

Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences

CAROL A. STEPIEN and JOSEPH E. FABER*

Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106–7080, USA

Abstract

Mitochondrial (mt) DNA control region sequences were used to test the genetic and phylogeographic structure of walleye *Stizostedion vitreum* populations at different geographical scales: among spawning sites, lake basins, lakes, and putative glacial refugia in the Great Lakes region. Sequencing 199 walleye revealed nucleotide substitutions and tandemly repeated sequences that varied in copy number, as well as in sequence composition, in \approx 1200 bp of the mtDNA control region. Variable numbers of copies of an 11-bp tandem repeat showed no geographical patterning and were not used in further analyses. Substitutions in the other areas of the control region yielded 19 haplotypes, revealing phylogeographic structure and significant differences among glacial refugia, lakes, basins and some spawning sites. Differences among spawning populations were consistent with reduced gene flow, philopatry and possible natal homing. Analysis of spawning populations showed consistency of genotypic frequencies among years and between males and females, supporting philopatry in both sexes. The unglaciated plateau in southern Ohio, USA housed a very different haplotype that diverged prior to the Missouri, Mississippi and Atlantic glacial refugia types. Haplotypes from the three refugia colonized the Great Lakes after retreat of the Wisconsin glaciers, and their present distribution reflects the geography of their prior isolation and differential colonization. Populations that became associated with spawning localities appear to have diverged further due to philopatry, resulting in fine-scale phylogeographic structuring.

Keywords: control region, Great Lakes, mtDNA, Percidae, *Stizostedion vitreum*, walleye

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Introduction

When barriers to gene flow disrupt the 'genetic glue' linking populations, genetic divergence usually occurs over time. Geographic, temporal and reproductive isolation are primary barriers that produce changes in genetic composition. The primary phylogeographic determinant that shaped modern-day populations in the North American Great Lakes region was their geographical isolation in southern refugia during the Pliocene and Pleistocene Ice Ages, and subsequent colonization of the newly formed Great Lakes about 12 000 years ago (Scott & Crossman

1973; Bolsenga & Herdendorf 1993; Bernatchez & Wilson 1998). Populations of fishes from the Great Lakes today are believed to be composed of a genetic admixture from the Missouri, Mississippi and Atlantic glacial refugia (Ward *et al.* 1989; Billington *et al.* 1992; Murdoch & Hebert 1997).

Considerable genetic diversity has been discerned in the walleye *Stizostedion vitreum vitreum* (Teleostei: Percidae) in the Great Lakes region. Broad-scale genetic structure among glacial refugia and some of the Great Lakes was revealed in studies of mitochondrial (mt) DNA restriction fragment length polymorphisms (RFLPs; Billington & Hebert 1988; Ward *et al.* 1989; Billington *et al.* 1992) and allozymes (Ward *et al.* 1989; Todd 1990; Todd & Haas 1993). Fine-scale patterning was discerned among two of three spawning sites in Lake Erie from mtDNA RFLP data of the control region and ND3/4 genes by Merker & Woodruff (1996). A preliminary study by

Correspondence: C. A. Stepien. Fax: +1-216-368-4672; E-mail: cas20@pop.cwru.edu

*Present address: West Virginia University, Parkersburg, West Virginia 26101, USA.

Faber & Stepien (1997) identified further variation among additional spawning sites in Lake Erie from sequencing the entire mtDNA control region. The present study tested for patterns of genetic divergence among glacial refugia, lakes, basins and spawning populations in order to link the previous broad- and fine-scale studies. The purpose was to provide a framework for interpreting the phylogeographic history of the Great Lakes region and to evaluate the factors regulating population genetic structure of fishes.

In addition to historic and present physiographic barriers to genetic mixing, natal homing (the tendency for individuals to reproduce at the sites where they were born), constitutes a behavioural barrier that reduces random mating and produces spatial patterns of population divergence. Natal homing and resulting population genetic divergence have been described for several aquatic animals, including: Atlantic and Pacific species of salmon (Hasler & Wisby 1951; Harden Jones 1968; Hasler & Scholtz 1983; Gyllensten & Wilson 1987; Stahl 1987; Billington & Hebert 1991), the rainbow trout *Oncorhynchus mykiss* (Nielsen *et al.* 1997), the oceanic loggerhead turtle *Caretta caretta* and green turtle *Chelonia mydas* (Bowen *et al.* 1994; Bowen & Karl 1997) and the humpback whale *Megaptera novaengliae* (Baker *et al.* 1990). Natal homing has been suspected for walleye on the basis of tagging data (Colby *et al.* 1994; Jennings *et al.* 1996). The question of whether fine-scale genetic divergences among natural spawning populations of walleye may be due to philopatry (site faithfulness) and natal homing was examined in this study.

Life history of walleye and the study area

The walleye's greatest population sizes and native distribution centre are in the Great Lakes (Scott & Crossman 1973). Spawning localities and ecologically separable regions, such as lake basins (Fig. 1), have been postulated to house genetically divergent groups of walleye (Todd 1990; Todd & Haas 1993; Colby *et al.* 1994; Jennings *et al.* 1996). Genetic studies of walleye prior to investigations of rapidly evolving regions of mtDNA (Stepien 1995; Merker & Woodruff 1996; Faber & Stepien 1997) lacked the resolving power to evaluate fine-scale structure (e.g. Billington & Hebert 1988; Ward *et al.* 1989; Todd 1990; Billington *et al.* 1992; Todd & Haas 1993; Billington & Strange 1995).

