Genetic divergence and connectivity among river and reef spawning groups of walleye (*Sander vitreus vitreus*) in Lake Erie

Rex Meade Strange and Carol A. Stepien

Abstract: Discerning population genetic structure is challenging for highly vagile open water animals, as contemporary gene flow may obscure historic phylogeographic patterns. We examined genetic variation among all 10 major river and reef spawning groups of walleye (*Sander vitreus vitreus*) in Lake Erie for evidence of isolation by distance, segregation by physiographic partitions, and natal site fidelity using 10 nuclear DNA microsatellite loci. Results revealed that although most spawning groups were distinguishable, relationships did not correspond with physiographic basins or distances among localities. Bayesian analyses showed connectivity among some southern shore spawning groups, which included the largest-sized groups. Significant genetic divergence was discerned among walleye spawning in the river systems of eastern Lake Erie, as well as in two sites in western Lake Erie, along with marked isolation from Lake St. Clair. Population structure of Lake Erie walleye thus appears to reflect the interaction of two different intrinsic factors: isolation due to natal site fidelity that maintains patterns of divergence, and connectivity due to individuals that stray from their natal sites to spawn.

Résumé : Il n'est pas facile de déterminer la structure génétique de la population chez les animaux très mobiles dans la masse d'eau, car le flux génique actuel peut obscurcir les patrons phylogéographiques du passé. Par l'analyse de 10 locus microsatellites d'ADN nucléaire, nous avons étudiéla variation génétique dans l'ensemble des dix principaux groupes de dorés jaunes (*Sander vitreus vitreus*) qui fraient dans les rivières et sur les récifs au lac Érié, à la recherche d'indices d'isolement par la distance, de ségrégation par partitions physiographiques et de fidélité au site de naissance. Nos résultats montrent que, bien qu'on puisse reconnaître la plupart des groupes de fraie, leurs relations ne correspondent pas aux bassins hydrographiques, ni aux distances entre les localités. Des analyses bayésiennes révèlent l'existence de connectivité entre certains groupes de fraie de la rive sud, qui incluent les groupes de plus grande taille. Nos données indiquent aussi une divergence génétique significative chez les dorés qui fraient dans les bassins versants de l'est du lac Érié, de même qu'à deux sites dans l'ouest du lac Érié; elles montrent aussi un isolement vis-à-vis le lac St-Clair. La structure de population des dorés du lac Érié semble donc refléter l'interaction de deux facteurs intrinsèques distincts, l'isolement due à la fidélité au site de naissance qui maintient les patrons de divergence et la connectivité due aux individus qui s'éloignent de leur site de naissance pour se reproduire.

[Traduit par la Rédaction]

Introduction

The walleye (*Sander* (= *Stizostedion*) *vitreus vitreus*; Percidae) is one of the most economically important fish species in the Great Lakes. The species is a large and highly vagile piscivore endemic to the riverine and lacustrine systems of eastern North America and attains greatest abundance in western Lake Erie (Hubbs and Lagler 2004). Only 10 primary spawning groups are recognized by Lake Erie fishery managers (Fig. 1), making the lake a geographically simple and relatively isolated system in which to assess mechanisms defining contemporary patterns of population connectivity and isolation. Understanding the ecological mechanisms regulating genetic divergences among spawning groups is important for successful resource management.

Adult walleye forage in open water habitats and separate into discrete spawning groups in the spring. Walleye mature at age 2–3 in Lake Erie (Wolfert 1963) and spawn at night in small groups shortly after the ice breaks up in spring, with females depositing their eggs in a single night and males staying on the spawning grounds for up to several weeks (Scott and Crossman 1973). Some walleye groups spawn in specific tributary rivers, while other groups spawn on shallow shoals or reefs of Lake Erie (Olson and Scidmore 1962; Todd and Haas 1993). Tagging studies indicate that individuals tend to return to their natal spawning locations (Regier et

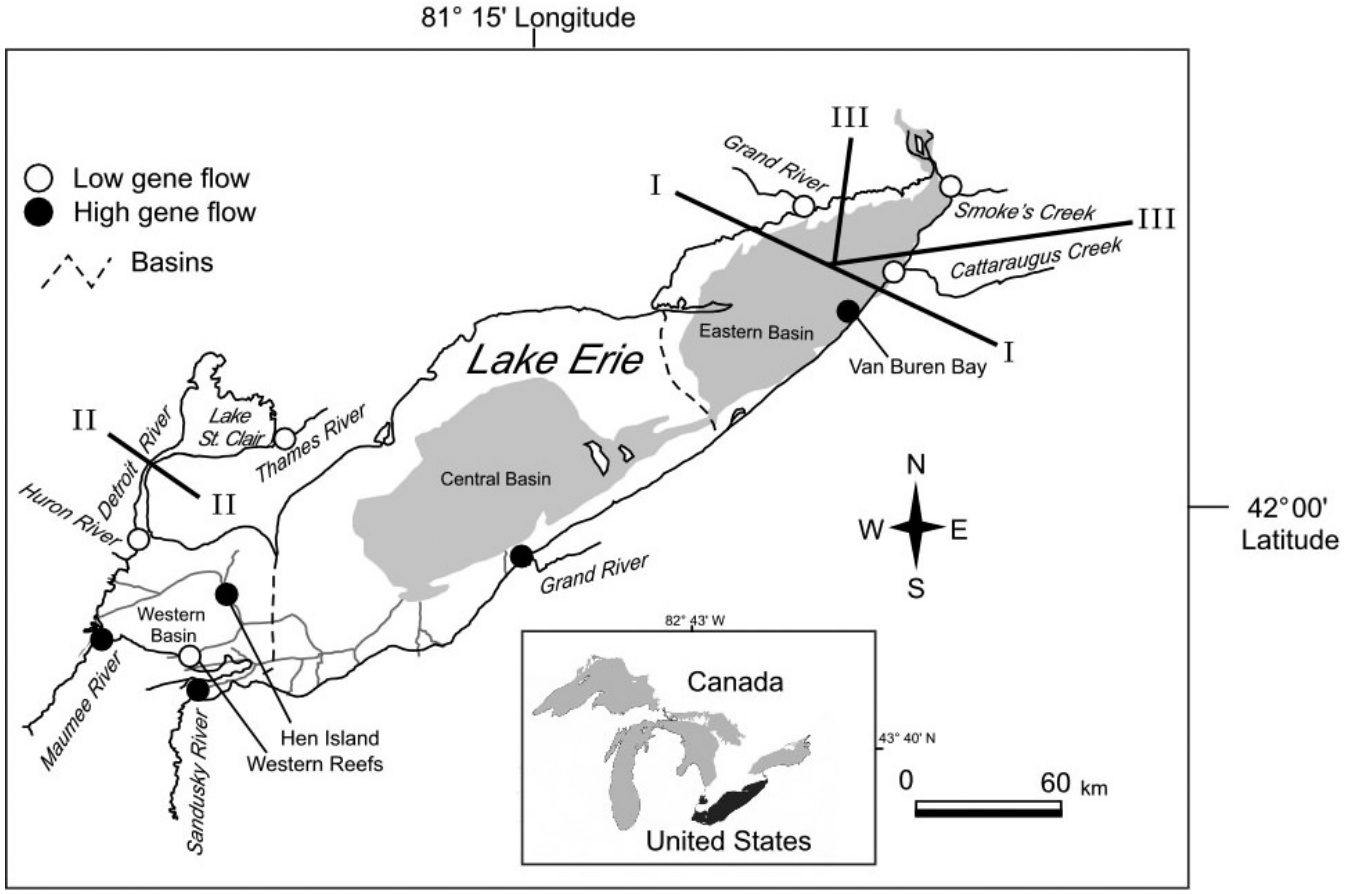
Received 31 July 2006. Accepted 14 December 2006. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 27 March 2007.
J19455

R.M. Strange[1] and C.A. Stepien.[2] Great Lakes Genetics Laboratory, Lake Erie Center and Department of Environmental Sciences, The University of Toledo, 6200 Bayshore Road, Toledo, OH 43618, USA.

