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# SPECIES FLOCK IN THE NORTH AMERICAN GREAT LAKES: MOLECULAR ECOLOGY OF LAKE NIPIGON CISCOES (TELEOSTEI: COREGONIDAE: *COREGONUS*)

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*Abstract.*—Studies on north temperate fish species indicate that new habitat availability following the last ice sheet retreat has promoted ecological speciation in postglacial lakes. Extensive ecophenotypic polymorphisms observed among the North American Great Lakes ciscoes suggest that this fish group has radiated through trophic adaptation and reproductive isolation. This study aims at relating the ecomorphological and genetic polymorphisms expressed by the Lake Nipigon ciscoes to evaluate the likelihood of an intralacustrine divergence driven by the exploitation of alternative resources. Morphological variation and trophic and spatial niches are characterized and contrasted among 203 individuals. Genetic variation at six microsatellite loci is also analyzed to appraise the extent of genetic differentiation among these morphotypes. Ecomorphological data confirm the existence of four distinct morphotypes displaying various levels of trophic and depth niche overlap and specialization. However, ecological and morphological variations were not coupled as expected, suggesting that trophic morphology is not always predictive of ecology. Although extensive genetic variability was observed, little genetic differentiation was found among morphotypes, with only one morph being slightly but significantly differentiated. Contrasting patterns of morphological, ecological, and genetic polymorphisms did not support the hypothesis of ecological speciation: the most ecologically different forms were morphologically most similar, while the only genetically differentiated morph was the least ecologically specialized. The low levels of genetic differentiation and the congruence between $\theta$ and $\phi$ estimates altogether suggest a recent (most likely postglacial) process of divergence and/or high gene flow among morphs A, C, and D, whereas higher $\phi$ estimates for comparison involving morph B suggest that this morph may be derived from another colonizing lineage exchanging little genes with the other morphs. Patterns of ecophenotypic and genetic diversity are also compatible with a more complex evolutionary history involving hybridization and introgression.

*Key words.*—*Coregonus*, genetic differentiation, microsatellites, morphology, species flock, trophic ecology.

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The ecological theory of adaptive radiation proposes several mechanisms that can promote rapid phenotypic divergence within a single clade (Huxley 1942; Mayr 1963). In most of these mechanisms, divergent natural selection leads to morphological and physiological differentiation if populations are submitted to different selection regimes when they exploit alternative resources and environments. Opportunities or incentives to exploit new resources are associated with relaxed competition regimes offered by the colonization/availability of novel suitable habitats or the acquisition of a key evolutionary innovation that can both provide access to unexploited niches. When reproductive isolation evolves as a consequence of resource-based divergent natural selection and/or resource competition, the process can be regarded as ecological speciation (Schluter 1996a,b).

Recent studies on north temperate fish species indicated that new habitat availability following the last ice sheet retreat (10,000–15,000 yr ago) has promoted ecological speciation in postglacial lakes (reviewed in Schluter 1996b). A postglacial speciation burst in north temperate lakes is corroborated by the negative correlation between the median latitude of distribution and the degree of genetic differentiation between fish sister species (Bernatchez and Wilson 1998). For several fish species, sympatric and/or allopatric ecotypes have long been recognized (Lindsey 1963; Fenderson 1964; Benhke 1972; Moodie and Reimchen 1976), and observed differences are most often related to traits potentially involved in the exploitation of alternative resources. Dimorphism for trophic traits such as the number of gill rakers (comblike gill arch projections involved in food sieving) is especially common (Schluter and McPhail 1993), but divergent life-history traits have also been documented (Schluter 1996b). Reproductive isolation between species pairs is generally incomplete, but varies greatly (Hindar 1994), and has likely evolved either locally (Taylor et al. 1996; Wood and Foote 1996; Pigeon et al. 1997) or following secondary contacts (Bernatchez and Dodson 1990; McVeigh et al. 1995; Bernatchez et al. 1996).

The North American Great Lakes (GL) ciscoes (*Coregonus* spp.) are the most phenotypically variable and taxonomically challenging fish of North American postglacial lakes (Scott and Crossman 1973). Several of their characteristics suggest that they have undergone a recent adaptive radiation involving ecological speciation. First, in each of these lakes, a planktivorous high-gill-rakered form coexists with one or several low-gill-rakered epibenthivorous forms, and differences in gill-raker counts among these species can be interpreted as adaptive divergences for contrasted feeding modes (Smith and Todd 1984). Second, although the species status of GL ciscoes is questionable (Todd 1981; Phillips and Ehlinger 1995), evidence for their reproductive isolation is indicated by differences in time and depth of reproduction among species (Koelz 1927; Todd and Smith 1980; Smith and Todd 1984). Third, phylogenetic relationships are con-

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sistent with recent postglacial divergences: unlike other coregonids, genetic distances estimated from allozymes, mtDNA, and rDNA among GL ciscoes are generally very low (Bernatchez et al. 1991; Bodaly et al. 1991; Snyder et al. 1992; Sajdak and Phillips 1997; Reed et al. 1998). Finally, differentiation must have occurred within one or a few evolutionary clades because it is improbable that many species independently colonized the GL watershed during such a short period. Altogether, these characteristics suggest that the GL ciscoes consitute a species flock because they correspond to the criteria originally advanced by Greenwood (1984) for a species flocks denomination, that is, endemism, geographical circumscription, and monophyly.

However, GL cisco radiation appears to be different from those suspected in other North American, postglacial fish systems. Unlike most cases where divergence led to pairs of ecotypes (reviewed in Schluter 1996b), ciscoes have differentiated into multiple morphotypes, five of which are endemic to the GL and three are widespread (Scott and Crossman 1973). Whereas ecological divergence among fish species pairs is principally associated with the trophic niche axis (Robinson and Wilson 1994), reproductive allochrony, foraging, and spawning depth allopatry appear to be as important as trophic morphology in the radiation of ciscoes. Smith and Todd (1984) have proposed that the GL ciscoes form an incipient species flocks whereby one (or two) colonizing lineage(s) radiated through intralacustrine diversification driven by food and/or depth niche specialization. Nonexclusive alternatives to this evolutionary scenario include: (1) lineage plasticity, whereby phenotypic variation is the mere expression of a plastic and unstructured gene pool; and (2) colonization by genetically differentiated lineages (secondary contacts) followed by persistence or hybridization/introgression (Smith and Todd 1984; Phillips and Ehlinger 1995). Unfortunately, these hypotheses are not assessable in the five major Great Lakes, for native cisco assemblages have been profoundly altered by various anthropogenic factors (overfishing, invasions, introductions). In contrast, Lake Nipigon is still inhabited by the pristine GL fish fauna, and ecological perturbations are limited to the recent invasion of rainbow smelt (*Osmerus mordax*) in 1976 (Salmon and van Ogtrop 1996). Cisco diversity in Lake Nipigon thus offers an opportunity to investigate the evolutionary processes involved in the establishment of its diverse cisco fauna.

This study is the first to thoroughly evaluate the hypothesis of an adaptive radiation driven by the exploitation of alternative resources in North American ciscoes (Smith and Todd 1984). This hypothesis predicts that morphotypes should differ by ecologically relevant morphological traits related to different modes of resource exploitation and that partial or complete reproductive isolation should exist among these ecotypes. We first verify that phenotypic polymorphisms of Lake Nipigon ciscoes are expressed as distinct morphotypes and identify discriminant sets of morphological traits. Ecological polymorphisms along trophic and depth niche axes are then simultaneously documented to verify the putative functional link between morphology and ecology. The same fish are also typed at polymorphic microsatellite loci to assess the extent of genetic differentiation among ecotypes. Genetic variation among Lake Nipigon ciscoes is then paralleled with patterns of ecomorphological variation.