Walleye spawn during the spring months in the Great Lakes region, migrating to historical spawning grounds shortly after the ice breaks up (Scott & Crossman 1973; Colby *et al.* 1994). Some populations migrate up rivers, whereas others spawn exclusively on rocky reefs in lakes (Colby *et al.* 1994; Jennings *et al.* 1996). A recent study by Jennings *et al.* (1996) experimentally supported natal homing of walleye in laboratory-reared released broodstock of river-spawning vs. reef-spawning populations.

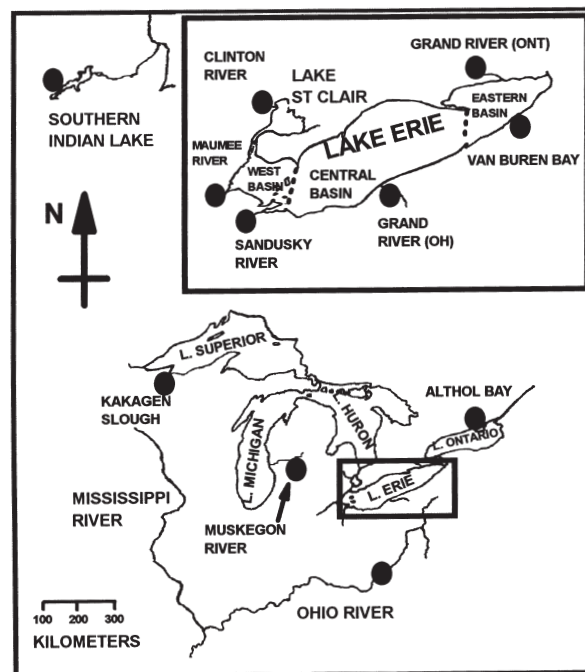


Fig. 1 Sampling sites (closed circles) for walleye (*Stizostedion vitreum*), including spawning sites in Lakes Erie and St Clair, and sites in the other Great Lakes, Southern Indian Lake (Manitoba, Canada) and the Ohio River.

That investigation found that natal homing in spawning walleye is governed by a genetically based response to environmental cues (Jennings *et al.* 1996). The eggs of walleye hatch in about 2 weeks and the larvae disperse into the open water. By late summer, the juveniles move into deeper areas of the lakes (Scott & Crossman 1973). Tagging studies indicate that walleye move readily between lakes during the summer months (Nepszy *et al.* 1991), including between Lakes St Clair and Erie (Todd 1990; Todd & Haas 1993) and among the three basins of Lake Erie (M. Turner and R. Knight, Ohio Division of Wildlife, personal communication).

Approach and objectives

This study examined sequence substitutions in the mtDNA control region (or D-loop) of walleye in Lakes Erie and St Clair, where Billington *et al.* (1992) identified the greatest mtDNA RFLP diversity in the Great Lakes. We linked prior mtDNA RFLP studies with the present data set by sequencing the three primary glacial refugia types identified in the Great Lakes (representing the Atlantic, Mississippi and Missouri refugia; Ward *et al.* 1989; Billington *et al.* 1992) and the same individuals tested by Merker & Woodruff (1996) for three spawning sites in Lake Erie. The objectives of the present investigation were to test the following: (i) phylogeographic patterns at hierarchical scales among the three

glacial refugia, an unglaciated region from the Ohio River, the Great Lakes and lake basins; (ii) genetic variability within and among spawning populations in order to evaluate possible natal philopatry; (iii) whether males and females differed in spawning site fidelity; (iv) whether the genetic composition at spawning sites varied between years; and (v) relationships of blue morphotypes. Blue-coloured walleye were tested due to continued speculation about the genetic identity of the extinct blue pike subspecies *S.v. glaucum*, which was endemic to the deeper areas of Lake Erie and Ontario prior to 1960 (Scott & Crossman 1973; Trautman 1981).

Materials and methods

Sampling

Walleye ($N = 179$) were analysed from six spawning sites in Lakes Erie and St Clair (Fig. 1) that have historic yearly spawning runs. Spawning sites included the Clinton River (Lake St Clair), the Maumee and Sandusky Rivers (the western basin of Lake Erie), the Grand River, Ohio (the central basin of Lake Erie), and the Grand River, Ontario and Van Buren Bay reef (the eastern basin of Lake Erie; Fig. 1). A previous study (Faber & Stepien 1997) evaluated the use of mtDNA control region sequences for discerning population differences among 117 individuals from the 1993 spawning run for five of these sites, and provided the basis for further and more comprehensive analysis in the present investigation. New specimens in the present study included the Grand River, Ontario site, additional males from all spawning sites (enabling differences among the sexes to be tested), and samples from the 1995 spawning runs (allowing tests for consistency of genetic composition in comparison to the groups spawning in 1993). Three blue-coloured walleye (one each from Lake Erie, Lake Huron, and Lake Nippising, Ontario) were tested for divergence from the common yellow phenotype. Eleven walleye were tested from the Ohio River (Fig. 1) to determine their relationship to samples from Lakes Erie and St Clair and to test their possible historic separation.

mtDNA samples from the three primary RFLP biogeographic types of Great Lakes walleye (Ward *et al.* 1989; Billington *et al.* 1992) were obtained from N. Billington. These included the Missouri refugium type 'C' from Southern Indian Lake, Manitoba ($N = 1$) and Kakagen Slough, Lake Superior ($N = 2$), the Mississippi refugium type 'B' from Muskegon River, Lake Michigan ($N = 2$), and the Atlantic refugium type 'A' from Althol Bay, Lake Ontario ($N = 1$; Fig. 1). DNA samples from the 1993 spawning runs in the Maumee ($N = 15$), Sandusky ($N = 19$) and Grand ($N = 15$) Rivers were provided by Merker & Woodruff (1996), enabling

us to test the same individuals. New specimens for our project were collected by trap net, electroshocking, or hook and line. Tissues (fin, muscle, eggs, or liver) were either immediately frozen at $-80\text{ }^{\circ}\text{C}$ or preserved in 95% ethanol in the field.