[1]Present address: Department of Biology, University of Southern Indiana, Evansville, IN 47712, USA.
[2]Corresponding author (e-mail: carol.stepien@utoledo.edu)

 doi:10.1139/F07-022

Fig. 1. Locations of the 11 primary walleye spawning sites in Lakes Erie and St. Clair and depiction of their genetic relationships. Solid circles denote sites with interconnectivity (i.e., the track), and open circles designate sites that significantly differ from all other groups. Heavy straight lines denote primary discontinuities in gene flow determined using BARRIER (Manni et al. 2004) and are sequentially numbered (I–III) in order of decreasing relative support. Grey regions (basins) and lines (rivers) inside modern-day Lake Erie depict its early development about 10 000 years before present (after Bolsenga and Herdendorf 1993).



al. 1969; Goodyear et al. 1982), which appears to have a genetic basis (Jennings et al. 1996). Thus, natal site fidelity is a likely mechanism for maintaining population structure in walleye.

Demographic mechanisms may further regulate genetic structure in Lake Erie walleye. The largest spawning runs occur in the west, and smaller groups spawn in rivers and reefs of the central and eastern basins (Wolfert 1963; Schneider and Leach 1977). Although tagging studies have revealed relatively little overall migration, some walleye move seasonally from the west to the east (Thomas et al. 2005) or west into Lake St. Clair (Ferguson and Derksen 1971; Todd and Haas 1993). Walleye in the western basin outnumber those in the east by an order of magnitude (Thomas et al. 2005); thus, connectivity may be driven by different intrinsic growth rates among spawning groups. In other words, patterns of gene flow may be dominated by the largest spawning groups, which have greater reproductive success (i.e., increased recruitment) and from which more migratory individuals may stray (e.g., owing to overcrowding) to spawn with other groups. Great year-class variation, faster growth, and earlier maturity characterize walleye in western Lake Erie from those in the east (Colby and Nepszy 1981). Moreover, genetic structure within Lake Erie may be complicated by the population crash of walleye (as measured by annual catches) from the late 1950s through 1970s because of exploitation and habitat loss and their later recovery (Colby and Nepszy 1981; Hatch et al. 1987; Knight 1997), which likely altered patterns of gene flow and divergence.

The objective of this study was to test whether population genetic structure exists within Lake Erie walleye and to infer its defining mechanisms. Although geographically isolated populations of walleye are known to be genetically distinct (Billington and Strange 1995; Stepien and Faber 1998), few studies have investigated isolation and connectivity in a single system with a comprehensive sampling design (but see Stepien 1995 and Stepien and Faber 1998 for an mtDNA sequence analysis of Lake Erie walleye). Here we employed a landscape genetics analytical approach (Manel et al. 2003) to test the spatial distribution of genetic diversity in relation to extrinsic environmental features and intrinsic behavior. This study examined genetic variation at 10 microsatellite loci among walleye from all primary river and lake spawning groups in Lake Erie for evidence of (*i*) isolation by geographic distance, (*ii*) physiographic differences, and

Table 1. Summary statistics for walleye spawning groups from Lakes Erie and St. Clair based on 10 microsatellite loci.

Spawning group	N	H_O	H_E	F_{IS}	N_A	N_{PA}	P_{PA}
Lake St. Clair							
Thames River, Ont. 2004	38	0.745	0.766	0.028	79	1	0.013
Western basin, Lake Erie							
Huron River, Mich. 2003	20	0.745	0.761	0.021	84	1	0.012
Maumee River, Ohio 2003	76	0.698	0.758	0.073*	107	3	0.028
Western Reefs, Ohio 2004	20	0.675	0.675	0.001	72	1	0.014
Hen Island Reef, Ont. 2003	82	0.671	0.735	0.087*	108	2	0.019
Sandusky River, Ohio 2003	20	0.775	0.780	0.007	82	2	0.024
Central basin, Lake Erie							
Grand River, Ohio 2003	30	0.660	0.793	0.168*	88	1	0.011
Eastern basin, Lake Erie							
Van Buren Bay, N.Y. 2003	77	0.716	0.779	0.082*	104	7	0.067
Cattaraugus Creek, N.Y. 2003	30	0.700	0.764	0.084*	92	3	0.033
Smoke's Creek, N.Y. 2003	20	0.780	0.787	0.009	84	2	0.024
Grand River, Ont. 2003–2004	35	0.674	0.779	0.134*	91	2	0.022
Total for Lake Erie	410	0.704	0.778	0.095*			
Total for all sites	448	0.707	0.779	0.093*			

Note: N, sample size; H_O, observed and H_E, expected heterozygosity; F_{IS}, deviation from Hardy–Weinberg (HW) proportions; N_A, number of alleles; N_{PA}, number of private alleles; P_{PA}, proportion of private alleles. Divergences from HW proportions prior to Bonferroni correction ($P < 0.05$) are denoted by asterisks (*), although all spawning groups conformed to equilibrium expectations following Bonferroni correction and exclusion of the *Svi*L8 locus ($P < 0.0056$).

(*iii*) natal site fidelity. Finally, we asked whether genetic structure is associated with or driven by demographic processes, and if so, to what extent.

Materials and methods

Study area

The Great Lakes are geologically young (<13 000 years) and formed during the retreat of the last of the Pleistocene glaciations (Bolsenga and Herdendorf 1993). Most fishes now present in the lower Great Lakes colonized the newly formed habitats from lotic systems located south of the glacial maximum. In particular, fishes are believed to have entered Lake Erie from the west through a connection between the Wabash River and Maumee River valleys, whereas others are hypothesized to have entered from an eastern glacial refugium (Bailey and Smith 1981; see also Billington et al. 1992). Such physical isolation of different glacial refugium groups within the Lake Erie system may have existed as recently as 7 000 – 10 000 years before present, when the central and eastern basins of Lake Erie were relatively isolated from one another and aquatic habitats in the western basin consisted of a river system draining a low lying plain (see shaded interior of Fig. 1; Holcombe et al. 2003).

Present-day Lake Erie is a relatively isolated system, and its drainage is separated by divides from adjacent river systems (e.g., the Ohio River system to the south, from Lake Ontario to the east by Niagara Falls, and from the upper Great Lakes to the west by the Detroit River that drains from Lake St. Clair) (see Fig. 1). The Detroit River presumably constrains gene flow between Lake Erie and the upper Great Lakes through a narrow connection and thus may represent a potential bottleneck relative to the open water connections of Lake Erie.

Lake Erie is the shallowest of the five Great Lakes, and its natural division into three physiographic basins is of considerable ecological importance. The western basin is the most biologically productive region of the Lake and houses many islands and associated shoals (Bolsenga and Herdendorf 1993). The western region of the Lake constitutes the most important walleye spawning and nursery area in Lake Erie (Nepszy 1977). Each year, river spawning groups of walleye enter the Huron (Michigan), Maumee (Ohio), and Sandusky (Ohio) rivers of the western basin, with the largest spawning run occurring in the Maumee River (Nepszy 1977). Walleye also spawn over an extensive reef system consisting of numerous shoals in the western basin (Jeffrey Tyson, Ohio Division of Wildlife, 305 E. Shoreline Drive, Sandusky, OH 44870, personal communication).

In contrast, the central basin is deeper, has few islands, and contains a single primary spawning location for walleye in the south (e.g., Grand River (Ohio)). Habitats along the northern coast of the Lake are unsuitable for walleye spawning. The eastern basin contains the deepest regions of the Lake, has few shoal areas, and is not as biologically productive as the western basin (Bolsenga and Herdendorf 1993). Resident populations of walleye are known to spawn at a single shoal area (Van Buren Bay, New York) and in three rivers of the eastern basin: Cattaraugus Creek (New York), Smoke's Creek (New York), and Grand River (Ontario). The shoal area at Van Buren Bay once housed the "blue pike" walleye subspecies *Sander vitreus glaucus*, which became extinct by the late 1950s because of exploitation (see Stone 1948; Scott and Crossman 1973; Stepien and Faber 1998). The physiographic differences in Lake Erie represent a heterogeneous suite of foraging habitats, which combined with the small number of spawning sites for walleye may result in the segregation of spawning groups situated in either the western or eastern basins.

Sample collection

A sound sampling strategy is necessary to avoid erroneous conclusions regarding population structure in highly migra-

tory fishes. Therefore, samples of fin clips in tag and release programs were taken from spawning walleye either entering or leaving or over their spawning locations, as recommended by the International Great Lakes Fishery Commission's Lake Erie Walleye Task Group (Roger Knight, Ohio Division of Wildlife, 305 E. Shoreline Drive, Sandusky, OH 44870, USA, personal communication). Most of the samples were taken by state, federal, and provincial agencies during their monitoring programs and included the 10 primary spawning groups in Lake Erie and a single spawning area in neighboring Lake St. Clair (Table 1; Fig. 1). The sampling sites did not have a known history of stocking, except Cattaraugus Creek and Smoke's Creek, New York, which are no longer being stocked (Donald Einhouse, New York Department of Environmental Conservation, Dunkirk, NY 14048, USA, personal communication) and whose brief stocking history was not successful and did not genetically affect the native genotypes (see Stepien et al. 2004).