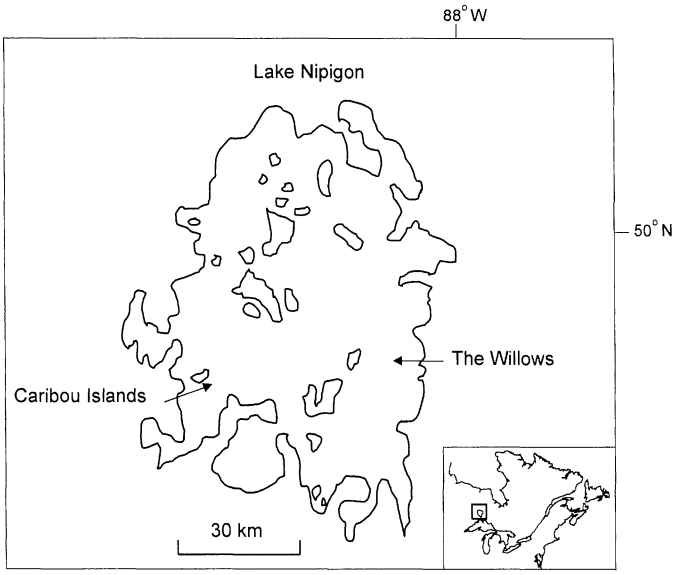


FIG. 1. Location map of Lake Nipigon (Ontario, Canada) and sampling sectors (Caribou Islands and the Willows).

## MATERIALS AND METHODS

### *Sampling of Biological Material*

Lake Nipigon (Ontario, Canada, 49°45′N 88°30′W) is a large lake (4500 km$^2$) located in the northwestern corner of the North American Great Lakes watershed (Fig. 1). In August 1997, 690 ciscoes (*Coregonus* ssp.) were captured in two lake sectors (Fig. 1). In each of these two sampling zones, fish were gillnetted at several sites in three depth strata (10–30 m, 31–60 m, and $>$ 60 m). Four morphotypes were recognized on the basis of external morphological features currently used in the taxonomy of *Coregonus* species: body shape, pigmentation, eye size, mouth morphology and orientation, and relative size of upper and lower jaws. These traits are essentially those used by Koelz (1927) for the original description of GL cisco species. A priori morphological assignation, gender, and depth of catch were individually recorded for all captured fish. Twenty-five individuals of each morphotype were kept from each sampling zone (when possible) to also test for interzone differentiation within morphotype. For each morphotype, random samples were chosen from each depth stratum so as to reflect the relative abundance of each morph in each zone. Individuals were filmed, heads were tagged and preserved in ethanol, and stomach contents were kept in 4% formaline. For Caribou Islands, 21, 44, 25, and 25 individuals assigned to morphotypes A, B, C, and D were analyzed, respectively. In the Willows, sampling sizes were 24 (A), 14 (B), 25 (C) and 25 (D).

### *Morphology*

Analysis of the field-collected video images was performed using the Optimas software: *xy* coordinates were determined for 15 homologous landmarks to calculate 11 morphometric

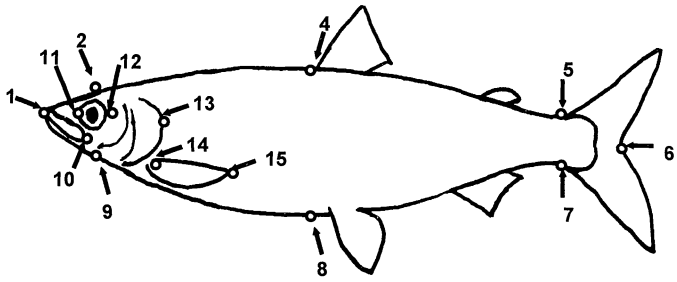


FIG. 2. Lateral view of a cisco indicating landmarks used for morphometric measurements (FKL, fork length, 1–6; HDL, head length, 1–13; HDD, head depth, 2–9; SNL, snout length, 1–11; EYA, eye area, 2 $\pi$ $(11–12)^2$; EYH, eye height, eye center–9; PCL, pectoral length, 14–15; MXL, maxillary length, 1–10; MXA, maxillary angle, angle between body axis and MXL; BDD, body depth, 4–8; PDH, peduncular height, 5–7).

variables (Fig. 2). Manual measurements with an electronic calliper provided values for three additional morphometrics (IOW: interorbital width; MDL: mandible length; MXW: maxillary width; Lindsey 1962). Two of the longest gill rakers (first and third most posterior rakers of the lower arch, GR1 and GR3, respectively), as well as the length of the lower and upper gill arches (LGL, UGL, respectively) were measured using the micrometer of a dissecting scope. Rakers were counted on the first lower (LGR) and upper (UGR) right gill arches. The average of GR1 and GR3 determined mean gill-raker length (GRL), whereas intergill-raker space (IGS) was estimated as (LGL + UGL)/(LGR + UGR − 1).

The univariate residual method was used to adjust each morphometric character for size heterogeneity among individuals (Thorpe 1976; Reist 1985; Flemming et al. 1994). For each character, raw values were logged and standardized; these were then used to establish the overall pooled sample regression line describing the relationship between this character and fork length (FKL). Residuals from the regression line were used as variables for all statistical analyses, along with two meristic variables (UGR, LGR) and maxillary angle with body axis (MXA). Residuals were also calculated for regression lines established separately for each morphotype; the results were essentially equivalent to those of the pooled sample analysis and are not presented in this paper.

Differences among a priori morphotypes were first assessed by univariate analyses of variance (ANOVA), adjusting significance level for simultaneous testing with the sequential Bonferroni procedures ($k_i$ = 18, Rice 1989). When a character showed among-morph differences, Scheffé's multiple comparisons were performed. Two multivariate methods were also used to identify patterns of covariation among morphological characters and to investigate the validity of the a priori classification. A principal component analysis (PCA) was first conducted to identify those characters contributing most to the overall variation. PCA also allowed to objectively examine patterns of individual clustering when there are no a priori group membership assignment. Discriminant analysis (DA) complemented the PCA in using assigned group membership to maximize the among-group relative to the within-group variation. DA also allowed to detect misclassified individuals and to determine the morph to which they most likely belong.

### *Depth and Food Niches*

Morphotype abundances were compared among depth strata in each sampling zone with Fisher exact tests performed with the STRUC module of GENEPOP 3.1 (Raymond and Rousset 1995) using field data for 690 captured individuals. Morphotype depth distribution was also used to calculate Schoener's index of niche overlap, $I_{n.o.} = \frac{1}{2} \Sigma p_i q_i$, where $p_i$ and $q_i$ are the proportions of morphotypes $p$ and $q$ in class (stratum) $i$ (Schoener 1968, cited in Naesje 1991). Niche overlap within morph (between zones) and zone (among morphs) were compared using Mann-Whitney $U$-test.

The trophic niche of each morphotype was characterized by identifying and enumerating prey items contained in the stomach of each individual. Occurrences of common prey items were compared among morphs with $\chi^2$ tests using Monte Carlo simulations (1000 iterations in the REAP package; McElroy et al. 1992). The diet of each fish was also described as the weight proportion of each food item after prey size/type correction into equivalent weight units (Culver et al. 1985; Tremblay and Magnan 1991; Sandlund et al. 1995; P. Magnan, unpubl. data). Average weight-adjusted diet composition of each morphotype was further adjusted for the occurrence of each prey type prior to being used to estimate and compare the extent of food niche overlap with Shoener's index and Mann-Whitney $U$-tests (as above).

### *Genetic Variation*

#### *Microsatellite Markers*

Five microsatellite markers cloned from ciscoes and one marker cloned from the congeneric *Coregonus nasus* (Patton et al. 1997) were used to characterize the genetic variability of the Lake Nipigon cisco assemblage (Table 1). Cisco markers were cloned from Lake Nipigon ciscoes following the detailed protocol of Estoup and Turgeon, which is available at the following address: http://www.inapg.inra.fr/dsa/microsat/microsat.htm. DNA was obtained from head muscle tissue using classical phenol extraction (Sambrook et al. 1989) or the rapid Chelex method (Estoup et al. 1996). Genotypes were visualized by migrating $\alpha$-$^{35}$S-labeled PCR fragments in 6% acrylamide gels following standard procedures (Sambrook et al. 1989). Allele sizes were determined by comparison to several internal standards, including the cloned allele for cisco loci and M13 phage sequence (Sambrook et al. 1989).