DNA extraction, amplification and sequencing

DNA was extracted and purified from the samples following methods previously described (Stepien 1995). The entire mtDNA control region was amplified in three sections using conserved primers (Kocher *et al.* 1989; Meyer *et al.* 1990) and the polymerase chain reaction (PCR). The 5' end or 'left' domain of the control region from tRNA-Pro to the central conserved section was amplified using the oligonucleotide primers L15926, 5'-TCAAAGCTTACCAGTCTTGTAACC-3' (Kocher *et al.* 1989) and H16498, 5'-CCTGAAGTAGGAACCAGATG-3' (Meyer *et al.* 1990). The 3' end or 'right' domain of the control region from the central conserved section to tRNA-Phe was amplified with the light-strand complement of H16498, L16498 5'-CATCTGGTTCCTACTTCAGG-3' and H503 5'-GCACGAGATTTACCAACCC-3' (Titus & Larson 1995). The central conserved section (167 bp) was amplified with custom primers designed from sequences conserved among species of the family Percidae; L16378, 5'-AATGTAGTAAGAGCCTA-3' and H16578, 5'-GGGTAAACGAGGAGTATG-3' (Faber & Stepien 1997). Heavy-chain primers were end-labelled with biotin (Hultman *et al.* 1989) and the DNA strands were separated using Dynabeads M-180 streptavidin (DynaL Corp.) for single-strand sequencing (Hultman *et al.* 1989; Uhlen 1989). Both strands were sequenced separately using diluted PCR primers with Sanger di-deoxy sequencing (Sanger *et al.* 1977) and Sequenase version 2.0 kits (Amersham/U.S. Biochemical). Sequencing reactions were run on 6% polyacrylamide gels for 2, 5 and 8 h in order to resolve ≈ 600 bp from the primer, and bands were visualized by autoradiography. Approximately 25% of the samples were sequenced more than once for verification.

Population genetic analysis

DNA sequences were read into a Macintosh computer using an IBI/Kodak digitizer and were aligned with other percid species (Faber & Stepien 1997, 1998). Levels of inter- and intrapopulation genetic diversity were quantified by indices of haplotype diversity (\hat{h} ; Nei & Tajima 1983; Nei 1987) and by the maximum-likelihood estimation of the average number of nucleotide substitutions per site within (nucleotide diversity, π ; Nei 1987) and among population groups (nucleotide divergence, d_{xy} ; Nei & Tajima 1983) using the program DA in REAP (restriction enzyme analysis package) version 4.0 (McElroy *et al.* 1992).

Relationships among mtDNA haplotypes were assessed using genetic distance analysis of percentage sequence divergence among haplotypes in the program MEGA (molecular evolutionary genetics analysis, version 1.01; Kumar *et al.* 1993). Kimura (1980) 2-parameter genetic distances were used to correct for multiple substitutions per site and different substitution rates between transitions and transversions (Kumar *et al.* 1993). Standard errors of distances were computed following Kumar *et al.* (1993). A distance neighbour-joining (NJ) tree (Saitou & Nei 1987) was constructed using MEGA (Kumar *et al.* 1993) and support for individual nodes was evaluated with 1000 bootstrap replicates (Rzhetsky & Nei 1992). The sauger, *Stizostedion canadense*, was used as the outgroup for rooting the phylogenetic trees, because previous studies showed that it is the sister species (Billington *et al.* 1991; Faber & Stepien 1998). Maximum parsimony analyses of relationships among haplotypes were conducted using PAUP* (version 4.0.0, d61; Swofford 1998) with the branch and bound algorithm, 50% majority rule consensus analysis of the most parsimonious trees and 1000 bootstrap replications (Swofford *et al.* 1996).

Genetic divergence times were calibrated with estimates from mtDNA RFLPs for walleye (Ward *et al.* 1989; Billington *et al.* 1992) and with those for the percid genus *Gymnocephalus* from mtDNA control region sequences (Stepien *et al.* 1998). In the absence of a fossil record, divergence times are often calibrated with related taxa and other data sets (Avice 1994). A calibration of 2% sequence divergence per million years appears to be accurate for areas of the mtDNA control region other than the tandemly repeated copies, for walleye and other percids (Faber & Stepien 1998; Stepien *et al.* 1998). This calibration matches estimates from Billington *et al.* (1992) for whole mtDNA RFLPs of walleye and corresponds to an average rate for the mtDNA molecule. This rate is slower than that of the mtDNA control region in mammals (reviewed in Avice 1994), but average for fishes, whose mtDNA evolves more slowly (Bernatchez *et al.* 1992; Bernatchez & Danzmann 1993; Stepien & Kocher 1997), apparently related to poikilothermy (Martin & Palumbi 1993; Avice 1994).

Geographic heterogeneity in frequency distributions of haplotypes was analysed using a Monte Carlo simulation approach with 10 000 randomizations to account for small sample sizes and empty cells in the contingency matrix (Roff & Bentzen 1989), using the MONTE option in REAP (version 4.0; McElroy *et al.* 1992). A Bonferroni correction that divided the probability value by the number of pairwise comparisons (Cooper 1968) was used for multiple *post hoc* tests (Fry *et al.* 1993) between pairs of sampling sites. Tests for differences in haplotype distribution between the sexes and between the sampling years 1993 and 1995 were conducted for tributary

spawning sites in the Maumee, Sandusky and Grand (Ohio) Rivers, and the reef site in Van Buren Bay.