To reduce possible complications in comparing spawning groups taken from different years, we restricted our Lake Erie samples to the 2003 spawning season, except for the Thames River (Lake St. Clair) and the Western Reefs, which were collected during the 2004 spawning season. The 2003 year class produced by walleye spawning in Lake Erie was the strongest for over 20 years (Roger Knight, Ohio Division of Wildlife, 305 E. Shoreline Drive, Sandusky, OH 44870, USA, personal communication), rendering the present study an important baseline. Sex information was recorded for seven of the spawning groups (e.g., Huron River, Sandusky River, Grand River (Ohio), Van Buren Bay, Cattaraugus Creek, Smoke's Creek, and Grand River (Ontario)), enabling us to test for differences in allelic distributions between males and females. Each sample consisted of a small (1–2 cm^2) piece of pectoral or caudal fin fixed in 95% ethanol (EtOH) and then stored at room temperature in the laboratory. Most specimens were subsequently released by agency personnel.

DNA extraction, amplification, and allelic determination

Genomic DNA was extracted from the EtOH-fixed tissues either following traditional phenol–chloroform procedures or with the Qiagen DNeasy kit, following the manufacturer's directions (Qiagen, Inc., Valencia, California). DNA extractions were frozen and archived in the Great Lakes Genetics Laboratory for future studies. The polymerase chain reaction (PCR) was used to amplify allelic length variants from 10 microsatellite loci developed by other investigators for walleye, including Borer et al. (1999: *Svi*4, *Svi*6, *Svi*17, *Svi*18, *Svi*33), Wirth et al. (1999: *Svi*L6, *Svi*L7, *Svi*L8), and Eldridge et al. (2002: *Svi*2 and *Svi*7). PCR reactions consisted of 50 $mmol \cdot L^{-1}$ KCl, 1.5 $mmol \cdot L^{-1}$ $MgCl_2$, 10 $mmol \cdot L^{-1}$ Tris–HCl, 50 $\mu mol \cdot L^{-1}$ of each deoxynucleotide, 0.5 $\mu mol \cdot L^{-1}$ of both forward and reverse primers, 5–30 ng DNA template, and 0.6–1.2 units of *Taq* polymerase per 10 microlitres of reaction volume. For each locus, the forward primer was synthesized with a 5′ fluorescent label to allow visualization on an ABI 3130 genetic analyzer (Applied Biosystems Inc., Fullerton, California), and four different dye labels were used to facilitate multi- and pool-plexing of loci. A sterile mineral oil overlay was added to ensure that the reaction volume remained constant throughout the PCR cycles. A thermal cycle of 2 min at 94 °C for initial denaturation was followed by 35 cycles of denaturation (94 °C, 30 s), primer annealing (1 min) at a primer-specific temperature, and polymerase extension (72 °C, 30 s). A final extension at 72 °C for 5 min was included to minimize the number of partial strands. Annealing temperatures were either 54 °C (*Svi*L6, *Svi*L7, *Svi*L8, *Svi*17) or 60 °C (*Svi*2, *Svi*3, *Svi*4, *Svi*6, *Svi*7, *Svi*18, *Svi*33). This procedure was found to be robust and allowed for multiplexing primer sets with similar annealing temperatures.

Amplification products were prepared prior to electrophoresis by diluting 1:50 with dH_2O; 1 µL of this dilution was then added to 13 µL of a solution containing formamide and Gene Scan 500 LIZ size standard (ABI) and loaded onto a 96-well plate. Finally, microsatellite products were denatured for 2 min at 95 °C prior to loading. Allelic variants were identified by size using GeneMapper 3.7 software (ABI).

Data analyses

The set of samples included in our data set was modeled as a spatial set of panmictic subpopulations within the total population of Lake Erie walleye. There is an ongoing debate as to which equivalents of F_{ST} (i.e., the degree of genetic divergence among putative subpopulations) are best suited for analyzing divergences based on microsatellite data (see Hedrick 1999; Balloux and Lugon-Moulin 2002). Since the relationships among recently diverged populations, such as those tested here for our fine-scale analyses of Lake Erie, have been shown to be better resolved in models with θ_{ST} (the F_{ST} estimate of Weir and Cockerham 1984; see Balloux and Lugon-Moulin 2002), that method was adopted here. Thus, *F* statistic analogs (Wright 1943; Weir and Cockerham 1984) and their associated levels of significance were used to quantify genetic heterogeneity using the programs FSTAT (Goudet 1995) and GENEPOP 3.3 (Raymond and Rousset 1995; also refer to http://genepop.curtin.edu.au/).

Genetic variability within subpopulations first was estimated as the number of alleles per locus (N_A), the number of private alleles (N_{PA}), the proportion of private alleles (P_{PA}), and estimates of the observed (H_O) and expected (H_E) heterozygosities (Table 1). Variation within each population was quantified as $F_{IS} = (1 - [H_O(H_E)^{-1}])$. Any departures from Hardy–Weinberg (HW) expectations were assessed by a Markov chain method analogous to Fisher's exact tests (Guo and Thompson 1992), as implemented by GENEPOP using 5000 dememorizations, 500 batches, and 5000 iterations per batch. In addition, the loci were tested for linkage disequilibrium. Possible occurrence of null (nonamplified) alleles was assessed using the procedure of van Oosterhout et al. (2004, 2006) in the program MICRO-CHECKER (also refer to http://www.microchecker.hull.ac.uk).

Comparative pairwise tests of allelic frequency heterogeneity also were conducted using Goudet et al.'s (1996) test for genetic differentiation based on an exact nonparametric procedure in GENEPOP, which was independent of F_{ST} and HW equilibrium expectations and employed the same parameters as above for the Markov chain. Critical values for all tests involving multiple comparisons were subjected to Bonferroni correction (Rice 1989). Similarly, we tested for

Table 2. Summary statistics for 10 microsatellite loci across the 11 spawning groups of walleye from Lakes Erie and St. Clair.

Locus	N_A	Size range (bp)	F_{IS}	F_{IT}	F_{ST}
*Svi*2	14	190–220	0.069	0.120***	0.016***
*Svi*4	11	104–122	0.072	0.079***	0.034***
*Svi*6	21	140–170	0.022	0.068***	0.017***
*Svi*7	13	156–172	0.055	0.116***	0.007*
*Svi*17	9	102–118	0.056	0.106***	0.019**
*Svi*18	10	116–130	0.199*	0.323***	0.154***
*Svi*33	12	82–104	0.046	–0.002	0.034***
*Svi*L6	19	108–136	0.044	0.043*	0.003***
*Svi*L7	22	192–226	0.045	0.054***	0.010***
*Svi*L8	18	120–144	0.266*	0.271***	0.008***
Total	149	—	0.093*	0.117***	0.029***

Note: N_A is the number of alleles, bp indicates base pairs, F_{IS} quantifies the average differentiation within a spawning group, F_{IT} quantifies deviation in the total sample (i.e., all walleye sampled in the two lakes), and F_{ST} represents the average genetic divergence between pairs of spawning groups. Significant contribution to population genetic structure is indicated by asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The *Svi*L8 locus was not used in further analyses, since it did not conform to Hardy–Weinberg (HW) equilibrium expectations because of null alleles.

possible differences in allelic representation between males and females using Goudet et al.'s (1996) genetic differentiation test, within the seven spawning groups with available sex data.

The distribution of allelic combinations (genotypes) within the total population of Lake Erie walleye was analyzed by means of exact tests and quantified as F_{IT}. The degree of genetic divergence among putative subpopulations (either spawning groups or basins) was estimated as F_{ST}, which assumes that divergences represent equilibrium between gene flow and genetic drift (Wright 1978). Values of F_{ST} that differ significantly ($P < 0.05$) from zero falsify the null hypothesis of panmixia between the putative subpopulations (spawning groups; Wright 1978).

