduction in effective population size. Nucleon diversities of *N. cornutus* mtDNAs for each of the southern transect populations sampled indicates that a severe reduction in size did not occur recently, since values are uniformly high across all populations (Fig. 6).

Notropis cornutus mtDNAs could also have become fixed in *N. chrysocephalus* if introgression is asymmetrical. Reduced production or fitness of F_1 progeny produced by matings between *N. cornutus* males and *N. chrysocephalus* females relative to the reciprocal mating and continued backcrossing of hybrids with *N. chrysocephalus* could eventually cause the elimination of *N. chrysocephalus* mtDNAs. Dowling et al. (1989) found that in some populations from western Michigan, strong unidirectional introgression of *N. cornutus* mtDNA into *N. chrysocephalus* has resulted in large numbers (approximately 60%) of morphologically and allozymically "pure" *N. chrysocephalus* with *N. cornutus* mtDNA. If the situation were similar in southern Ohio and northern Kentucky, it is easy to visualize *N. cornutus* mtDNA becoming fixed in hybrid populations without a significant reduction in population size. Such a genetic interaction would also limit the reduction in population size necessary for elimination of *N. chrysocephalus* mtDNA by drift, making it possible that the interaction between reduction in population size and unidirectional introgression were responsible for the loss of *N. chrysocephalus* mtDNAs in southern localities.

Conclusions

In this study, genetic characters collected from along transects north and south of the contact zone between *Notropis cornutus* and *N. chrysocephalus* were used to assess the potential for and extent of introgression outside of that contact zone. Since only single transects were used, it is impossible to extrapolate these conclusions to other, distant transects; however, the genetic data presented here allow us to address the historical position of the contact zone between *Notropis cornutus* and *N. chrysocephalus* near its longitudinal midpoint.

Estimates of gene flow from allozyme and mtDNA data suggest that these species are capable of considerable dispersal; yet, the boundaries of the zone appear concordant for diagnostic allozymic and morphological characters (and mtDNA for the northern transect). The lack of allozyme and mtDNA introgression north of the hybrid zone is consistent with either recent establishment of northern populations or selection maintaining the northern boundary. The pattern of mtDNA introgression south of the contact zone provided evidence for a more southward position of the contact zone in previous times, with a relatively recent northward shift (Trautman, 1981) and elimination of *N. cornutus* alleles from the nuclear genome. Evidence for ecological (Smith et al., 1981) and physiological (Radforth, 1944; Hart, 1952) differentiation of these species and selection against hybrids (Dowling and Moore, 1985*a*) along with the genetic data of this study suggests that the distributional limits, and hence the boundaries of the contact zone, may be maintained by an environmentally based selection gradient in the face of considerable levels of gene flow.

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