AMOVA (analysis of molecular variance, version 1.55; Excoffier 1995) was used to test the hierarchical partitioning of genetic variability among spawning localities, basins and lakes. AMOVA analyses utilized the number of mutational events among haplotypes as a Euclidian distance measure, *F*-statistic analogues (Φ_{ST} ; Reynolds *et al.* 1983) and 1000 permuted matrices to test their significance (Excoffier *et al.* 1992). Populations (spawning sites) and groups (basins and lakes) were considered significantly different from one another if the measured variance was lower than 95% of the variance in the null distribution (Excoffier *et al.* 1992). Separate AMOVA runs (Excoffier 1995) tested for hierarchical partitioning between lakes and spawning sites, and for lakes and basins.

Results

Phylogenetic and geographical relationships among haplotypes

The complete mtDNA control region consensus sequence for walleye was reported in GenBank (Accession no. U90617). A single PCR band and one DNA sequence was found for every individual sampled. The mtDNA control region of the 199 walleye tested varied from 1184 to 1262 bp in length, due to different numbers of copies of tandemly repeated sequences (Buroker *et al.* 1990; Fumagalli *et al.* 1996) located near the light-chain 5' terminus. Variation in numbers of tandem repeats among individual walleye did not show geographical patterns with chi-square tests and the Monte Carlo distribution ($\chi^2 = 3.52$, $P = 0.85$), and are not treated further here. The sequences of the repeats and their evolutionary patterns are described in Faber & Stepien (1998).

Nineteen mtDNA control region haplotypes based on the sequence polymorphisms in the imperfect repeat-2 section (which did not vary in copy number; Faber & Stepien 1998) and the nonrepeated sequences were identified for 199 walleye sampled and were used in the present analyses (Table 1). In our study, both Kimura (1980) 2-parameter distances and pairwise (*p*) distances (Nei 1987) were calculated, which differed by less than 0.001. The neighbour-joining (NJ) genetic distance tree is shown in Fig. 2, along with relative branch lengths and bootstrap support. A PAUP* (Swofford 1998) branch and bound maximum parsimony search yielded 478 most parsimonious trees. Relationships among the sauger, the Ohio River haplotype and all haplotypes from the Great Lakes were identical in the NJ and PAUP* trees (supported by 100% of the PAUP* trees and 100% of the bootstrap replications). Additional relationships on the NJ

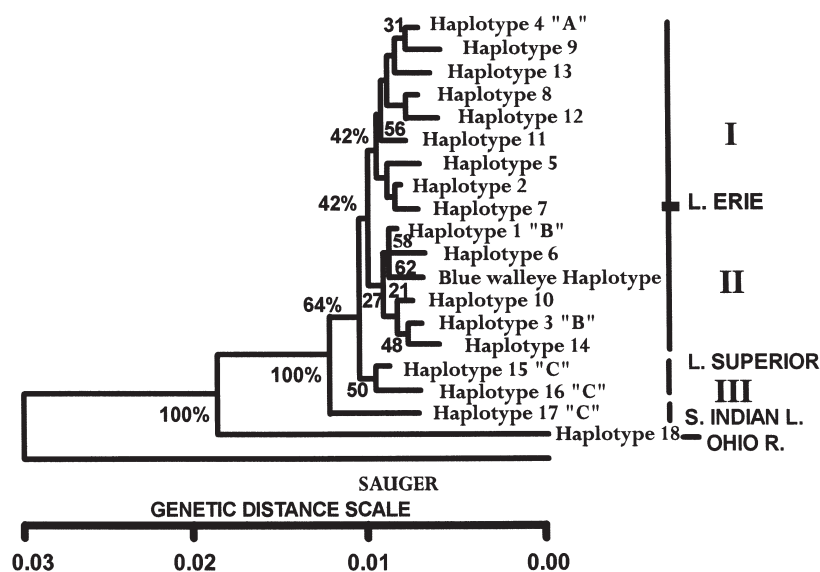


Fig. 2 MEGA neighbour-joining tree (Saitou & Nei 1987) from Kimura (1980) 2-parameter distances between pairs of walleye (*Stizostedion vitreum*) haplotypes from Lakes Erie and St Clair, Lake Superior, Southern Indian Lake (Manitoba) and the Ohio River, and the sauger (*S. canadense*). Hypothesized refugium groups in the Great Lakes are indicated by Roman numerals: I, Atlantic; II, Mississippi; and III, Missouri. Sequence haplotypes for refugium representatives identified from RFLP studies of Ward *et al.* (1989) and Billington *et al.* (1992) are lettered: A, Atlantic; B, Mississippi; and C, Missouri. Bootstrap values above 20% are indicated at nodes.

tree (Fig. 2) that also were resolved with PAUP* were Group II (the hypothesized Mississippi refugium) with 61% consensus support, the relationship among haplotypes 3 and 14 with 61% consensus, and the relationship among haplotypes 8 and 12 with 65% consensus and 79% bootstrap support. Other relationships were not resolved with PAUP* due to a greater number of haplotypes than phylogenetically informative sites and many haplotypes being distinguished by a single autapomorphy (Table 1).

Three of the 11 samples of walleye from the Ohio River (Fig. 1) had a single divergent haplotype that varied from the other walleye sampled in base substitutions at 27 nucleotide positions, five of which were shared with sauger (Table 1). The Ohio River haplotype was located basally on the NJ (Fig. 2) and PAUP* trees and formed the sister taxon to all other walleye surveyed, with 100% bootstrap support and 100% consensus of the most parsimonious trees (Fig. 2). The Ohio River type diverged from the other walleye by an average Kimura (1980) 2-parameter genetic distance of 0.029 ± 0.003 , corresponding to an estimated 1.5×10^6 years before present (Myr BP). The remaining eight samples from the Ohio River included haplotypes 1 ($N = 3$), 2 ($N = 2$), 3 ($N = 2$) and 4 ($N = 1$), that were also common in Lake Erie (Tables 2 and 3).