#### *Data Analysis*

The first step consisted of identifying panmictic units suitable for further analyses. Genotype distributions were compared to those expected under Hardy-Weinberg equilibrium (HWE) for different grouping schemes taking into account morphological and/or spatial factors (GENEPOP 3.1, option 1.1; exact test of $H_0$ = random union of gametes, with a rejection zone defined as $H_1$ = heterozygote deficit evaluated with a $U$-test). Grouping schemes considered were as follows:

TABLE 1. Cisco microsatellite markers: description and amplification conditions. All amplifications were performed in a total reaction volume of 12.5 μl with 25–50 ng of genomic DNA; 75 μM of CTP, GTP, and TTP; 5 μM ATP; 1.0–1.2 mM MgCl; 400 nM of each primer; 0.2 μCi $^{35}$S-ATP; and 0.25 unit of *Taq* polymerase. PCR cycles were as follows: 1 × (3 min at 95°C); 5 × (45 sec at 95°C, 40 sec at $T_a$, 40 sec at 72°C); 23–25 × (30 sec at 95°C, 40 sec at $T_a$, 40 sec at 72°C); 1 × (2 min at 72°C).

| Microsatellites description | | | | PCR conditions | | |
|---|---|---|---|---|---|---|
| Name | Core sequence | Primer sequence 5′–3′ | Cloned allele (bp) | $T_a$ (°C) | Number of cycles | $MgCl_2$ (mM) |
| Cisco-90 | $(AC)_{10}ATAT(AC)_3$ | 1: CAG ACA TGC TCA GGA ACT AG<br>2: CTC AAG TAT TGT AAT TGG GTA C | 116 | 55 | 30 | 1.2 |
| Cisco-126 | $(TC)_{10}N_{84}(GT)_8$ | 1: GCC AGA GGG GTA CTA GGA GTA TG<br>2: GCA GAG AAA GAG CCT GAT TGA AC | 202 | 60 | 30 | 1.2 |
| Cisco-157 | $(GT)_{17}$ | 1: CTT AGA TGA TGG CTT GGC TCC<br>2: GGT GCA ATC ACT CTT ACA ACA CC | 147 | 60 | 30 | 1.2 |
| Cisco-181 | $(GATA)_{36}$ | 1: GGT CTG AAT ACT TTC CAA ATG CAC<br>2: CCA TCC CTT TGC TCT GCC | 200 | 60 | 27 | 1.0 |
| Cisco-200 | $(GT)_{45(interrupted)}$ | 1: GGT TAG GAG TTA GGG AAA ATA TG<br>2: GTT GTG AGG TAG GCC TGG | 215 | 60 | 28 | 1.0 |
| BWF2* | $(CA)_{25}$ | 1: CGG ATA CAT CGG CAA CCT CTG<br>2: AGA CAG TCC CCA ATG AGA AAA | — | 55 | 30 | 1.0 |

* Developed for *Coregonus nasus,* Patton et al. 1997.

all genotyped individuals as a single gene pool (one group, $n = 203$); individuals grouped by sampling zone (two groups: the Willows and Caribou Islands); individuals pooled by a priori morphological classification (four groups: morphs A, B, C, and D); and individuals pooled by morph and by sampling zone (eight groups). Significant global (multilocus) heterozygote deficits were interpreted as indicative of a substructuration in the unit examined (Wahlund effect). Grouping of individuals on genetic characteristics alone was also assessed by examining the topology of a neighbor-joining tree using the shared-allele distance among individual genotypes ($D_{as}$, Chakraborty and Jin 1993) and by computing index of classification as described in Estoup et al. (1995a). The programs performing the neighbor-joining algorithm and calculating classification indices were written by J.-M. Cornuet (Laboratoire de modélisation et de biologie évolutive, INRA, Montpellier, France).

Once panmictic units were defined, exact tests of genic differentiation were performed globally and between pairs of units (GENEPOP 3.1, options 3.1 and 3.2, respectively) to compare both within- and between-zone differentiation among morphotypes. The results of the pairwise tests provided multilocus probabilities adjusted with the Fisher's method; to maintain the Type I probability error at $\alpha = 0.05$, these probability values were further adjusted by applying a Bonferroni-type correction (Rice 1989), that is, by dividing by the number of pairwise comparisons ($k$) computed for a given test.

The extent of genetic differentiation among units then was measured by hierarchical analyses of genetic variance using two estimators of the classical parameter $F_{ST}$ (Wright 1951), one that only considers allele frequency variation (θ of Weir and Cockerham 1984) and one that considers both allele frequency and allele size variation (φ of Excoffier et al. 1992). We used the Arlequin 1.1 software package (Schneider et al. 1997) to compute these two estimators, with morph and zone effects successively used as upper and lower hierarchical levels. Pairwise $\theta_{ST}$ and $\phi_{ST}$ values between units were also calculated with Arlequin 1.1; the $P$-values associated with $H_o$: $\theta = 0$ were determined by 2000 genotype permutations and were corrected for multiple comparisons (sequential Bonferroni procedure, cf above). Global θ- and φ-values were compared with a Wilcoxon matched pairs test performed with Statistica, version 5 (Statsoft 1997).

All mutation models except the infinite allele model (IAM) generate size homoplasy, and stepwise markers such as microsatellites are particularly prone to size homoplasy because all or a large proportions of newly arisen alleles are identical in size (but not by descent) to previously existing alleles. The extent of homoplasy is thus a consequence of the way new alleles are created, that is, of the mutation model. Compound and interrupted microsatellites are useful to detect size homoplasy (e.g., Estoup et al. 1995b; Garza et al. 1995), but because interrupted microsatellites show more deviation from the single-step mutation model (SMM) than perfect ones, they may actually be less homoplasious than perfect ones. Moreover, for a given mutation model, size homoplasy will be positively correlated to mutation rate and time since divergence between populations.

Indices of differentiation incorporating allele size differences (such as φ) indirectly assume SMM and it is still unclear how departure from this model and size homoplasy affect their calculation. Although it appears improbable that measures of genetic differentiation involving closely related and recently diverged populations such as in this study suffer from these potential biases (Angers and Bernatchez 1998; Estoup and Angers 1998), we have also performed the analyses of genetic differentiation separately on perfect (Cisco-157, Cisco-181, and BWF2) and imperfect (Cisco-90, Cisco-126, and Cisco-200) loci.

Finally, to ensure that our conclusions based on units defined by a priori morphological assignations were not biased by the imperfect morphological classification performed on the field, we conducted the genetic analyses using the a posteriori classification generated by the discriminant analysis as well as with a subset of individuals ($n = 175$) defining nonoverlapping clusters in the multivariate space.

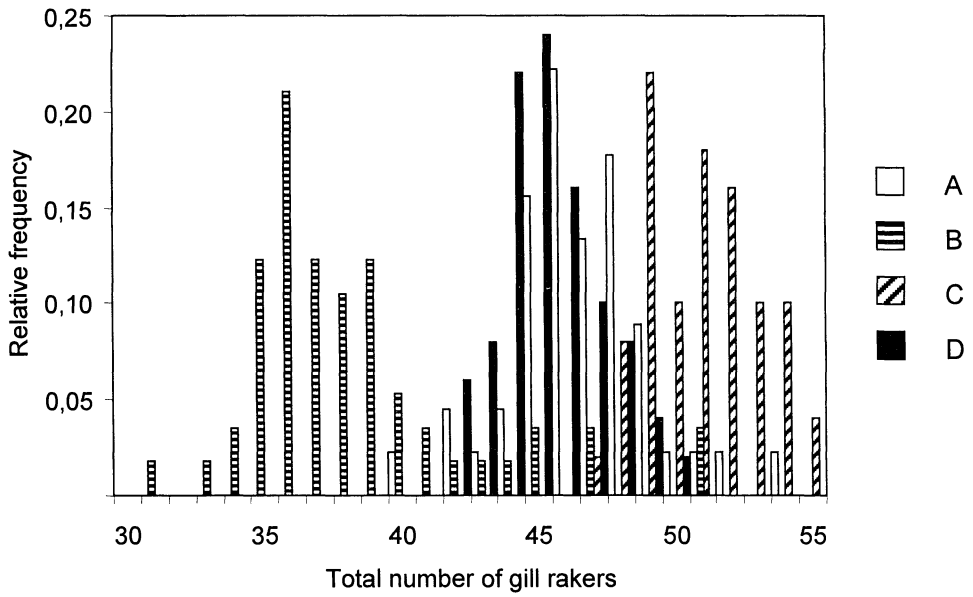


FIG. 3. Distribution of total number of gill rakers among cisco morphotypes of Lake Nipigon.