Although evidence suggests that adult walleye tend to return to their natal sites to spawn, it is reasonable to assume that some may stray and join other spawning groups. If this is true, it may be hypothesized that migrant walleye either spawn at localities close to their natal sites or randomly join other spawning groups in Lake Erie. Isolation by distance would be consistent with the elongate structure of the Lake (i.e., a stepping-stone model of gene flow). Therefore, we tested whether or not the stepping-stone model is an appropriate model for walleye in Lake Erie through the regression of $F_{ST}(1 - F_{ST})^{-1}$ against the natural logarithm of geographical distance (which is expected to be linear under isolation by distance in two dimensions; Rousset 1997). Geographical distance was measured as the shortest waterway distance (km) between pairs of spawning sites. The significance of the regression was tested using the procedure of Mantel (1967). Pairwise gene flow between spawning groups was estimated using Slatkin's (1985) private allele measure of Nm (migration rate m of N individuals exchanged between sites per generation) under the rationale that private alleles are likely to attain higher frequencies in sites when gene flow is low.

The relative magnitude of genetic structure among walleye spawning groups was further investigated by combining the philosophy of continuous track analysis developed by Alroy (1995) with an analytic approach based on computational geometry using algorithms implemented in the program BARRIER 2.2 (Manni et al. 2004; also refer to www.mnhn.fr/mnhn/ecoanthropologie/software/barrier.html). The locations of genetic discontinuities in these analyses were made without reference to a priori knowledge of landscape features that might define population structure (e.g., basins). A track is defined as a geographically continuous assemblage of sampling sites (i.e., spawning groups here) that are linked by appreciable gene flow (Wright 1978). Pairwise estimates of F_{ST} were mapped onto a matrix of their geographic coordinates (latitude and longitude), and a Monmonier maximum-difference algorithm identified which of the borders between neighboring populations exhibited the highest level of genetic divergence (Manni et al. 2004). Initial estimates of genetic discontinuities were made with a multilocus F_{ST} matrix, followed by a second analysis that incorporated single-locus F_{ST} values. This procedure ranked each identified barrier in relative magnitude, according to respective support from individual loci F_{ST} values.

To further distinguish populations, we used a Bayesian-based clustering algorithm, which is independent of assumptions about mutation process, as implemented in the program STRUCTURE (vers. 2.2; Pritchard et al. 2000; also refer to http://pritch.bsd.uchicago.edu). Briefly, this analysis assigns individual fish to putative groups on the basis of distinctive genotypic frequencies without prior knowledge of their true spawning population identity. We analyzed correspondence to spawning groups by specifying number of groups (K) in independent runs of the algorithm, ranging from $K = 1$ (thus testing the null hypothesis of panmixia) to $K = 10$ (the total N of spawning sites sampled in Lake Erie). The program assigned individuals to one or more groups, with their relative frequency of predicted membership in groups totaling 1.00. The burn-in period consisted of 100 000 replications, after which 700 000 Markov chain Monte Carlo (MCMC) simulations were run on pooled samples for $K = 1$–10 under a

Table 3. Pairwise estimates of migration (N_m; above diagonal) and F_{ST} values (below diagonal) among spawning populations of walleye from Lakes Erie and St. Clair based on nine microsatellite loci.

	Thames River, Ont.	Huron River, Mich.	Maumee River, Ohio	West Reefs, Ohio	Hen Island, Ont.	Sandusky River, Ohio	Grand River, Ohio	Van Buren Bay, N.Y.	Cattaraugus Creek, N.Y.	Smoke's Creek, N.Y.	Grand River, Ont.
Thames River	—	2.36	1.15	2.86	1.48	1.07	1.26	0.84	4.77	1.49	2.30
Huron River	0.046***	—	1.20	2.02	0.95	1.19	1.17	0.87	1.47	3.24	1.74
Maumee River	0.051***	0.038***	—	1.65	4.61	5.04	4.34	8.09	1.28	1.03	1.96
West Reefs	0.018***	0.061***	0.057***	—	1.58	1.96	1.52	1.09	2.41	2.06	2.43
Hen Island	0.049***	0.031***	0.004*	0.058***	—	4.61	3.70	3.70	1.27	1.20	2.43
Sandusky River	0.051***	0.045***	0.001	0.060***	0.003	—	2.75	3.52	1.54	1.10	1.58
Grand River, Ohio	0.043***	0.033***	0.001	0.051***	0.002	0.002	—	3.85	1.12	0.93	1.64
Van Buren Bay	0.059***	0.044***	0.002	0.063***	0.011*	0.001	0.008	—	0.90	0.83	1.70
Cattaraugus Creek	0.006	0.032***	0.048***	0.016***	0.047***	0.046***	0.037***	0.050***	—	1.71	2.50
Smoke's Creek	0.044***	0.001	0.033***	0.055***	0.027***	0.031***	0.033***	0.031***	0.034**	—	1.45
Grand River, Ont.	0.032***	0.051***	0.051***	0.055***	0.041***	0.045***	0.042***	0.052***	0.039***	0.029***	—

Note: F_{ST} values represent Weir and Cockerham's (1984) correction of F_{ST}. Significance of divergence between spawning groups is indicated by asterisks after the F_{ST} value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

model assuming admixture. We then examined the consistency among 10 independent runs at each K, the comparative probabilities of individuals assigning to one or more groups, the log-likelihood and posterior probability values from each run, and their respective grouping patterns, following the program methodology. In addition, we calculated ΔK according to the procedure recommended by Evanno et al. (2005), which is based on the rate of change in the log probability of data between successive K values. The magnitude of ΔK over the 10 replicate runs was graphed against K for K = 2–10, and the height of the modal value of the distribution was used to additionally verify the correct K value. Results of the STRUCTURE analyses then were compared with spawning group relationships derived from the BARRIER and genetic divergence analyses.

Results

Variation within spawning groups

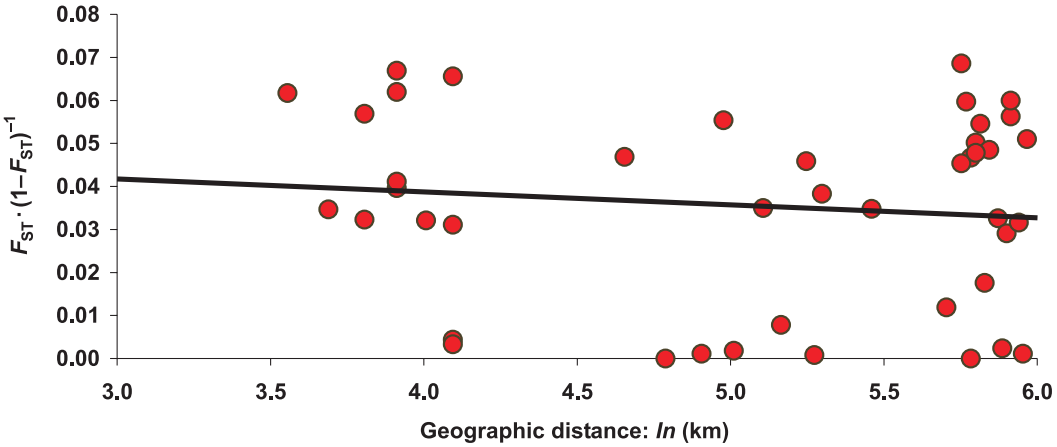
Genotypic data were collected for 448 walleye from 10 locations within Lake Erie and a single location in Lake St. Clair (Table 1; Fig. 1). Loci were unlinked and were highly polymorphic, ranging from 9 (*Svi*17) to 22 (*Svi*L7) alleles per locus (Table 2). Within each spawning group sample, each locus was represented by two to four common alleles and a larger number of alleles at lower frequencies. The average number of alleles per spawning group was 90.18 (range = 79–108); including one to three private alleles, except for Van Buren Bay where seven uniquely occurring alleles were present (Table 1). Walleye spawning at Van Buren Bay thus had the highest overall proportional representation of private alleles (P_{PA} = 0.067), whereas the sample from Grand River (Ohio) had the smallest proportion (P_{PA} = 0.011).