Walleye from Lake Superior ($N = 2$) and Southern Indian Lake ($N = 1$), representing the Missouri refugium (type 'C' from the RFLP studies of Ward *et al.* 1989; Billington *et al.* 1992), had different unique haplotypes in our study (Table 1). The Missouri refugium types were located as the sister taxa basal to the other walleye in the Great Lakes on the NJ and PAUP* trees (Fig. 2). The cluster

from Lake Superior was denoted as Group III on the NJ tree. The genetic distances between the haplotypes in Southern Indian Lake and Lake Superior averaged 0.0046 ± 0.0020 , corresponding to about $230\,000 \pm 100\,000$ years BP. Genetic distances between the haplotypes from Lake Superior vs. Lakes Erie and St Clair averaged 0.0030 ± 0.0016 , about $150\,000 \pm 80\,000$ years BP.

Pairwise divergences among the walleye haplotypes in Lakes Erie and St Clair averaged 0.002 ± 0.001 , about $100\,000 \pm 50\,000$ years BP. Haplotypes in Lakes Erie and St Clair clustered in two groups, designated I and II on the NJ tree, with relatively low bootstrap support (Fig. 2). The Atlantic refugium specimen (type 'A' from the RFLP studies of Ward *et al.* 1989; Billington *et al.* 1992) from Lake Ontario corresponded to our haplotype 4 (Table 1) in Group I. Representatives of known RFLP types (Ward *et al.* 1989; Billington *et al.* 1992) sequenced from the Mississippi refugium were identified as haplotypes 1 and 3 in group II. Groups I and II may correspond to hypothesized Atlantic and Mississippi refugia, although there were no defining synapomorphies. Numbers of individuals from Groups I and II were significantly different between Lakes Erie and St Clair, and among the three basins of Lake Erie (Table 3).

One blue-coloured walleye from Lake Erie had a unique haplotype, distinguished by a transition (Table 1). It clustered in Group II on the NJ and the PAUP* consensus trees, near the known Mississippi refugium representatives (Fig. 2). The blue walleye from Lake Nipissing and Lake Huron were haplotype 1 (Tables 1 and 3). Haplotype 1 was common in Lakes Erie and St Clair (Table 1), and was an identified Mississippi refugium representative (Billington *et al.* 1992).

Table 2 Distribution of mtDNA control region sequence haplotypes and haplotype diversity (\pm standard error) of walleye among sites in Lakes Erie and St Clair

Haplotype	Sampling site						Total
	St Clair			Erie			
	Clinton River, MI	Maumee River, OH	Sandusky River, OH	Grand River, OH	Van Buren Bay, NY	Grand River, ONT	
1	22	10	16	10	11	6	75
2	1	8	2	10	9	10	40
3	5	8	4	6	4	1	28
4	0	2	3	6	5	6	22
5	0	0	1	0	1	0	2
6	0	0	1	0	0	0	1
7	0	0	1	0	0	0	1
8	0	0	1	0	0	0	1
9	0	1	0	0	0	0	1
10	0	2	0	0	1	0	3
11	0	1	0	0	1	0	2
12	0	0	1	0	0	0	1
13	0	0	1	0	0	0	1
14	0	0	1	0	0	0	1
Total	28	32	32	32	32	23	179
Haplotype diversity	0.36 \pm 0.10	0.79 \pm 0.03	0.75 \pm 0.08	0.76 \pm 0.03	0.78 \pm 0.04	0.71 \pm 0.05	0.69 \pm 0.001

Diversity and divergences among spawning groups

The spawning site in Lake St Clair contained only haplotypes 1, 2 and 3 and its haplotypic diversity was lower than that of the spawning sites in Lake Erie (Table 2). Lake St Clair housed significantly more individuals of haplotype 1 than did Lake Erie (35%; $\chi^2 = 18.33$, $P < 0.001$). Confirmed Mississippi refugium types (haplotypes 1 and 3, located in Group II of the NJ tree; Fig. 2) comprised 97% of the individuals spawning in the Lake St Clair site (Table 3). Representation of the hypothesized Atlantic (Group I of Fig. 2) and Mississippi refugia (Group II) differed significantly between Lakes Erie and St Clair (Table 3).

The number of haplotypes per spawning site in Lake Erie averaged 6.6, ranging from four to 11 (Table 2). Haplotypes 1, 2, 3 and 4 were found in all Lake Erie sites (Tables 2 and 3). Haplotypes 1 and 3 (identified Mississippi refugium types) were considerably more common than other haplotypes in the western basin of Lake Erie, and decreased in the central and the eastern basins (Table 3). Representation of the hypothesized Mississippi refugium group (II) significantly decreased from west to east. Haplotypes 4 (the known Atlantic refugium representative) and 2 (which clustered with the Atlantic group) increased from the western through the eastern basins. Similarly, overall representation of the hypothesized Atlantic refugium group (I) significantly increased from west to east (Table 3).

Nucleotide divergences among spawning populations were higher in comparisons between Lake St Clair and sites in Lake Erie (averaging 0.00040 ± 0.00001 and ranging from 0.00008 to 0.00050) than among sites in Lake Erie (averaging 0.000030 ± 0.000001 and ranging from 0.000020 to 0.000140). Haplotype representation was significantly different between Lakes Erie and St Clair (Tables 4 and 5), among the eastern and western basins of Lake Erie (Table 4), and among some spawning sites (Table 5) in modified chi-square (McElroy *et al.* 1992) and AMOVA (Excoffier 1995) tests.