## RESULTS

### *Morphology*

Statistical analyses revealed significant differences among the four a priori morphotypes, indicating that the number of gill rakers and a subset of head characteristics were useful to discriminate among these morphotypes. Size-adjusted morphological characters were statistically different among morphs for all but two morphometric variables (UJW and GRL, all other variables: $P < 0.001$ for $\alpha = 0.05$ and $k_i = 16$), and lower and upper number of gill rakers were also significantly different ($P < 0.001$). Post hoc Scheffé pairwise comparisons suggest discriminant morphological characters among morphs: morph C has a longer snout, a longer head, and bigger eyes than all three other morphs, whereas morph D has a more vertical maxillary, a deeper body, and eyes located higher up on the head. With regard to gill-raker counts, morphs C and B are significantly different from each other as well as from A and D, which do not have significantly different numbers of rakers on either the upper or lower gill arches. The overall distribution of total gill-raker counts appears closer to a bimodal than a multimodal distribution (Fig. 3). The high variance in morph B total gill-raker counts is noteworthy (TGR: 31 to 51).

The first three components of the PCA accounted for 61% of the variation captured by the 18 morphological variables. Results of the PCA confirm that the most variable characters (highest loadings) are related to gill-raker number and spacing (PC1: LGR, UGR; PC3: IGS) and head characteristics (PC2: SNL, HDL, HDD, MXL, EYA). Discriminant analysis imposing the a priori classification confirmed the trends revealed by PCA. Individual clustering in the multivariate space defined by the first two discriminant roots shows good separation between morphs C and B on the basis of gill-raker counts, while A's and D's are totally overlapping with each other and partially with either C or B (Fig. 4). However, body depth, eye position, and maxillary orientation allowed to differentiate the latter two morphs on the second discriminant function (Fig. 4). The a posteriori classification matrix indicated that over 90% of morphs B, C, and D were correctly assigned to their group, whereas 77% of morph A were properly classified. Misclassified morph D were grouped with A; morph B and C were mostly assigned to morph A or D; and misclassified morph A grouped principally with morphs C or D. Geographical origin of individuals did not influence the proportion or type of misclassification. It is worth noting that individuals of morph B that stand out as outliers in the DA space are those possessing a high number of gill rakers (TGR $> 44$; Figs. 3, 4).

### *Depth and Trophic Niches*

Comparisons of ciscoes depth distribution of each morph revealed the distinct and deeper niche of morph D (Table 2A, Fig. 5A). Relative abundance of the four morphs in each depth stratum were significantly different in both sampling zones ($P < 0.0001$). Patterns of distribution somewhat differ between sampling zones, but trends are similar in each (Fig. 5A). Morphs A, B, and C were extremely rare in the deepest stratum, whereas morph D was more frequent in these deepest waters and totally absent above 50 m. Morph C was concentrated in midwater depth in both zones, whereas morphs A and B were more common in the shallows but also present in midwater layers. Depth niche overlap was higher when comparing a given morph between zones than a pair of morphs within a zone (mean $I_{n.o.} = 0.46$ and 0.22, respectively; $U = 6.0$, $P = 0.03$). In both zones, morphs A, B, and C shared a considerable portion of their depth niche, whereas morph D was strikingly differentially distributed (Table 2A, lowest niche overlap index).

Food items were identified and enumerated in 163 individuals proportionally distributed among morphs and sampling zones. The diet of Lake Nipigon ciscoes was dominated by five prey items, including plankton (Cladocera, Copepo-

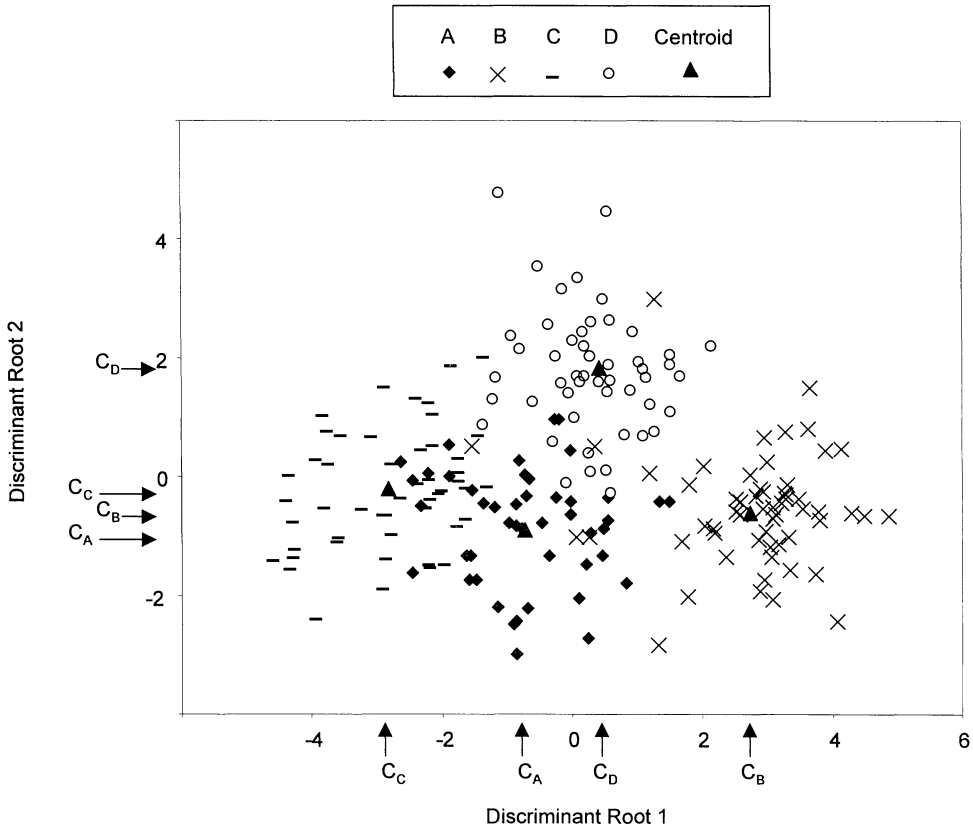


FIG. 4. Distribution of Lake Nipigon cisco individuals of four morphotypes in the multivariate space defined by the first and second discrimininant root functions describing variation in morphological traits. $C_A$, $C_B$, $C_C$, and $C_D$ refer to $x$ and $y$ coordinates of centroid positions (▲) for morphs A, B, C, and D, respectively.

da), epibenthos (Mysidae, Diporeidae), and benthos (Chironomidae larvae) (Fig. 5B). A variety of benthic prey were observed (insect larvae Odonata and Ephemeroptera, Hirudinae, Gasteropoda, Bivalvia, and fish larvae); although individuals of each morphotype had foraged on benthos, each benthic prey type was found in less than 10 individuals and usually at a very low occurrence. Occurrences of Mysidae and pooled miscellaneous benthos were not different among morphs in either bay, whereas occurrences of the remaining prey types were significantly different in at least one sampling zone ($P < 0.005$). When expressed in equivalent weight units corrected for occurrence, further diet differentiation was revealed among morphs (Fig. 5B). Although all morphs fed on epibenthic mysids, the diet of morphs A and D were more specific in including a substantial proportion of plankton and Diporeidae, respectively (Table 2B, Fig. 5B). Both morphs B and C had diets dominated by Mysidae, but morph C also fed on plankton around Caribou Islands. Pairwise comparisons of niche overlap based on corrected mean diets indicated that the differentiation is stronger among morphs than between sampling zones (mean $I_{n.o.}$ = 0.43 and 0.73, respectively), but these differences were not significant ($U$ = 10.0, $P$ = 0.09). Morph A occupied the most specific trophic niche (plankton), which was especially well differentiated from that of morph D.

TABLE 2. Schoener's index of (A) depth and (B) trophic niche overlap among Lake Nipigon cisco morphotypes (within zone: Caribou Island and the Willows above and below diagonal, respectively).

A

| Morpho-type | Depth niche overlap: Between zones | Within zone, among morphs: A | B | C | D |
|---|---|---|---|---|---|
| A | 0.60 | — | 0.45 | 0.48 | 0.13 |
| B | 0.23 | 0.34 | — | 0.25 | 0.02 |
| C | 0.51 | 0.43 | 0.37 | — | 0.10 |
| D | 0.51 | 0.03 | 0.02 | 0.07 | — |

B

| Morpho-type | Trophic niche overlap: Between zones | Within zone, among morphs: A | B | C | D |
|---|---|---|---|---|---|
| A | 0.51 | — | 0.12 | 0.52 | 0.12 |
| B | 0.93 | 0.36 | — | 0.59 | 0.62 |
| C | 0.60 | 0.30 | 0.93 | — | 0.60 |
| D | 0.88 | 0.29 | 0.51 | 0.50 | — |

### *Genetic Variation*

Genetic polymorphism at six microsatellite loci covered a wide range of variability, with four to 32 alleles per locus (Table 3). Observed heterozygosity within units (morph by zone) was generally high, ranging from 0.43 (Cisco-90) to 1.0 (Cisco-200).