Observed heterozygosities for each spawning group were similar, ranging from 0.660 to 0.780 (Table 1). Out of the 11 spawning locations sampled, F_{IS} values for six (e.g., Hen Island, Maumee River, Grand River (Ohio), Van Buren Bay, Cattaraugus Creek, and Grand River, (Ontario)) showed significant deviations from HW equilibrium after Bonferroni correction (critical P for 10 loci = 0.005). All deviations from HW involved a single locus (*Svi*L8), which was due to the presence of null alleles for those spawning groups at that locus (based on analysis using MICRO-CHECKER (van Oosterhout et al. 2004)). After removal of the *Svi*L8 locus, all populations were in HW equilibrium using the remaining nine loci. Therefore, all subsequent genetic analyses were performed without the *Svi*L8 locus. We found no significant differences in allelic distributions between males and females collected from the same locality within the seven samples for which sex information was available.

Divergence patterns among spawning sites

Combining data from all 10 microsatellite loci for all sites combined revealed a significant departure from HW proportions (F_{IT} = 0.117, $P < 0.0001$), thus falsifying the null hypothesis of a single panmictic population. Similar significant results were obtained when data from the *Svi*L8 locus was then excluded from the analysis. Based on nine loci, significant allelic frequency differences were observed for 45 of the 55 pairwise comparisons among the 11 spawning sites

Fig. 2. Pairwise relationship between genetic divergence and geographic distance expressed as $F_{ST}(1 - F_{ST})^{-1}$ versus the natural logarithm of kilometres. Linear regression and correlation coefficients are $b = -0.003$ and $r = -0.12$, respectively (Mantel $P = 0.827$).



(Table 3). Using a critical $P = 0.05 \times 55^{-1}$ for Bonferroni correction, 42 pairwise F_{ST} values remained significant. Pooling genotypic data from each spawning group into respective physiographic regions (Table 1) revealed a statistically significant divergence between Lakes St. Clair and Erie ($F_{ST} = 0.026$, $P = 0.039$). Overall distinctions between the western and eastern basins of Lake Erie were not significant ($F_{ST} = 0.006$, $P = 0.341$) and suggest that walleye populations are not partitioned according to physiographic differences. Comparative pairwise tests of allelic frequency heterogeneity (Goudet et al. 1996) were congruent with the F_{ST} results shown in Table 3.

Genetic divergences were independent of spatial distances between pairs of spawning sites, as revealed by the regression of pairwise $F_{ST}(1 - F_{ST})^{-1}$ against the natural logarithm of geographical distance ($b = -0.003$, $r = -0.12$, $P = 0.827$; see Fig. 2). Likewise, pairwise estimates of the number of migrants per generation (N_m) varied unevenly across the Lake and did not correspond to physiographic regions (Table 3). Mean migration among all 11 samples after correction for sample size was $N_m = 6.94$, suggesting moderate levels of gene flow. However, pairwise estimates (Table 3) ranged from 0.83 to 8.09 between sites and most values were less than 2.00, thus indicating little gene flow among spawning groups from Lake St. Clair, two sites in western Lake Erie, and riverine locations in eastern Lake Erie. Interestingly, the highest pairwise rate of gene flow in Lake Erie ($N_m = 8.09$) occurred between the geographically distant spawning groups in the Maumee River (western basin) and Van Buren Bay (eastern basin).

Continuous track analysis of pairwise N_m estimates revealed a pattern of connectivity and isolation among walleye spawning groups uncorrelated with physiographic partitions in the Lake. In particular, a pathway of gene flow (= track) is suggested among some walleye spawning groups along the southern shore, including some western basin sites (Hen Island, Maumee River, and Sandusky River), the central basin location (Grand River (Ohio)), and Van Buren Bay in the eastern basin (designated by solid circles in Fig. 1). Spawning groups in the Huron River and Western Reefs (both in the western basin) fell outside the track and were genetically distinguishable from all other sites (shown with open circles in Fig. 1). BARRIER analysis of F_{ST} values (designated by straight lines in Fig. 1) confirmed the limits of the track; the first inferred barrier (i.e., the strongest, labeled I) separated the spawning groups from the western, central, and Van Buren Bay from the three rivers sampled in the eastern basin (Cattaraugus Creek, Smoke's Creek, and Grand River (Ontario)) and was supported by significant allelic divergences at eight separate loci. The second barrier (II) was supported by divergences at four of the loci and separated Lake Erie from the walleye spawning group in the Thames River (Lake St. Clair). The third barrier (III) separated walleye spawning in each of the three eastern rivers (Cattaraugus Creek, Smoke's Creek, and Grand River (Ontario)) from one another.

Bayesian analysis of genetic structure among all spawning groups included in this study was largely consistent with the results of the BARRIER analysis. The STRUCTURE analysis showed evidence for three population groups ($K = 3$ with posterior probability = 0.998 and maximum ΔK vs. K modal distribution peak height; see Fig. 3). The null hypothesis of panmixia across Lake Erie thus was rejected for $K = 1$, when all populations were considered in the overall analysis. Strongest assignments to single population groups were found for spawning groups from the Thames River, Lake St. Clair (0.848 for $K = 3$; Fig. 3), western Lake Erie reefs (0.813), and the eastern Lake Erie basin sites at Smoke's Creek (0.740), Cattaraugus Creek (0.724), and Grand River (Ontario) (0.672).

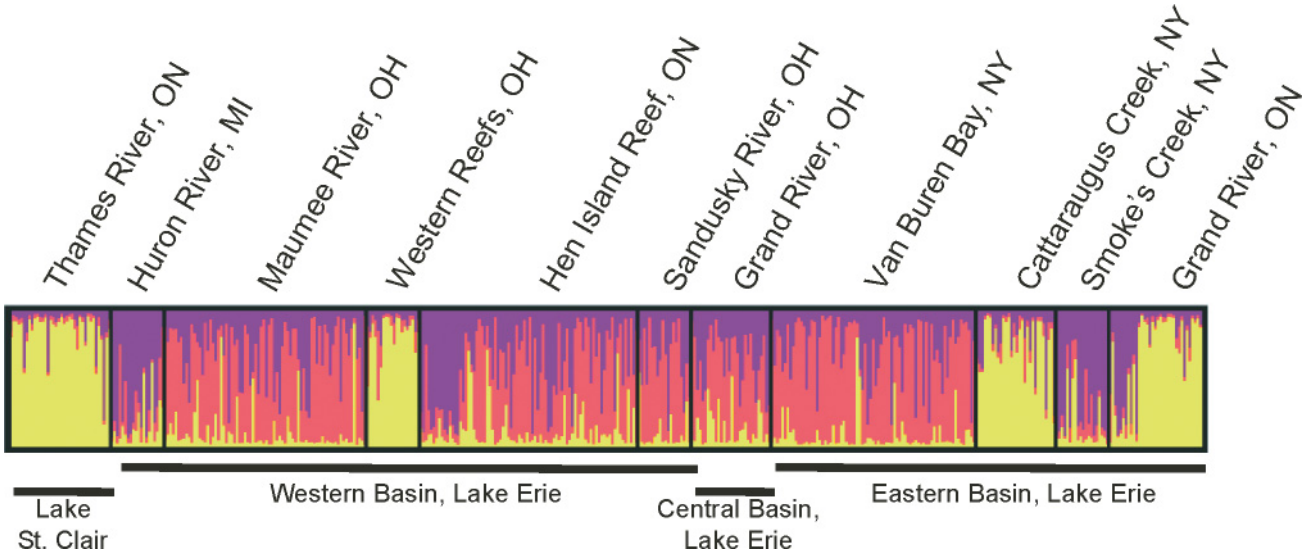
Moreover, the null hypothesis of panmixia was not rejected when a separate STRUCTURE analysis included only the five spawning groups comprising the track identified in the BARRIER analysis (posterior probability = 0.999 for $K = 1$). This restricted analysis thus indicated that spawning groups from the Maumee River, Sandusky River, Hen Island, Grand River (Ohio), and Van Buren Bay comprise a single interbreeding population of walleye.

Discussion

Patterns of microsatellite heterogeneity

Microsatellite DNA variation of Lake Erie walleye reveals greater diversity than that reported for the same loci in walleye populations from Minnesota (e.g., Borer et al. 1999; Eldridge et al. 2002) and Quebec (Wirth et al. 1999), likely

Fig. 3. Estimated population composition from Bayesian STRUCTURE analysis for $K = 3$ groups (posterior probability = 0.998). Each individual is represented by a thin vertical line, which is partitioned into three colored segments that represent the individual's estimated group membership fractions. Black lines separate individuals from different spawning sites. Ten STRUCTURE runs at each K produced nearly identical individual membership coefficients. Moreover, $K = 3$ was verified by calculation of the rate of change of the likelihood distribution and graphing ΔK vs. K, following Evanno et al. (2005).



reflecting the greater number of spawning groups and larger spatial scale included in our data set. Cena et al. (2006) reported similar levels of microsatellite variation in their survey of 46 inland populations of walleye from Ontario, which may also represent such mixing. Cena et al. (2006) further noted that deviations from HW equilibrium in Ontario walleye involved a single (unspecified) locus per population. In the present study, all deviations from HW equilibrium within spawning groups from Lake Erie also involved a single locus (*Svi*L8), for which the presence of null alleles was indicated. Thus, we conclude that our microsatellite data (with the possible exception of *Svi*L8) appear to represent an unbiased estimator for genetic distinctions among spawning groups of Lake Erie walleye.