Most genetic variation occurred within populations and there was significant partitioning of variation between Lakes St Clair and Erie, between the lake basins overall and among the spawning sites (Table 6; AMOVA; Excoffier 1995). Haplotype frequencies did not differ significantly between the sampling years of 1993 vs. 1995 in the spawning sites tested, with the exception of the Van Buren Bay reef (Table 7). There were no significant differences in the distribution of haplotypes between males and females in any of the spawning sites (Table 7).

Discussion

Population genetic relationships

Despite considerable migration of tagged walleye between Lakes St Clair and Erie during nonspawning

Haplotype	Lake St Clair	Western Basin Lake Erie	Central Basin Lake Erie	Eastern Basin Lake Erie	Total for all sites
A. Group II (Mississippi types)					
1	22 79%	26 40%	10 31%	17 31%	75 42%
3	5 18%	12 19%	6 19%	5 9%	28 15%
6,10,14	0 0%	4 6%	0 0%	1 2%	5 3%
N Group II (total)	27 97%	42 66%	16 50%	23 42%	108 60%
B. Group I (Atlantic types)					
2	1 3%	10 15%	10 31%	19 34%	40 23%
4	0 0%	5 8%	6 19%	11 20%	22 12%
5,7,8,9,11,12,13	0 0%	7 11%	0 0%	2 4%	9 5%
N Group I (total)	1 3%	22 34%	16 50%	32 58%	71 40%
N per site (overall)	28	64	32	55	179

*denotes a significant difference in frequency of refugia types in χ^2 contingency test (Sokal & Rohlf 1981) at $P < 0.05$.

χ^2 between Lakes St Clair and Erie = 17.3, $P < 0.001^*$; χ^2 among basins in Lake Erie = 21.4, $P < 0.001^*$.

	Lake St Clair (N = 28)	Western Basin of Lake Erie (N = 64)	Central Basin of Lake Erie (N = 32)	Eastern Basin of Lake Erie (N = 55)
Lake St Clair	–	$\chi^2 = 14.8$ $P = 0.004^{**}$	$\chi^2 = 17.8$ $P = 0.001^{**}$	$\chi^2 = 24.7$ $P = 0.001^{**}$
Western basin of Lake Erie	$\phi_{ST} = 0.120$ $P < 0.001^{**}$	–	$\chi^2 = 10.7$ $P = 0.040^*$	$\chi^2 = 13.8$ $P = 0.001^{**}$
Central basin of Lake Erie	$\phi_{ST} = 0.230$ $P < 0.001^{**}$	$\phi_{ST} = 0.025$ $P = 0.102$	–	$\chi^2 = 3.3$ $P = 0.540$
Eastern basin of Lake Erie	$\phi_{ST} = 0.243$ $P < 0.001^{**}$	$\phi_{ST} = 0.038$ $P = 0.028^*$	$\phi_{ST} = 0.016$ $P = 0.782$	–

*denotes a significant difference at $P < 0.05$; **denotes a significant difference at $P < 0.008$ in multiple post hoc χ^2 tests, using a Bonferroni correction (Cooper 1968; Fry *et al.* 1993).

Contingency tests (Sokal & Rohlf 1981) for overall differences among the basins:

χ^2 among all four basins = 44.7, $P < 0.001^*$; χ^2 among the three basins in Lake Erie = 19.3, $P = 0.001^*$.

Table 3 Representation of Group I (hypothesized Atlantic glacial refugia types; from Fig. 2) and Group II (hypothesized Mississippi glacial refugia types) among spawning walleye in the basins of Lakes Erie and St Clair. Rarer haplotypes are listed together

Table 4 Tests for differences in the distribution of mtDNA control region sequence haplotypes of walleye between pairs of basins in Lakes Erie and St Clair. Above the diagonal are modified χ^2 values and their probabilities (P) from Monte Carlo tests (Roff & Bentzen 1989; McElroy *et al.* 1992). Below the diagonal are ϕ_{ST} values from AMOVA tests and the probability (P) of obtaining a random number greater than the value (Excoffier 1995). Below the table are contingency tests (Sokal & Rohlf 1981) for overall differences among the basins

Table 5 Tests for differences in the distribution of mtDNA control region haplotypes among pairs of spawning sites in Lakes Erie and St Clair for walleye. Above the diagonal are modified χ^2 values and their probabilities (P) from Monte Carlo tests (Roff & Bentzen 1989; McElroy *et al.* 1992). Below the diagonal are ϕ_{ST} values from AMOVA tests and the probability (P) of obtaining a random number greater than the value (Excoffier 1995)