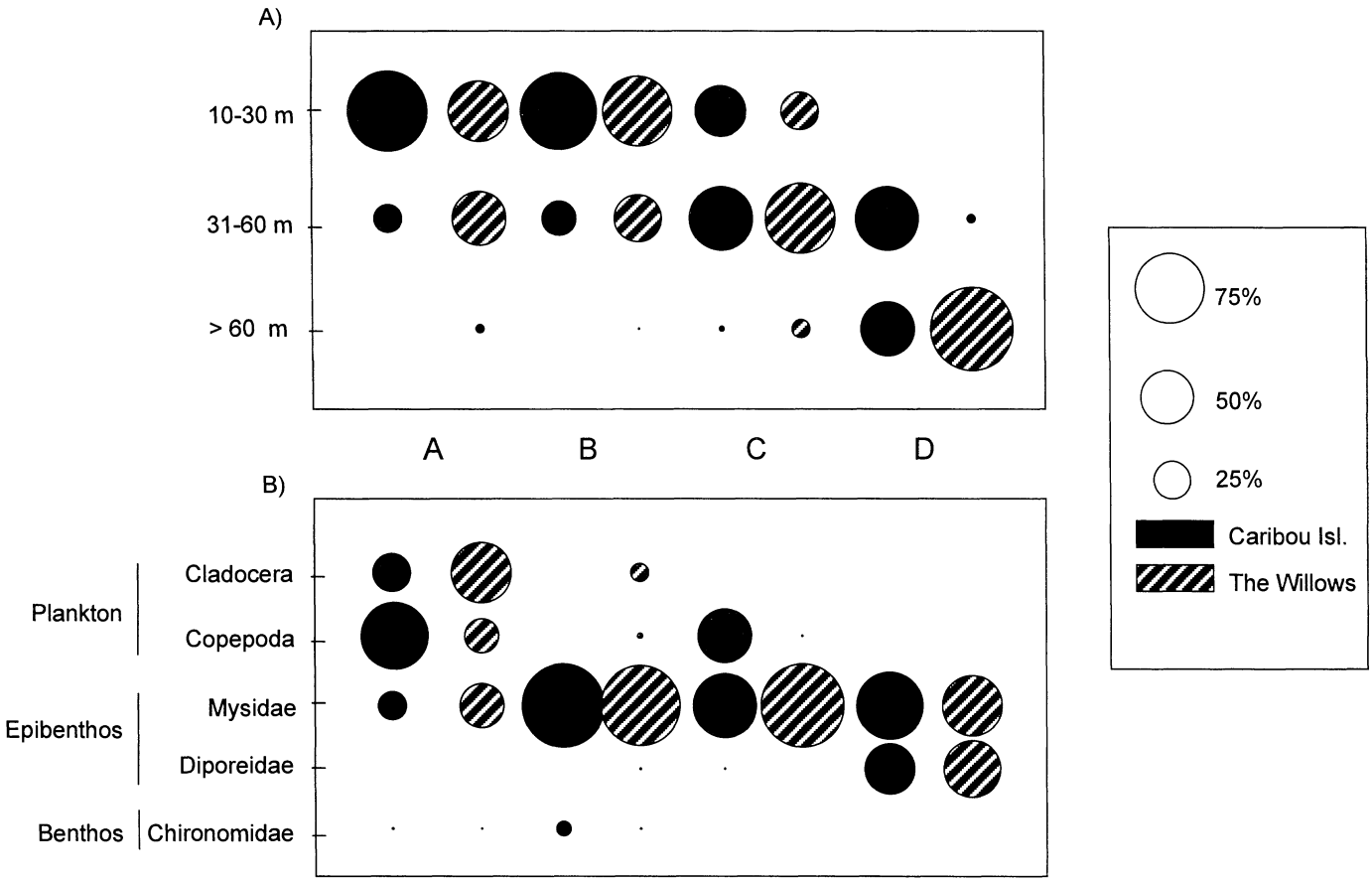


FIG. 5. Depth and trophic niches of Lake Nipigon cisco morphotypes from Caribou Islands and the Willows. (A) Depth niche: abundance per stratum corrected for equal sample sizes, $N_{tot} = 690$. (B) Trophic niche: mean diet composition expressed in equivalent weight units and corrected for the occurrence of each prey type.

The individual-based, neighbor-joining tree constructed with microsatellite data was densely and deeply branched and there were no distinct clusters (phenogram not shown). When crossed with a priori morphological assignations, classification indices were always very low, with only individuals of morph B grouping better than expected by chance ($I_c$ = 0.14, $P$ = 0.004 for morph B vs. $I_c < 0.05$, $P > 0.15$ for A, C, and D).

Table 4 indicates that the ciscoes of Lake Nipigon did not form a single homogeneous gene pool (morph and zone pooled, $P$ = 0.002). When a priori morphotype assignations were not considered (morph pooled), individuals from The Willows could constitute a panmictic unit (Table 4; $P$ = 0.106), but the significant heterozygote deficit in Caribou Islands (Table 4; $P$ = 0.002) is suggestive of a structure by morphotype at least in that last sampling zone. When grouped by morphotype only, morphs A, B, and C are in HWE. However, the test for heterozygote deficiency for morph D approached the adjusted probability level allowing rejection of $H_0$ ($k_i$ = 4), suggesting that spatial structuration may exist for that morph. Because both morphological and geographical factors cannot be clearly rejected as structuring factors in all cases, comparisons of individuals on the basis of both criteria were pertinent. This grouping scheme produced units that were all in HWE with no significant excess of homozygotes (Table 4, shaded area) and was retained for further analysis. In addition to defining panmictic units, this grouping scheme offered the possibility to track and evaluate the relative importance of each grouping criterion by using between zone comparisons for a given morph (zone effect) and among-morph comparisons within each zone (morph effect). Note that this grouping scheme was also supported by the ecological data, which suggest that differences in depth distribution and diet occur mainly among morphs but also between zones.

Results of the exact tests of genic differentiation are reported in Table 5A. Globally, morphs were highly differentiated in both zones ($P < 0.0001$). Differences in allele occurrences between zones were all nonsignificant, although the morph B and D comparisons were nearly significant. Within each zone, all pairwise comparisons involving morph B were significant or markedly near significance level. In the Willows, morphs A and D also had significantly different allelic arrays. When zones are pooled for each morph, morph B is significantly different from morph A, C, and D ($P <$ 0.004) whereas all comparisons not involving morph B are nonsignificant ($P > 0.03$).

Hierarchical analyses indicated that most genetic variation was within morph × zone units. For both hierarchical schemes, a low but significant proportion of the allelic variance could be attributed to differences among morphotypes

TABLE 3. Number of alleles (A), range of allele size (bp), observed heterozygosity ($H_o$: proportion of heterozygous individuals per sample), gene diversity ($H_e$, Nei 1987), and number of scored individuals ($n$) for each cisco morphotype and sampling zone of Lake Nipigon. W, the Willows; C, Caribou Islands.