Combining the information from all Lake Erie spawning groups into a single population group showed significant heterozygote deficiencies (positive F_{IT} values) at most loci, indicating that allelic and genotypic distributions are incompatible with a single panmictic population. This conclusion is supported by both the number of significant F_{ST} values among spawning groups and an unequal distribution of N_m estimates. Further, the significant overall F_{IT} value is larger than the comparable F_{ST} value, suggesting that localized patterns of inbreeding are more important determinants of genetic structure in Lake Erie than are distinctions among random assemblages of spawning groups. In other words, some spawning groups are more closely related than others. Moreover, there were no significant differences in allelic distributions between males and females within spawning groups (similar to findings based on mtDNA for several of these sites; Stepien and Faber 1998; Stepien et al. 2004). Thus, both male and female walleye have similar migration and divergence patterns.

Patterns of divergence and connectivity

Metapopulation structure among a connected series of subpopulations can assume different forms, depending on the unique ecology of the species and contexts in which it occurs (Pulliam 1988; Hanski 1999; Strange 1999). In Lake Erie, walleye spawning groups could potentially be segregated by extrinsic factors such as habitat differences in the Lake (east versus west) or type of spawning site (lake versus river). The predominant feature of genetic structure in Lake Erie walleye is the divergence of spawning groups in the eastern basin. We found no evidence to suggest that population structure in walleye is constrained by contemporary physiographic partitions in Lake Erie (e.g., basins rather than individual spawning sites), nor did lake spawning groups form a unique assemblage exclusive of those spawning in rivers.

Another notable feature of population structure in Lake Erie is the presence of a track of gene flow (inferred from N_m estimates) connecting most of the walleye spawning sites along the southern shoreline (i.e., Hen Island, Maumee River, Sandusky River, Grand River (Ohio), and Van Buren Bay), punctuated by a genetic barrier (inferred from F_{ST} values) with walleye that spawn in the three eastern tributaries (i.e., Cattaraugus Creek, Smoke's Creek, and Grand River (Ontario)). This pattern of gene flow and divergence is largely congruent with relationships among many of the same walleye spawning populations inferred from mtDNA sequences (Stepien and Faber 1998; Stepien et al. 2004). Although sample sizes in the earlier studies may have precluded some detection of fine-scale divergences, microsatellite data lend credence to the hypothesis that some walleye spawning groups are connected by contemporary patterns of gene flow, whereas others likely maintain ancient (Pleistocene or earlier) patterns of differentiation.

The genetic discontinuity within the eastern basin is intriguing, as Van Buren Bay was formerly occupied by the blue pike (*S. vitreus glaucus*), an extinct subspecies endemic to the deeper habitats of eastern Lake Erie (Stone 1948). Stepien et al. (2004) reported a distinctive assemblage of mtDNA haplotypes in the Cattaraugus Creek population, and

our analyses further identified divergence between Cattaraugus Creek and each of the three eastern rivers. It is possible that the walleye populations in the eastern rivers are older, resident populations, whereas the spawning group in Van Buren Bay contains strays from the western basin that began to exploit this lake spawning habitat after the demise of the blue pike in the late 1950s. Prior hybridization between the two forms of walleye (*S. v. vitreus* and *S. v. glaucus*) may account for the higher number of private alleles documented from Van Buren Bay in our study.

Correspondence of patterns in walleye with other fishes

Many of these patterns are also evident in other Lake Erie fishes, indicating similar biogeographic history. Notably, several species of Lake Erie fishes show west–east patterns in allelic frequencies: walleye *S. vitreus* (Stepien and Faber 1998; Stepien et al. 2004; this study), brown bullhead *Ameiurus nebulosus* (Murdoch and Hebert 1997), yellow perch *Perca flavescens* (Ford and Stepien 2004), and smallmouth bass *Micropterus dolomieu* (Stepien et al. 2007). Each of these species represents a different suite of life history attributes, including reproductive strategies, vagility, and habitat affinities. Shared patterns among species as different as these have been used to postulate the role of Pleistocene–postPleistocene events responsible for the structure of freshwater fish faunal assemblages (e.g., Wiley and Mayden 1985; Strange and Burr 1997).

Unlike walleye, subpopulations of smallmouth bass across Lake Erie correspond to a broadscale isolation by geographic distance pattern (Stepien et al. 2007). Smallmouth bass spawning groups are linked by a smaller track along the southern Lake shore, which does not extend to Van Buren Bay. Also, additional population units were discerned among Lake Erie spawning populations of smallmouth bass using a similar STRUCTURE analysis of microsatellite data ($K = 4$, posterior probability = 0.997; Stepien et al. 2007). Differences between smallmouth bass and walleye likely reflect their respective life histories and vagilities, with smallmouth bass being largely confined to a limited home range throughout its life history (Scott and Crossman 1973).

As in walleye, natal site fidelity appears to maintain reproductive isolation in a number of species, including rainbow smelt (*Osmerus mordax*; Bernatchez 1997), Atlantic herring (*Clupea harengus*; Bekkevold et al. 2005; Jorgensen et al. 2005), walleye pollock (*Theragra chalcogramma*; O'Reilly et al. 2004), and mackerels (*Scomber* spp.; Zardoya et al. 2004). Other species within the genus *Sander* are known to migrate to specific rivers to spawn (Koed et al. 2000, 2002), and this behavior may be a general pattern in this genus. Although the mechanism for homing behavior is unknown in percids, a study by Gerlach et al. (2001) suggests that Eurasian perch *Perca fluviatilis* may recognize kin through olfactory cues. Thus, it is possible that walleye return to their natal locations guided by olfactory information imprinted during early stages of their life history, which may be a primary mechanism for maintaining divergence among the respective spawning groups.

Mechanisms: patterns of gene flow and divergence in walleye

Population genetic structure is the consequence of localized patterns of gene flow punctuated by isolation and divergence. Lake Erie walleye exhibit patterns of genetic variability consistent with both phenomena: a track running along the southern shore and across the Lake that ends abruptly at a border separating the three relatively isolated eastern rivers (e.g., Cattaraugus Creek, Smoke's Creek, and Grand River (Ontario)). Thus, there are two different attributes of population structure that must be considered to better understand the mechanisms responsible for genetic relationships in Lake Erie walleye: (*i*) genetic divergence in the rivers to the east and west and (*ii*) connectivity within the track.

Our results suggest that river spawning groups in the eastern basin of Lake Erie remain distinct because most adults return to their natal locations to reproduce. Similar to many anadromous fishes, walleye form seasonal mixed stocks in open water habitats, but then separate at spawning and return to their natal sites to spawn. This pattern of repeat homing to specific spawning locations is well documented in walleye (Olson et al. 1978), and it is widely accepted that most individuals display spawning-site philopatry (Regier et al. 1969; Colby and Nepszy 1981; Horrall 1981).

Whereas natal homing as a mechanism for divergence among walleye spawning groups from the eastern rivers is relatively easy to postulate, the dynamics of gene flow throughout the Lake is more challenging. Tagging studies indicate that walleye from the western basin tend to migrate eastward into the central and eastern basins, whereas those tagged in Van Buren Bay are nonmigratory (Thomas et al. 2005). The number of private alleles per spawning group within the track ranged from one (Grand River (Ohio)) to seven (Van Buren Bay), whereas the remaining three populations had two to three unique alleles each. A larger number of private alleles in the spawning group from Van Buren Bay may reflect an accumulation of private alleles from recipient migratory spawning groups (Kawecki and Holt 2002; Kennington et al. 2003). In other words, asymmetrical gene flow may result from a greater number of individuals in a sink subpopulation (here, Van Buren Bay), whose alleles trace to immigrants from source subpopulations (e.g., sites to the west along the track); this merits further testing in walleye. Alternatively, the additional private alleles in the Van Buren Bay spawning group may trace their ancestry to the now-extinct blue pike that is hypothesized to have introgressed with walleye (see Stone 1948 and Stepien and Faber 1998), which is being tested by the Great Lakes Genetics Laboratory.

