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Signatures of vicariance, postglacial dispersal and spawning philopatry: population genetics of the walleye *Sander vitreus*

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Abstract

Population genetic relationships reveal the signatures of current processes such as reproductive behaviour and migration, as well as historic events including vicariance and climate change. We analyse population structure of native walleye *Sander vitreus* across North America, encompassing 10 nuclear DNA microsatellite loci, 26 spawning sites and 921 samples from watersheds across the Great Lakes, Lake Winnipeg, upper Mississippi River, Ohio River and Mobile Bay of the Gulf Coast. Geographical patterning is assessed using phylogenetic trees, pairwise F_{ST} analogues, hierarchical partitioning, Mantel regression, Bayesian assignment and Monmonier geographical networks. Results reveal congruent divergences among population groups, corresponding to historic isolation in glacial refugia, dispersal patterns and basin divisions. Broad-scale relationships show genetic isolation with geographical distance, but reproductive groups within basins do not – with some having pronounced differences. Greatest divergence distinguishes outlying Gulf Coastal and northwest populations, the latter tracing to dispersal from the Missourian refugium to former glacial Lake Agassiz, and basin isolation ~7000 ya. Genetic barriers in the Great Lakes separate groups in Lakes Superior, Huron's Georgian Bay, Erie and Ontario, reflecting contributions from Mississippian and Atlantic refugia, and changes in connectivity patterns. Walleye genetic patterns thus reflect vicariance among watersheds and glacial refugia, followed by re-colonization pathways and changing drainage connections that established modern-day northern populations, whose separations are maintained through spawning site fidelity. Conservation management practices should preserve genetic identity and unique characters among these divergent walleye populations.

Keywords: glacial refugia, Great Lakes, natal homing, Percidae, population genetics, *Sander vitreus*, walleye

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Introduction

The evolution of genetic structure in a subdivided population is a central problem in evolutionary studies, with critical implications for conservation. Mechanisms driving or constraining gene flow may be extrinsic or intrinsic to the species (or an interplay between both) and operate on either ecological (10–1000 generations) or evolutionary time scales (>1000 generations; see Ricklefs 1989 & Avise 2004). For example, broad-scale landscape genetic patterns in riverine fishes often are products of extrinsic barriers to gene flow (i.e. vicariance and allopatric isolation) that originated from ancient (i.e. Pleistocene or earlier) drainage patterns (Wiley & Mayden 1985; Strange & Burr 1997; Poissant *et al.* 2005; Burridge *et al.* 2008). Conversely, fishes inhabiting large lacustrine systems may exhibit relatively shallow genetic

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structures attributable to contemporary patterns of colonization and gene flow (see Turgeon & Bernatchez 2001; Miller 2003). Their populations often display relatively homogenous genetic compositions across large spans of interconnected habitats, which may be punctuated by differentiation among some demes (=subpopulations) inhabiting specific basins or reef systems (Stepien *et al.* 2007; Parker *et al.* 2009; VanDeHey *et al.* 2009). It is often unclear whether these shallow genetic divergences are the result of recent isolation or restricted gene flow.

Possible intrinsic mechanisms for fine-scale divergences in the absence of extrinsic barriers to gene flow include: (i) low vagility, (ii) natal site fidelity and (iii) metapopulation structure (Marjoram & Donelly 1994; Hudson 1998; Wakely & Aliacar 2001). A clinal pattern may form among closely spaced demes connected by some gene flow if the average individual dispersal rate is relatively low (Irwin 2002). This genetic structure fits a pattern of isolation by geographical distance, which would not occur in most highly migratory species. Natal site fidelity, on the other hand, is known to occur in many anadromous fishes (those that mature in open waters and then return to rivers to spawn), and promotes isolation in proportion to the accuracy of the mechanism underlying homing activity. However, it is often difficult to distinguish between these intrinsic mechanisms and extrinsic factors that impede gene flow, such as physiographical features that define suitable habitats. The central question is to identify which factors (e.g. historical isolation, contemporary landscape features and/or intrinsic behaviours) are responsible for genetic structure among demes connected by gene flow and in what time frame they operate (e.g. along ecological or evolutionary time scales).

Broad-scale genetic patterns among population groups largely are shaped by extrinsic historical factors, notably geography and climate, which regulate divergence or connectivity. Fine-scale relationships usually stem from contemporary intrinsic mechanisms, such as migration and reproductive behaviour, promoting either gene flow or genetic drift. This interplay among geographical, temporal and behavioural influences on the barriers or cohesion among populations thus reveals patterns observable at different spatial scales, which emerge over evolutionary time. However, it is especially problematic to detect and interpret fine-scale structure, particularly in systems that have few geographical separations and among groups that display intermediate levels of migration. Such populations/subpopulations often display some evidence for departure from panmixia, yet retain considerable connectivity (see summary by Waples & Gaggiotti 2006). Delineating this fine-scale structure may be critically important for conserving native genetic diversity and divergence patterns.

Geographical, life history and behaviour features that may lead to genetic divergence

The primary phylogeographic determinant that shaped modern-day aquatic populations in the North American Great Lakes region was their geographical isolation in southerly refugia during the late Wisconsinan glaciations (reaching their maxima ~18 kya), followed by their dispersal to found populations in the modern Great Lakes system ~13–4 kya (Scott & Crossman 1973; Bolsenga & Herdendorf 1993; Bernatchez & Wilson 1998). Over a period of nearly 5 ky, the Wisconsinan glaciations advanced and retreated, creating a complicated series of lakes with radically different drainage patterns; whose varying connections shaped the modern Great Lakes fauna (Underhill 1986; Mandrak & Crossman 1992; Larson & Schaetzl 2001). Populations of fishes from the Great Lakes today are believed to comprise a genetic admixture originating from several areas – including the Missourian, Mississippian and Atlantic Coastal glacial refugia (Ward *et al.* 1989; Billington *et al.* 1992; Murdoch & Hebert 1997; Turgeon & Bernatchez 2001). The historic genetic signatures of the descendents from these refugia may have been reinforced by vicariant barriers among lakes, their basins and drainages, as well as obscured by gene flow among interconnected sites. These scenarios are explored in this study.