| | Morphotype (sampling zone) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | | B | | C | | D | | |
| Locus | W | C | W | C | W | C | W | C | Global |
| Cisco-90 | | | | | | | | | |
| A | 4 | 3 | 4 | 4 | 3 | 4 | 5 | 5 | 9 |
| Range | 102–110 | 102–110 | 102–110 | 102–114 | 102–110 | 102–110 | 102–118 | 102–110 | 102–118 |
| $H_o$ | 0.542 | 0.667 | 0.429 | 0.591 | 0.640 | 0.480 | 0.560 | 0.680 | 0.581 |
| $H_e$ | 0.630 | 0.563 | 0.582 | 0.614 | 0.566 | 0.642 | 0.576 | 0.507 | 0.591 |
| $n$ | 24 | 21 | 14 | 44 | 25 | 25 | 25 | 25 | 203 |
| Cisco-126 | | | | | | | | | |
| A | 3 | 2 | 3 | 3 | 4 | 4 | 2 | 3 | 4 |
| Range | 167–202 | 167–202 | 167–202 | 167–202 | 167–206 | 167–206 | 167–202 | 167–202 | 167–206 |
| $H_o$ | 0.667 | 0.476 | 0.462 | 0.535 | 0.480 | 0.480 | 0.640 | 0.625 | 0.550 |
| $H_e$ | 0.546 | 0.511 | 0.568 | 0.578 | 0.490 | 0.518 | 0.465 | 0.536 | 0.530 |
| $n$ | 24 | 21 | 13 | 43 | 25 | 25 | 25 | 24 | 200 |
| Cisco-157 | | | | | | | | | |
| A | 8 | 11 | 6 | 9 | 8 | 9 | 8 | 11 | 15 |
| Range | 133–161 | 121–163 | 145–159 | 141–161 | 145–161 | 145–161 | 139–159 | 133–163 | 121–163 |
| $H_o$ | 0.652 | 0.810 | 0.714 | 0.762 | 0.760 | 0.960 | 0.840 | 0.875 | 0.799 |
| $H_e$ | 0.765 | 0.860 | 0.789 | 0.839 | 0.825 | 0.855 | 0.682 | 0.805 | 0.815 |
| $n$ | 23 | 21 | 14 | 42 | 25 | 25 | 25 | 24 | 199 |
| Cisco-181 | | | | | | | | | |
| A | 20 | 23 | 11 | 24 | 18 | 21 | 15 | 20 | 32 |
| Range | 176–288 | 168–292 | 172–236 | 180–304 | 180–280 | 168–256 | 176–248 | 168–252 | 168–304 |
| $H_o$ | 0.875 | 0.900 | 0.923 | 0.927 | 0.920 | 0.960 | 0.880 | 0.958 | 0.919 |
| $H_e$ | 0.946 | 0.968 | 0.945 | 0.941 | 0.923 | 0.956 | 0.929 | 0.941 | 0.946 |
| $n$ | 24 | 20 | 13 | 41 | 25 | 25 | 25 | 24 | 197 |
| Cisco-200 | | | | | | | | | |
| A | 14 | 17 | 15 | 21 | 16 | 16 | 17 | 16 | 32 |
| Range | 197–229 | 195–257 | 195–247 | 193–265 | 195–249 | 197–249 | 199–251 | 199–241 | 193–265 |
| $H_o$ | 0.955 | 0.900 | 0.909 | 0.950 | 1 | 0.880 | 0.920 | 0.870 | 0.926 |
| $H_e$ | 0.888 | 0.953 | 0.925 | 0.932 | 0.923 | 0.925 | 0.957 | 0.894 | 0.935 |
| $n$ | 22 | 20 | 11 | 40 | 22 | 25 | 25 | 23 | 188 |
| BWF2 | | | | | | | | | |
| A | 3 | 5 | 5 | 6 | 3 | 4 | 4 | 6 | 9 |
| Range | 153–159 | 147–159 | 149–163 | 151–161 | 153–157 | 149–157 | 151–157 | 149–161 | 147–163 |
| $H_o$ | 0.542 | 0.714 | 0.571 | 0.545 | 0.640 | 0.520 | 0.600 | 0.640 | 0.591 |
| $H_e$ | 0.765 | 0.860 | 0.789 | 0.839 | 0.825 | 0.855 | 0.682 | 0.805 | 0.588 |
| $n$ | 24 | 21 | 14 | 44 | 25 | 25 | 25 | 25 | 203 |
| Global | | | | | | | | | |
| Mean A | 8.7 | 10.2 | 7.3 | 11.2 | 8.7 | 9.7 | 8.5 | 10.2 | 16.8 |
| Mean $H_o$ | 0.705 | 0.744 | 0.668 | 0.718 | 0.740 | 0.713 | 0.740 | 0.775 | 0.728 |
| Mean $H_e$ | 0.757 | 0.786 | 0.766 | 0.791 | 0.759 | 0.792 | 0.175 | 0.748 | 0.734 |
| Mean $n$ | 23.5 | 20.7 | 13.2 | 42.3 | 24.5 | 25.0 | 25.0 | 24.2 | 198.3 |

(Table 6; $\theta = 0.016$, $P = 0.007$ and $\theta = 0.013$, $P = 0.0005$ for morph and zone as upper level, respectively), whereas variation between zones was not significant (Table 6: $\theta = 0$, $P = 0.623$ and $\theta = 0.002$, $P = 0.443$ for morph and zone as upper level, respectively). When the nonsignificant zone effect was removed (pooled zones for each morph), genetic variation among morphotypes remained significant ($\theta = 0.016$, $P < 0.0001$). Pairwise $\theta$ indicated that most of the global differentiation can be attributed to morph B (Table 5B). Regardless of whether zone is considered as a grouping criterion, all significant $\theta$-values concerned comparisons involving morph B; $\theta$-values involving morph B are small (range: 0.011–0.029), but are nevertheless larger than all others by nearly one order of magnitude (range: 0–0.004). When individuals of morph B were removed from the analysis, there was no significant differentiation among morphs or zones nor significant pairwise $\theta$s. Global analysis taking into account allele size differences ($\phi$) also showed that genetic variation was significant among morphs only (Table 6; $\phi = 0.018$, $P = 0.013$). However, none of the pairwise $\phi$-values were significant (results not shown), a result that may be explained by the higher variance of estimators considering allele size differences (Slatkin 1995, Estoup and Angers 1998). Global $\phi$-values were slightly larger than global $\theta$ values (Wilcoxon matched pairs test, $P = 0.046$) when all morphs are considered, but not so when morph B was excluded from the comparison ($P = 0.225$).

Tests conducted separately with perfect and interrupted

TABLE 4. Identification of panmictic units among Lake Nipigon ciscoes. Morph × zone units show no significant heterozygote deficit ($H_0$ = HWE and $H_1$ = heterozygote deficit). Asterisks indicate significant sequential Bonferroni-adjusted *P*-value).

| | Zone | | |
|---|---|---|---|
| Morph | Pooled | The Willows | Caribou Islands |
| Pooled | 0.002* | 0.106[a] | 0.002[a]* |
| A | 0.154[b] | 0.103[c] | 0.077[c] |
| B | 0.282[b] | 0.347[c] | 0.489[c] |
| C | 0.302[b] | 0.378[c] | 0.335[c] |
| D | 0.032[b] | 0.394[c] | 0.253[c] |

Bonferroni corrections:
[a] $k_i = 2$, $P = 0.025$.
[b] $k_i = 4$, $P = 0.0125$.
[c] $k_i = 8$, $P = 0.00625$.

TABLE 5. Genetic differentiation among Lake Nipigon ciscoes between and within sampling zone (Caribou Islands and the Willows above and below diagonal, respectively). (A) Pairwise multilocus *P*-values from Fisher exact tests. (B) Pairwise multilocus θ. Asterisks indicate significant sequential Bonferroni-adjusted *P*-values.

A

| | Between | Within zone, among morphs[b] | | | |
|---|---|---|---|---|---|
| Morph | zones[a] | A | B | C | D |
| A | 0.369 | — | 0.050 | 0.446 | 0.150 |
| B | 0.038 | 0.038 | — | 0.003* | $< 2 \times 10^{-5}$* |
| C | 0.703 | 0.214 | 0.089 | — | 0.191 |
| D | 0.050 | 0.005* | 0.038 | 0.150 | — |

B

| | Between | Within zone, among morphs[b] | | | |
|---|---|---|---|---|---|
| Morph | zones[a] | A | B | C | D |
| A | 0.007 | — | 0.011 | 0.001 | 0.002 |
| B | 0.005 | 0.016 | — | 0.029* | 0.025* |
| C | 0 | 0.002 | 0.018 | — | 0 |
| D | 0 | 0.004 | 0.020 | 0 | — |

[a] $k_i = 4$, $P = 0.0125$.
[b] $k_i = 6$, $P = 0.008$.

microsatellite loci, as well as those using the a posteriori morphological classification or a subset of individuals unambiguously representing each morph revealed the same patterns and extent of genetic differentiation (results not shown).