The mechanism(s) responsible for the track may be demographic. For example, Fraser et al. (2004) reported evidence that asymmetric migration proceeds from sites with a larger effective population size in brook trout (*Salvelinus fontinalis*) from a postglacial lake in Quebec, and a similar situation may exist in Lake Erie walleye. Walleye population sizes and recruitment (as estimated by management agencies during spawning runs; Roger Knight, Ohio Division of Wildlife, 305 E. Shoreline Drive, Sandusky, OH 44870, USA, personal communication) are significantly higher in the western basin than in the eastern basin. The Lake Erie Walleye Task Group of the Great Lakes Fishery Commission estimates that western basin populations range from 30 to 40 million spawning individuals, whereas those in the eastern basin number about 0.5–2 million (Thomas et al. 2005). Thus, it is possible that the greater population size of

walleye in the western basin contributes more individuals to a migrant pool than does the spawning group from Van Buren Bay.

Origins of the pattern

Population structure in Lake Erie walleye appears to reflect contemporary differences in natal site fidelity and demographic processes (e.g., population sizes); however, the overall pattern (most notably the west–east distinction) may be attributable to postPleistocene colonization of the Lake. Tributaries of eastern Lake Erie were isolated from the rest of the Lake by ice sheets during the Pleistocene, and it is notable that the eastern and central basins remained isolated during the earliest stages of Lake Erie (about 7 000 to 10 000 years before present; Holcombe et al. 2003; see internal shading in Fig. 1). The spawning groups of the three eastern tributaries were separated from other walleye population groups until this barrier broke down, and this genetic discontinuity has been maintained by natal site fidelity as discussed above. On the other hand, the track consists of some spawning sites in the western basin and along the southern shore of the Lake. During the early stages of Lake Erie, the western basin was dominated by a large river system that included the present Maumee and Sandusky rivers as its headwaters. Kitchell et al. (1977) noted that the walleye is primarily a large river fish and occupies habitats similar to the pools of large rivers when it occurs in lakes. Western Lake Erie is such a habitat in terms of shallow depth, minimal flow, and appropriate spawning substrates (Kitchell et al. 1977, p. 1938), and it is likely that the spawning groups dominating the track are descendent from those occurring in the rivers of the western basin.

In conclusion, walleye in Lake Erie represent a classical subdivided population in which differences among spawning groups are likely the result of two contemporary intrinsic mechanisms: (*i*) demographic patterns in which the differential productivity and concomitant contributions to a common gene pool result in gene flow and (*ii*) natal site fidelity that maintains isolation of specific river spawning groups. These contemporary patterns have modified but not obscured historical patterns of divergence attributable to postglacial colonization by different source populations, which is being further investigated in a broader-scale study using both microsatellite and mtDNA (C.A. Stepien, R.L. Lohner, and D.J. Murphy, unpublished data). Although walleye do not segregate on the basis of physiographic differences in Lake Erie or on distinctions between subpopulations that spawn in rivers versus shoals, we found evidence that Lake Erie walleye appear to retain historic patterns of genetic diversity despite recent patterns of exploitation and habitat destruction.

Acknowledgements

This is publication No. 2007-006 from the Lake Erie Center. This project was developed by the Great Lakes Genetics Laboratory in conjunction with the Lake Erie Walleye Task Force of the Great Lakes Fishery Commission and Great Lakes fishery managers — especially the Ohio Division of Wildlife, the Michigan Department of Natural Resources, the Ontario Ministry of Natural Resources, the Pennsylvania Fish and Boat Commission, and the New York Department of Environmental Conservation. The study was supported by National Oceanic and Atmospheric Administration (NOAA) Ohio Sea Grants Nos. R/LR-4 and R/LR-8, an award from the US Fish and Wildlife Service Restoration Act through the Great Lakes Fishery Commission, and US Environmental Protection Agency (USEPA) grant No. CR-83281401-0 (all to CAS). Walleye were collected with the help of D. Einhouse, R. Haas, T. Johnson, K. Kayle, C. Knight, R. Knight, E. Roseman, M. Turner, M. Thomas, J. Tyson, C. Vandergoot, and C. Wilson. DNA technicians D. Murphy, C. Taylor, and R. Lohner provided valuable help with laboratory and data analysis. The manuscript additionally benefited from proofreading and suggestions by M. Neilson, R. Lohner, and D. Murphy.

References

Alroy, J. 1995. Continuous track analysis: a new phylogenetic and biogeographic method. Syst. Biol. **44**: 152–178.

Bailey, R.M., and Smith, G.R. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Can. J. Fish. Aquat. Sci. **38**: 1539–1561.

Balloux, F., and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. **11**: 155–165.

Bekkevold, D., Andre, C., Dahlgren, T.G., Clausen, L.A.W., Tortensen, E., Mosegaard, H., Carvalho, G.R., Christensen, T.B. Norlinder, E., and Ruzzante, D.E. 2005. Environmental correlates of population differentiation in Atlantic herring. Evolution, **59**: 2656–2668.

Bernatchez, L. 1997. Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt *Osmerus mordax* and their reproductive isolation in the St. Lawrence River estuary (Quebec, Canada). Mol. Ecol. **6**: 73–83.

Billington, N., and Strange, R.M. 1995. Mitochondrial DNA analysis confirms the existence of a genetically divergent walleye population in northeastern Mississippi. Trans. Am. Fish. Soc. **124**: 770–776.

Billington, N., Barrette, R.J., and Hebert, P.D.N. 1992. Management implications of mitochondrial DNA variation in walleye stocks. N. Am. J. Fish. Manag. **12**: 276–284.

Bolsenga, S.J., and Herdendorf, C.E. 1993. Lake Erie and Lake St. Clair handbook. Wayne State University Press, Detroit, Mich.

Borer, S.O., Miller, L.M., and Kapuscinski, A. 1999. Microsatellites in walleye *Stizostedion vitreum*. Mol. Ecol. **8**: 335–346.

Cena, C.J., Morgan, G.E., Malette, M.D., and Heath, D.D. 2006. Inbreeding, outbreeding, and environmental effects on genetic diversity in 46 walleye (*Sander vitreus*) populations. Mol. Ecol. **15**: 303–320.

Colby, P.J., and Nepszy, S.J. 1981. Variation among stocks of walleye (*Stizostedion vitreum*): management implications. Can. J. Fish. Aquat. Sci. **38**: 1814–1831.

Eldridge, W.H., Bacigalupi, M.D., Adelman, I.R., Miller, L.M., and Kapuscinski, A.R. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. Can. J. Fish. Aquat. Sci. **59**: 282–290.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14**: 2611–2620.

Ferguson, R.G., and Derksen, A.J. 1971. Migrations of adult and juvenile walleyes (*Stizostedion vitreum vitreum*) in southern Lake Huron, Lake St. Clair, Lake Erie, and connecting waters. J. Fish. Res. Board Can. **28**: 1133–1142.

Ford, A.M., and Stepien, C.A. 2004. Genetic variation and spawning population structure in Lake Erie yellow perch, *Perca flavescens*: a comparison with a Maine population. *In* Proceedings of Percis III, the 3rd International Symposium on Percid Fishes, 20–24 July 2003, University of Wisconsin, Madison, Wisc. *Edited by* T.P. Barry and J.A. Malison. University of Wisconsin Sea Grant Institute, Madison. pp. 131–132.

Fraser, D.J., Lippe, C., and Bernatchez, L. 2004. Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). Mol. Ecol. **13**: 67–80.

Gerlach, G., Schardt, U., Eckmann, R., and Meyer, A. 2001. Kin structured subpopulations in Eurasian perch (*Perca fluviatilis*). Heredity, **86**: 213–221.

Goodyear, C.S., Edsall, T.A., Dempsey, D.M., Moss, G.D., and Polanski, P.E. 1982. Atlas of spawning and nursery areas of Great Lakes fishes. Vols. 1–14; Vol. 8: Detroit River. US Fish and Wildlife Service, Washington, D.C. FWS/OBS-82/52.

Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate *F*-statistics. J. Hered. **86**: 485–486.