Spawning Site (Sample size)	Clinton River (N=28)	Maumee River (N=32)	Sandusky River (N=32)	Grand River (Ohio) (N=32)	Van Buren Bay (N=32)	Grand River (Ontario) (N=23)
Clinton River	–	$\chi^2=16.4$ $P=0.001^{**}$	$\chi^2=11.2$ $P=0.014^*$	$\chi^2=17.8$ $P=0.002^{**}$	$\chi^2=18.0$ $P=0.002^{**}$	$\chi^2=24.9$ $P=0.001^{**}$
Maumee River	$\phi_{ST}=0.207$ $P<0.001^{**}$	–	$\chi^2=7.3$ $P=0.130$	$\chi^2=6.5$ $P=0.180$	$\chi^2=2.9$ $P=0.601$	$\chi^2=11.5$ $P=0.018^*$
Sandusky River	$\phi_{ST}=0.163$ $P<0.001^{**}$	$\phi_{ST}=0.014$ $P=0.232$	–	$\chi^2=15.1$ $P=0.002^{**}$	$\chi^2=7.5$ $P=0.110$	$\chi^2=18.7$ $P=0.002^{**}$
Grand River (Ohio)	$\phi_{ST}=0.278$ $P<0.001^{**}$	$\phi_{ST}=0.002$ $P=0.383$	$\phi_{ST}=0.049$ $P=0.001^{**}$	–	$\chi^2=3.6$ $P=0.500$	$\chi^2=3.2$ $P=0.380$
Van Buren Bay	$\phi_{ST}=0.276$ $P<0.001^{**}$	$\phi_{ST}=0.187$ $P=0.461$	$\phi_{ST}=0.006$ $P=0.297$	$\phi_{ST}=0.003$ $P=0.381$	–	$\chi^2=5.1$ $P=0.300$
Grand River (Ontario)	$\phi_{ST}=0.554$ $P<0.001^{**}$	$\phi_{ST}=0.083$ $P=0.027^*$	$\phi_{ST}=0.100$ $P=0.005^*$	$\phi_{ST}=0.011$ $P=0.314$	$\phi_{ST}=0.003$ $P=0.381$	–

*denotes a significant difference at $P \leq 0.05$; **denotes a significant difference at $P \leq 0.003$ for multiple *post hoc* χ^2 tests, using a Bonferroni correction (Cooper 1968; Fry *et al.* 1993).

Contingency test (Sokal & Rohlf 1981) for overall differences among sites: χ^2 among sites in Lakes St Clair and Erie = 56.94, $P=0.001^*$.

Variance component	Variance	% total	ϕ_{ST}	P value
Among Lakes Erie and St Clair	0.059	14.3	0.131	<0.001*
Within lakes	0.352	85.7	0.153	<0.001*
Among Lake Erie basins	0.026	7.0	0.025	0.047*
Within Lake Erie basins	0.346	93.0	0.154	<0.001*
Among spawning sites in Lake Erie	0.011	7.5	0.031	0.031*
Within spawning sites in Lake Erie	0.343	92.5	0.086	<0.001*

Table 6 Analysis of variance of pairwise genetic distances among walleye mtDNA control region sequences estimated by AMOVA (Excoffier 1995). P is the probability that the random distance (ϕ_{ST}) > the observed distance from 1000 iterations (Excoffier *et al.* 1992)

Sampling site	N males	N females	χ^2	P value
A. Tests between sexes				
Clinton River	18	10	4.2	0.08
Maumee River	14	18	1.9	0.77
Sandusky River	14	18	6.5	0.16
Grand River (Ohio)	10	22	3.8	0.32
Van Buren Bay	21	11	1.9	0.83
Overall	77	79	2.0	0.73
	N 1993	N 1995	χ^2	P value
B. Tests between years				
Maumee River	19	13	1.8	0.75
Sandusky River	19	13	6.3	0.15
Grand River (Ohio)	20	12	2.4	0.48
Van Buren Bay	13	19	13.7	0.01*
Overall	71	57	5.8	0.23

Table 7 Monte Carlo χ^2 tests (Roff & Bentzen 1989; McElroy *et al.* 1992) for differences in the distributions of walleye mtDNA control region sequence haplotype (A) between the sexes and (B) between sampling years (1993 and 1995). *denotes a significant difference at $P \leq 0.05$

months (Todd & Haas 1993), allozyme studies (Todd 1990; Todd & Haas 1993) and our data demonstrated that their spawning populations were genetically divergent (see Tables 3, 4, 5 and 6). The relative proportions of Mississippi and Atlantic refugia types in Lakes St Clair and Erie found in our study and from mtDNA RFLPs (Ward *et al.* 1989; Billington *et al.* 1992) were similar. Analogous proportions of RFLP haplotypes in Lakes Erie and St Clair for the two refugia were found for the brown bullhead *Ameiurus nebulosus* (Murdoch & Hebert 1997), suggesting a common phylogeographic history. Our results suggest that walleye populations in Lake St Clair, western Lake Erie, and eastern Lake Erie either were founded by significantly different proportions of the two refugium groups and/or their proportions changed over time as spawning groups diverged.

Genetic divergence among basins and spawning sites within Lake Erie (Tables 4, 5 and 6) supported spawning philopatry and nonrandom mating to tributary sites, with consistency between sampling years (Table 7). The reef-spawning subpopulation at Van Buren Bay appeared less philopatric than many of the tributary spawning sites (Table 5) and was different between the sampling years (Table 7), suggesting less specificity. There may be a fundamental difference in relative philopatry of tributary and reef-spawning subpopulations, which should be investigated further. Divergence between groups spawning in the eastern and western basins of Lake Erie (Tables 3, 4, 5 and 6) was congruent with differences in life history (i.e. age to reaching maturity) and morphology (differences in adult sizes and body shapes; Nepszy *et al.* 1991).

Significant differences in the frequencies of common haplotypes between the Grand (Ohio) and the Sandusky Rivers suggested divergence (Table 5), supported by tag returns of walleye in other studies (Colby *et al.* 1994; C. Knight, Ohio Division of Wildlife, personal communication). In our study, frequencies of haplotypes from the Grand River (Ohio) did not differ significantly from other spawning sites in Lake Erie (Tables 4 and 5), although representation of the hypothesized refugia groups was different (Table 2). The group of walleye that presently spawns in the central basin may have been founded from the eastern and/or western basins (R. Knight, Ohio Division of Wildlife, personal communication), following extinction of the blue pike subspecies that formerly dominated the central basin (Trautman 1981).