## DISCUSSION

### *Ecomorphology*

This study aimed to document the existence of different morphological types of ciscoes in Lake Nipigon and to verify the link between their morphology and ecology. Our data suggested that ecological differences parallel morphological distinctiveness. However, it revealed that gill-raker number alone may be misleading to predict cisco trophic ecology and that habitat must also be taken into consideration.

Global analysis of morphological characters supported the recognition of four cisco morphotypes in Lake Nipigon. On the basis of morphological characters, morphs A, B, C, and D can be equated to the description of Koelz (1927) and Scott and Crossman (1973) for *Coregonus artedi, C. zenethicus, C. nigripinnis*, and *C. hoyi* of Lake Nipigon, respectively. Atypical individuals of morphotype B may represent specimens of *C. nipigon*, although their gill raker counts were not as high as in the original description (TGR seldom less than 54; Koelz 1927). However, the poor genetic differentiation among these morphotypes does not support specific denominations, and letter code designations are retained hereafter.

As documented in other phenotypically polymorphic fish assemblages, the number of gill rakers was of the utmost importance for discrimination. It allowed us to clearly distinguish morphs B and C, but morphs D and A were characterized by similar number of gill rakers. Unexpectedly, morphs with most similar trophic morphology occupied the most distinct food niches (morphs A and D), indicating that it is not always possible to infer feeding habits from a given morphology. In many other fish species pairs, gill-raker traits have proven to be associated with specific trophic niches: small planktivorous prey are more easily captured by numerous long rakers, whereas larger benthic prey are amenable with fewer short and distant rakers (e.g., Larson 1976; Bodaly 1979; Bentzen and McPhail 1984; Malmquist et al. 1992). Our results confirmed that small planktonic prey are found almost exclusively in the diet of forms with numerous gill rakers (A and C), but also underscored the fact that these forms (e.g., morph D) are not necessarily foraging on small prey. Pelagic forms with numerous gill rakers are known to also forage on benthos in other sympatric species pairs (Larson 1976; Bodaly 1979; Malmquist 1992). Similar lack of association between gill-raker number and prey size has been documented for other coregonids (Sandlund et al. 1987), and cases are known of morphotypes with similar numbers of gill rakers that are segregated by food and depth (Hindar and Jonsson 1982). Numerous gill rakers may simply allow a wider range of potential prey by permitting retention of prey of any size, whereas few distantly spaced rakers may exclude smaller prey. In any case, caution should be taken to avoid inferences on foraging habits based solely on putatively determinant morphological features such as gill rakers. Differences in gill-raker counts between sympatric forms of ciscoes have been documented in several interior lakes in Canada (Clarke 1973; Hénault and Fortin 1989), but without proper

TABLE 6. Hierarchical analysis of genetic variance among Lake Nipigon ciscoes. θ considers allele frequency variation and ϕ considers both allele frequency and size variation. (A) Morphology as upper level. (B) Sampling zone as upper level.

A

| Source of variation | θ | *P* | ϕ | *P* |
|---|---|---|---|---|
| Among morphs | 0.016 | 0.007 | 0.018 | 0.013 |
| Between zones, within morph | 0 | 0.623 | 0 | 0.599 |
| Within units | 0.014 | < 0.0001 | 0.014 | 0.035 |

B

| Source of variation | θ | *P* | ϕ | *P* |
|---|---|---|---|---|
| Between zones | 0.002 | 0.412 | 0 | 0.443 |
| Among morphs, within zone | 0.013 | 0.0005 | 0.010 | 0.014 |
| Within units | 0.011 | < 0.0001 | 0.013 | 0.048 |

verification of the actual diet of these forms, care should be taken to avoid equating occurrence of sympatric morphotypes with that of trophic ecotypes. More importantly, these cases should not be readily used as supportive evidence for important evolutionary processes such as character displacement (e.g., Schluter and McPhail 1993). Lack of correspondence between morphology and trophic ecology warns against generalized inference because the latter may reflect developmental correlations and/or ancestry as much as ecology.

Crossing information on food and depth niches also confirmed the importance of vertical habitat segregation in ciscoes. As for morphs A and D in Lake Nipigon, contrasted depth distribution was one of the clearest discriminating criteria among ciscoes of the other Great Lakes, where it apparently constituted a factor of reproductive isolation (reproductive allopatry, Smith and Todd 1984). Maximum lake depth was also the only environmental variable correlated with the occurrence of multiple cisco forms in the "*C. artedi* complex" of interior lakes in Canada (Clarke 1973). Deep waters offer the ecological opportunity of a competition-free habitat that has never been conquered by any other coregonids, and these additional epibenthic niches may have constituted avenues for intralacustrine divergence at time of colonization. Speciation involving ecological differentiation or isolation-by-distance seems to be particularly common in deep lacustrine environments (Yampolski et al. 1994; Väinöla 1995; Martens 1997). The physiological ability of ciscoes to exploit the very deep sections of large lakes may be a key factor in explaining why multiple ciscoes forms exist, while most other fish species have one or two ecotypes. However, this hypothesis remains to be evaluated.

### *Genetic Variation*

Although characterized by high levels of polymorphism, microsatellite loci did not detect distinct gene pools corresponding to the four morphotypes identified in Lake Nipigon. However, the genotypic composition of all sampled individuals allowed us to reject the hypothesis of a single gene pool in Lake Nipigon and indicated that spatial and ecomorphological factors must be taken into account. The greater importance of ecomorphological over spatial factors was supported by the fact that genetic variation among morphs was significant, whereas that between zones was not (Table 6). This differentiation persisted when morphotypes (defined by a priori, a posteriori, or forming nonoverlapping multivariate clusters) from different zones were pooled, indicating that differentiation among morphs was not an artefact of small sample sizes or morphological assignation criterion.

However, the observed level of differentiation was low, and only morph B could be clearly identified as genetically distinct. The low levels of differentiation among Lake Nipigon cisco morphotypes may be attributed to a combination of molecular, biological, and historical factors. Differentiation indices are expected to be lower with hypervariable loci such as microsatellites relative to those obtained with less polymorphic markers such as enzymatic loci (Jin and Chakraborty 1995; Slatkin 1995; Rousset 1996; Estoup and Angers 1998; Estoup et al. 1998). However, microsatellites have successfully been used to detect a wide range of differentiation levels, revealing strong genetic differentiation at both local (e.g., Angers and Bernatchez 1998; Estoup et al. 1998) and large (e.g., Paetkau et al. 1995; Brunner et al. 1998) geographical scales, as well as contrasted levels of differentiation at the intraspecific level (Estoup et al. 1995a; Tessier et al. 1997; Goodman 1998). More importantly, significant but low levels of differentiation comparable to those in this study have also been documented in other systems analyzed with microsatellite markers (Allen et al. 1995; García de León et al. 1997; van Oppen et al. 1997). In the case of the Lake Malawi mbuna cichlids, low differentiation values characterized color morphs that were effectively reproductively isolated, as indicated by positive assortative mating (Van Oppen et al. 1997, 1998).

The small values of differentiation indices among Lake Nipigon cisco morphotypes are likely to reflect a very recent process of divergence and/or substantial gene flow among morphs. Divergence postdating the last glacial retreat (10,000–15,000 years ago) should be sufficiently recent to yield congruent estimation between $\theta$ and $\phi$, whereas higher $\phi$-values are expected for more ancient divergences with low gene flow (Slatkin 1995; Estoup et al. 1998; Goodman 1998). Such comparisons among populations of postglacial fishes indeed reveal divergence dating from distinct periods (Bernatchez et al. 1998; Estoup and Angers 1998; Estoup et al. 1998; Tessier and Bernatchez, unpubl. ms.). In Lake Nipigon, $\theta$- and $\phi$-values were similarly low when morph B was excluded from the comparison of $\theta$ and $\phi$, indicating that few mutations have shaped differences among morph A, C, and D due to a very recent divergence time with any level of gene flow or to an older divergence with high gene flow. The slightly higher values of $\phi$ when morph B was included in this $\theta$- vs. $\phi$-comparison suggest that this morph may be derived from a relatively more divergent lineage that subsequently exchanged genes with morphs A, C, and D at a lower rate.

Alternatively, but not exclusively, it is possible that detection of little differentiation is due to substantial (current or past) gene flow preventing rapid genetic differentiation among morphs. Contrary to several species where sympatric ecotypes are genetically distinct (Foote et al. 1989; Vespoor and Cole 1989; McPhail 1991; Hindar and Jonsson 1993), ciscoes do not exhibit assortative mating behavior (courtships, nest building, and defense), and their broadcast spawning may retard the development of reproductive isolation (Dominey 1984).