Goudet, J., Raymond, M., de Meeus, T., and Rousset, F. 1996. Testing differentiation in diploid populations. Genetics, **144**: 1933–1940.

Guo, S.W., and Thompson, E.A. 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics, **48**: 361–372.

Hanski, I. 1999. Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. Oikos, **87**: 209–219

Hatch, R.W., Nepszy, S.J., Muth, K.M., and Baker, C.T. 1987. Dynamics of the recovery of the western Lake Erie walleye (*Stizostedion vitreum vitreum*) stock. Can. J. Fish. Aquat. Sci. **44**: 15–22.

Hedrick, P.W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution, **53**: 313–318.

Holcombe, T.L., Taylor, L.A., Reid, D.F., Warren, J.S., Vincent, P.A., and Herdendorf, C.E. 2003. Revised Lake Erie postglacial lake level history based on new detailed bathymetry. J. Gt. Lakes Res. **29**: 681–704.

Horrall, R.M. 1981. Behavioral stock-isolating mechanisms in Great Lakes fishes with special reference to homing and site imprinting. Can. J. Fish. Aquat. Sci. **38**: 1481–1496.

Hubbs, C.L., and Lagler, K.F. 2004. Fishes of the Great Lakes region. *Revised by* G.R. Smith. The University of Michigan Press, Ann Arbor, Mich.

Jennings, M.J., Claussen, J.E., and Philipp, D.P. 1996. Evidence for heritable preferences for spawning habitat between two walleye groups. Trans. Am. Fish. Soc. **125**: 978–982.

Jorgensen, H.B.H., Hansen, M.M., Bekkevold, D., Ruzzante, D.E., and Loeschcke, V. 2005. Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol. Ecol. **14**: 3219–3234.

Kawecki, T.J., and Holt, R.D. 2002. Evolutionary consequences of asymmetric dispersal rates. Am. Nat. **160**: 333–347.

Kennington, W.J., Gockel, J., and Partridge, L. 2003. Testing for asymmetrical gene flow in a *Drosophila melanogaster* body-size cline. Genetics, **165**: 667–673.

Kitchell, J.F., Johnson, M.G., Minns, C.K., Loftus, K.H., Greig, L., and Oliver, C.H. 1977. Percid habitat: the river analogy. J. Fish. Res. Board Can. **34**: 1936–1940.

Knight, R.L. 1997. Successful interagency rehabilitation of Lake Erie walleye. Fisheries, **22**: 16–17.

Koed, A., Mejlhelde, P., Balleby, K., and Aarestrup, K. 2000. Annual movement of adult pikeperch in a lowland river. J. Fish Biol. **57**: 1266–1279.

Koed, A., Balleby, K., and Mejlhelde, P. 2002. Migratory behavior of adult pikeperch (*Stizostedion lucioperca*) in a lowland river. Hydrobiologia, **483**: 175–184.

Manel, S., Schwartz, M.K., Luikart, G., and Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trend. Ecol. Evol. **18**: 189–197.

Manni, F., Guerard, E., and Heyer, E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". Hum. Biol. **76**: 173–190.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. **27**: 209–220.

Murdoch, M.H., and Hebert, P.D.N. 1997. Mitochondrial DNA evidence of distinct glacial refugia for brown bullhead (*Ameiurus nebulosus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. **54**: 1450–1460.

Nepszy, S.J. 1977. Changes in percid populations and species interactions in Lake Erie. J. Fish. Res. Board Can. **34**: 1861–1868.

Olson, D.E., and Scidmore, W.J. 1962. Homing behavior in spawning walleyes. Trans. Am. Fish. Soc. **91**: 355–561.

Olson, D.E., Schupp, D.H., and Macins, V. 1978. An hypothesis of homing behavior of walleyes as related to observed patterns of passive and active movement. *In* Selected coldwater fishes of North America. *Edited by* R.L. Kendall. Am. Fish. Soc. Special Publ. 11. Bethesda, Md. pp. 52–57.

O'Reilly, P.T., Canino, M.F., Bailey, K.M., and Bentzen, P. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. **13**: 1799–1814.

Pritchard, J.K., Stephens, M., and Donelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**: 945–959.

Pulliam, H.R. 1988. Sources, sinks and population regulation. Am. Nat. **132**: 652–661.

Raymond, M., and Rousset, F. 1995. GENEPOP. Version 1.2. Population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Regier, H.A., Applegate, V.C., and Ryder, R.A. 1969. The ecology and management of the walleye in western Lake Erie. Great Lakes Fish. Comm. Tech. Rep. **15**: 1–101.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics, **145**: 1219–1228.

Schneider, J.C., and Leach, J.H. 1977. Walleye (*Stizostedion vitreum vitreum*) fluctuations in the Great Lakes and possible causes, 1800–1975. J. Fish. Res. Board Can. **34**: 1878–1889.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. Bull. 184. pp. 767–774.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution, **39**: 53–65.

Stepien, C.A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. *In* Symposium 17: Evolution and the Aquatic Ecosystem: Defining Unique Units in Group Conservation, Monterey, California, May 1994. *Edited by* J. Neilson. American Fisheries Society, Bethesda, Md. pp. 263–287.

Stepien, C.A., and Faber, J.E. 1998. Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. Mol. Ecol. **7**: 1757–1769.

Stepien, C.A., Taylor, C.D., and Einhouse, D.W. 2004. An analysis of genetic risk to a native spawning stock of walleye *Sander vitreus* (*Stizostedion vitreum*) due to stocking in Cattaraugus Creek. *In* Proceedings of Percis III, the 3rd International Symposium on Percid Fishes, 20–24 July 2003, University of Wisconsin, Madison, Wisc. *Edited by* T.P. Barry and J.A. Malison. University of Wisconsin Sea Grant Institute, Madison. pp. 93–94.

Stepien, C.A., Murphy, D., and Strange, R.M. 2007. Broad- to fine-scale genetic patterning in smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: an interplay of behavior and geography. Mol. Ecol. **16**. In press.

Stone, F.L. 1948. A study of the taxonomy of the blue and yellow pikeperches (*Stizostedion*) of Lake Erie and Lake Ontario. Ph.D. dissertation, University of Rochester, N.Y.

Strange, R.M. 1999. Historical biogeography, ecology, and fish distributions: conceptual issues for establishing IBI criteria. *In* Assessing the sustainability and biological integrity of water resources using fish communities. *Edited by* T.P. Simon. CRC Press, Boca Raton, Fla. pp. 65–78.

Strange, R.M., and Burr, B.M. 1997. Intraspecific phylogeography of North American highland fishes: a test of the Pleistocene vicariance hypothesis. Evolution, **51**: 885–897.

Thomas, M., Einhouse, D., Isermann, D., Kayle, K., Turner, M., Vandergoot, C., Belore, M., Cook, A., Johnson, T., Ryan, P., Wright, E., and Kenyon, R. 2005. Report for 2004 by the Lake Erie Walleye Task Group as presented to the Standing Technical Committee, Lake Erie Committee, Great Lakes Fishery Commission, Niagara Falls, Ont.

Todd, T.N., and Haas, R.C. 1993. Genetic and tagging evidence for movement of walleyes between Lake Erie and Lake St. Clair. J. Gt. Lakes Res. **19**: 445–452.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**: 535–538.

van Oosterhout, C., Weetman, D., and Hutchinson, W.F. 2006. Estimation and adjustment of microsatellite null alleles in nonequilibrium populations. Mol. Ecol. Notes, **6**: 255–256.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wiley, E.O., and Mayden, R.L. 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Missouri Bot. Gard. **72**: 596–635.

Wirth, T., Saint-Laurent, R., and Bernatchez, L. 1999. Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross species amplification within the family Percidae. Mol. Ecol. **8**: 1957–1969.

Wolfert, D.R. 1963. The movements of walleyes tagged as yearlings from the eastern and western basins of Lake Erie. Trans. Am. Fish. Soc. **92**: 414–420.

Wright, S. 1943. Isolation by distance. Genetics, **28**: 139–156.

Wright, S. 1978. Evolution and the genetics of populations. Vol. 4. Variability within and among populations. University of Chicago Press, Chicago, Ill.

Zardoya, R., Castilho, R., Grande, C., Favre-Krey, L., Caetano, S., Marcato, S., Krey, G., and Patarnello, T. 2004. Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the cub mackerel (*Scomber japonicus*), in the Mediterranean Sea. Mol. Ecol. **13**: 1785–1798.