Frequency differences in two mtDNA RFLP haplotypes (Merker & Woodruff 1996) suggested that walleye spawning in the Sandusky River in 1993 were genetically divergent from those in the Maumee River. In our study, populations from the two rivers were not significantly different (Table 5), despite increased sample sizes. This may be due to different evolutionary rates and/or a need for even greater sample sizes in our study due to more

haplotypes. Haplotype frequencies in the Sandusky River spawning group were significantly divergent from those in the Grand River, Ohio and the Grand River, Ontario (Table 5).

Although we measured maternally inherited mtDNA, both males and females showed similar patterning among sites (Table 7). If males were not philopatric, then their DNA would have originated from females (their mothers) in a variety of spawning sites and their genetic composition would be different than that of the females. There were no significant differences in the distributions of haplotypes between males and females at the spawning sites, indicating that both sexes are philopatric (Table 7). These hypotheses of fine-scale genetic divergences and philopatry should be further tested with nuclear DNA variation.

Phylogeography of walleye in the Great Lakes region and the Ohio River

Genetic divergence of the unique haplotype in the Ohio River (by about 1.5 Myr; Fig. 2) suggested its long-term separation from haplotypes in the Great Lakes (Fig. 2). Geological evidence indicates that the upper portion of the Ohio River drainage and its predecessor (the Teays River drainage) have been geographically isolated from the Great Lakes drainages for at least 1 Myr, predating the Pleistocene Ice Ages (Flint 1971). Other walleye (eight of 11) sampled from the Ohio River had haplotypes identical to those found in Lake Erie, which may be due to a common phylogeographic history or from historically prevalent stocking with walleye from Lake Erie broodstock (White & Schell 1995).

Genetic divergences of the Ohio River type from the other types of walleye identified in our study were similar to those determined for other North American freshwater fishes. For example, mtDNA divergences of fish groups in unglaciated areas of the southeastern USA ranged from pairwise distances of 0.002–0.061 (100 000 years to 3.1 Myr BP) for the spotted sunfish *Lepomis punctatus*, 0.001–0.061 (50 000 years to 3.1 Myr BP) for the warmouth *L. gulosus* and from 0.001 to 0.009 (50 000–450 000 years BP) for the bowfin *Amia calva* (Bermingham & Avise 1986).

Genetic distances among our mtDNA control region sequence haplotypes (Fig. 2) were congruent with those calculated from mtDNA RFLPs for walleye (reviewed by Billington & Strange 1995). Genetic distances among walleye from the Great Lakes also were similar to estimates calculated for other fishes that are believed to have diverged in the same glacial refugia (reviewed by Billington & Hebert 1991; Wilson & Hebert 1996; Murdoch & Hebert 1997). For example, mtDNA RFLP analyses identified sequence divergences less than 0.02 (< 1 Myr BP) for lake trout *Salvelinus namaycush* (Grewe & Hebert 1988; Wilson & Hebert 1996), from 0.0003 to 0.0172

(15 000–340 000 years BP) for lake whitefish *Coregonus clupeaformis* (Bernatchez & Dodson 1990a), 0.0008–0.0103 (40 000–515 000 years BP) for the cisco *Coregonus artedii* (Bernatchez & Dodson 1990b) and 0.001–0.067 (50 000 years to 3.35 Myr BP) for brown bullhead (Murdoch & Hebert 1997).

Haplotype divergence of walleye probably pre-dated the Pleistocene Ice Ages, i.e. the ancestral populations were polymorphic. Different glacial refugium groups then became partitioned due to the effects of genetic drift and population bottlenecks. Distributions of the haplotypes (Tables 2 and 3; Fig. 2) suggest that walleye from the different refugia colonized different regions of the Great Lakes and that they did not freely mix. Haplotypes of walleye from the Missouri refugium founded Southern Indian Lake and Lake Superior. Individuals from the Mississippi refugium colonized and remain predominant in Lakes Michigan, St Clair, and western Lake Erie. Walleye from the Atlantic refugium founded Lakes Ontario and eastern Lake Erie, where they remain more common. Further genetic analysis of walleye from other areas of the Great Lakes and throughout their range in North America would help to further trace their phylogeographic history.

The presence of identical haplotypes in blue walleye from Lake Huron and Lake Nippising, and in yellow walleye from Lake Erie, suggests that they are not different subspecies. A single unique substitution was found in the blue walleye from Lake Erie, which was no more divergent than the other Lake Erie haplotypes (Fig. 2) and belonged to Group II of the NJ tree (Fig. 2). The relationship of blue walleye to the extinct blue pike (*S. vitreum glaucum*) is difficult to determine without reference DNA sequence. Testing of preserved blue pike samples may resolve this question. If the blue pike evolved in the Mississippi refugium or within the short geological history of Lake Erie (Bailey & Smith 1981; Trautman 1981), little or no genetic differences may have accumulated between the morphological variants.

Conclusions

Sequences of the mtDNA control region resolved phylogeographic divergences among hypothesized glacial refugia, lakes, basins and spawning groups of walleye. Walleye haplotypes isolated in Missouri, Mississippi and Atlantic glacial refugia (Ward *et al.* 1989; Billington *et al.* 1992) diverged due to genetic drift and bottlenecks. The three refugia founded different regions of the Great Lakes after the last Wisconsin glacial retreat (Bolsenga & Herdendorf 1993). Significant differences in genotypic frequencies among spawning sites are consistent with the hypothesis of natal homing for both sexes. Returns of spawning walleye from generation to generation have reduced gene flow among sites, resulting in genetically

divergent subpopulations. In order for walleye to withstand continued pressures from exploitation, environmental degradation and habitat loss, it may be important to delineate and conserve such genetically different groups.

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