### *Evolutionary History*

The ecological theory of adaptive radiation predicts that phenotypically differentiating taxa should be distinguished by characters associated with resource exploitation and that partial or complete reproductive isolation should exist among ecotypes. In that context, Smith and Todd (1984) envisioned that intralacustrine ecological divergence within two colonizing lineages of ciscoes explained the extensive ecophenotypic diversity of the GL cisco fauna. This evolutionary scenario was only partly supported by the present study. As expected, morphotypes were identified and their trophic and

depth niches were contrasted at various levels. However, there was no evidence of strong reproductive isolation among most of these morphotypes. Although the lack of genetic differentiation does not formally exclude current reproductive isolation, most other fish species pairs where there are behavioral indications of reproductive isolation have generated significant values of genetic differentiation statistics (Foote et al. 1989; Vespoor and Cole 1989; McPhail 1991; Hindar and Jonsson 1993). Extensive morphological and/or ecological variation among morphs A, C, and D can thus be regarded as plasticity within a single gene pool, although a very recent ecological divergence among morphs A, C, and D remains plausible because morphological differentiation coincides with contrasting diets and/or depth preferences. The low but significant genetic differentiation of morph B cannot readily be attributed to current ecological divergence because this morph did not show strong trophic or depth niche specialization. However, the global $\theta$ vs. $\phi$ comparison including morph B is suggestive of a historical differentiation and could give credence to the hypothesis of Smith and Todd (1984), who proposed that the two lineages of ciscoes having colonized the Great Lakes basin correspond to *C. zenethicus* (morph B) and *C. artedi* (morph A). Indeed, the identity of these two colonizing lineages is supported on one hand for *C. zenethicus* (morph B) by its apparently older divergence from all other morphs and on the other hand for *C. artedi* (morph A) by its broad distribution in North American interior lakes. The low but significant current genetic differentiation of morph B could either be due to the low level of genetic divergence between the colonizing lineages prior to their secondary contact and/or to recent gene flow since then.

Based on our data, the most parsimonious scenario thus consists in secondary contacts between a B and an ACD lineage and phenotypic plasticity within the latter. Ecological speciation could not be confirmed due to the absence of strong reproductive isolation and the lack of strong relationship between morphology, ecology, and the degree of genetic differentiation. However, ecological divergence was obvious, and incipient ecological divergence leading to reproductive isolation and speciation by depth habitat and trophic niche partitioning among morphs A, C, and D cannot be dismissed. Consequently, if the (complete) speciation criterion is decoupled from that of invasion of multiple ecological niches accompanied by phenotypic divergence in the definition of adaptive radiation (Givnish 1997), the GL ciscoes certainly qualify as demonstrating this evolutionary phenomenon. Their ecomorphological polymorphisms are associated with the invasion of an overall adaptive zone that is certainly wider than that of most other postglacial fish groups. For freshwater fish, the occurrence of a geographically localized (intralacustrine or intrabasin) adaptive radiation is implicit to the concept of species flock, which is mainly defined on the basis of three criteria: endemism, geographical circumscription, and monophyly (Greenwood 1984). Although our data question the monophyletic origin of the Lake Nipigon ciscoes assemblage, the GL ciscoes compare with the small *Cyprinodon* flock of Laguna Chichancanab (Humphries and Miller 1981; Strecker et al. 1996). Both include a low number of species/morphotypes that are incompletely differentiated morphologically (intermediate phenotypes are noted) and ecologically. In both flocks, physical evidence of a very recent habitat origin is congruent with low genetic differentiation that cannot easily be parted from incomplete lineage sortage, plasticity and hybridization. These two small flocks contrast with the old habitat harboring the Lake Baikal cottoid and the African Rift Lakes cichlid species flocks, which contain several and phenomenal numbers of species, respectively. In the Lake Baikal Cottoids, phylogenetic grouping coincides with an obvious habitat partitioning by depth and an adaptive shift affecting vision under low illumination (Bowmaker 1994; Hunt et al. 1997). The East African cichlids species group likewise segregate by habitat (substrate) but have also invaded an incredible variety of trophic niches thanks to their innovative trophic apparatus (pharyngeal jaw; Fryers and Iles 1972). Moreover, unlike in any other fish flocks, it appears that sexual selection has fostered rapid phenotypic divergence within several cichlid subclades (Dominey 1984; Seehausen et al. 1997; Van Oppen et al. 1998).

These comparisons suggest that the many factors promoting adaptive radiation (e.g., ecological opportunity, competitive release, key evolutionary innovations) are not exclusive and that the combination of these factors influence the scale of extant fish species flocks. With regard to the criterion of monophyly in species flocks, that of younger flocks is difficult to assess for technical and temporal reasons, but it is also plausible that hybridization play a determinant role in the early stages of these radiations. In lieu of being the driving forces of divergence, as proposed by the ecological speciation theory, the extensive morphological and ecological polymorphisms observed in young flocks such as the GL cisco assemblage could result from the admixture of differentiated lineages whereby increased genetic variance produced the phenotypic variability necessary for the exploration and invasion of a breadth of niches not accessible to the original forms (Anderson 1953; Arnold 1997). Invasion of unoccupied niches by hybrids is well documented by botanists (e.g., Cruzan and Arnold 1993; Rieseberg and Wendel 1993; Baldwin 1997), and evidence of hybridization/introgression is documented in many animal species assemblages where an adaptive radiation is suspected (Carson et al. 1989; Grant 1993; Colbourne et al. 1997). Moreover, DeMarais et al. (1992) have clearly demonstrated that the minnow *Gila seminuda* originated through the hybridization of *G. robusta* and *G. elegans*, and there is evidence that introgressive hybridization has been a diversifying agent in the evolution of this morphologically polymorphic fish genus (Dowling and DeMarais 1993).

Several observations indicate that hybridization and introgression have also played a crucial role in the evolutionary history of coregonids. On the basis of contrasting patterns of phenotypic variation following transplant experiments involving sympatry or allopatry, Svärdson (1957, 1970) proposed that introgression was the main factor in the postglacial evolution of *Coregonus*. Smith (1964) also suspected that increasing difficulties in species identification were due to hybridization among ecologically stressed cisco taxa of Lake Michigan in the 1960s. Interfertility among some of the GL ciscoes has been experimentally documented (Garside and Christie 1962; T. Todd, pers. comm.), and natural occurrence of hybridization between GL cisco species is plausible, as

hypothesized by Todd and Stedman (1989) on the basis of merging gill-raker counts in *C. artedi* and *C. hoyi* in Lake Huron following ecological and demographic perturbations. Finally, extremely biased sex ratios observed in *C. hoyi* of Lake Michigan (Brown 1970; Bowen et al. 1991) can also be interpreted as an indication of hybridization and lower viability in the heterogametic sex (Haldane's rule; Phillips and Erhlinger 1995). In our study, morphological similitudes in gill raker-counts between morphs A and D, as well as skewed female:male ratios (5:1 and 15:1 for morph A and D, respectively) are congruent with an evolutionary scenario involving exchange between genetically differentiated cisco lineages.

Contrasting genetic signatures are expected in an ecomorphologically diverse assemblage resulting from (ecological) divergence and multilineage contact/hybridization. The best possible evidence should be provided by the characterization of additional populations of sympatric and allopatric cisco forms with complementary mitochondrial and nuclear markers to compare their genetic structure and establish their phylogenetic relationships. Because previous studies using mtDNA have not documented species-specific markers (Sajdak and Phillips 1997; Reed et al. 1998) and revealed limited intraspecific polymorphisms among regional populations (Bernatchez and Dodson 1990; Shields et al 1990; Snyder et al. 1992), a wide geographic survey may be required to sort out the evolutionary phenomena that have shaped the remarkable cisco phenotypic diversity. Although the cisco assemblage that once existed in the other North American Great Lakes has practically vanished, occurrences of sympatric forms of the "*C. artedi* complex" are still reported in several interior lakes. Morphological and genetic characterizations of several sympatric and allopatric populations are currently being achieved in our laboratory. We believe that the results from these analyses will shed light on the unusual evolutionary history of this puzzling group of temperate fish.

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