The walleye *Sander vitreus* (Teleostei: Percidae) is a popular commercial and sport fish that is widely distributed across much of North America, and a large piscivore that aggregates to spawn in gravel along river riffles or lake reefs (Colby *et al.* 1994; Jennings *et al.* 1996). Prior genetic studies of walleye have uncovered population differences among lakes and their drainages dating back to glacial refugia (Billington *et al.* 1992; Stepien 1995; Stepien & Faber 1998), whose patterns are comprehensively explored in this study. Walleye are vagile at nonreproductive times, and tagging studies report that they move among lake systems (Ferguson & Derkson 1971; Todd & Haas 1993) and then return to spring spawning locations (Olson & Scidmore 1962; Wolfert & Van Meter 1978; Todd & Haas 1993). Tagging results indicate that individuals tend to return to their natal spawning sites (Regier *et al.* 1969; Horrall 1981; Goodyear *et al.* 1982), which appears to have a genetic basis (Jennings *et al.* 1996; Stepien & Faber 1998). Ours is the first reported study based on high-resolution microsatellite markers to analyse the genetic structure of walleye across its natural range, as well as the first to compare broad- vs. fine-scale divergence patterns.

Genetic patterns of walleye in the Great Lakes and beyond have probably been altered by anthropogenic changes in their habitats during the past 50 years, including loss of wetlands, channelization of major streams and dams, oxygen depletion, shoreline modification, siltation of spawning areas, nutrient enrichment, deterioration in water quality, and sand and gravel extraction (Trautman 1981; Bolsenga & Herdendorf 1993; Ryan *et al.* 2003). Our study was designed to avoid areas of known 'stocking' or augmentation with fish from other regions, where possible, concentrating on natural spawning population areas. However, some areas, such as the St. Louis River in Lake Superior and Saginaw Bay in Lake Huron, have been stocked for many years (Fielder 2002; Fielder *et al.* 2008), which may have obscured historical genetic distinctiveness of reproductive groups and is examined in this study. We anticipate that our results will provide a baseline for comparing today's walleye populations with those in the future.

Objectives, hypotheses and approach

Our study tests for genetic structure and diversity patterns among spawning populations of walleye across its native range, to understand the interplay among historic divergences and dispersal, contemporary connectivity and behavioural reproductive isolation factors. We use methods from population genetics and landscape genetics (Manel *et al.* 2003; Storfer *et al.* 2007), to analyse how environmental features have shaped the historical and present-day population genetic structure of walleye. This approach allows us to evaluate the genetic linkages among populations in relation to likely barriers to gene flow, as well as connectivity among watersheds and their features (see summaries by Manel *et al.* 2003; Diniz-Filho *et al.* 2008). We thus identify the primary genetic discontinuities across the native range of North American walleye and relate these to patterns of gene flow, population structure and possible local adaptation.

We analyse allelic variation at 10 nuclear microsatellite loci to test the general null hypothesis of panmixia among populations against the alternative hypotheses for genetic patterning resulting from behavioural and/or geographical isolation, specifically:

1 Are reproductive groups of walleye in interconnected waterways genetically separable and how do these relate to other taxa with similar distributions?
2 Do genetic divergences correspond to geographical distances?
3 What is the degree of population delineation across and among watersheds and lakes?
4 Are historic colonization patterns from glacial refugia retained?

Population genetic relationships for walleye are then compared with those characterizing other taxa from freshwater systems, to aid interpretation of common influences on genetic divergence and gene flow.

Factors leading to genetic divergence patterns are explored – including: (a) historical (e.g. population differences arising during isolation in southerly glacial refugia during the late Wisconsinan glaciations, followed by their colonization of the modern Great Lakes region), (b) geographical (patterns corresponding to isolation by spatial distance among interconnected waterways and vicariant biogeographical barriers formed by drainage systems and lake basins) and (c) behavioural (tendency to return to spawn in natal localities from generation to generation). Outlying populations include watersheds from the upper Mississippi River, the Ohio River and the Tombigbee River/Mobile Bay/Gulf Coast drainages. We additionally compare these biogeographical patterns with those for taxa from other previously glaciated areas of the world. This investigation is anticipated to serve as a baseline for additional and future studies of the genetic variation of walleye and relatives, and constitutes a valuable comparison with population genetic patterns in other freshwater taxa.

Materials and methods

Sample locations and preparation

Walleye collections focused on native lacustrine and river spawning sites, including the Great Lakes (Lakes Superior, Huron, Michigan, Erie and Ontario) and river drainages (Detroit River/Lake St. Clair, upper Mississippi River, Ohio River, New River and Tombigbee River), encompassing broad to fine geographical scales. Geographical distribution of sampling sites is shown in Fig. 1 and Table 1, and totalled 921 individuals among 26 locations. Most of the sampling was conducted by fishery agencies (see Acknowledgements) at known spawning sites during the reproductive season, which clipped a small portion of pectoral fin about 1–2 cm^2 from adult fish and placed the clips in 95% ethanol.

Microsatellite loci procedure

Genomic DNA was extracted and purified from the ethanol-fixed fin clips with a DNeasy Qiaquick kit (Qiagen), then frozen and archived. The polymerase chain reaction (PCR) was used to amplify allelic length variants from 10 microsatellite loci developed for walleye by Borer *et al.* (1999) (*Svi* 4, 6, 17, 18 and 33), Wirth

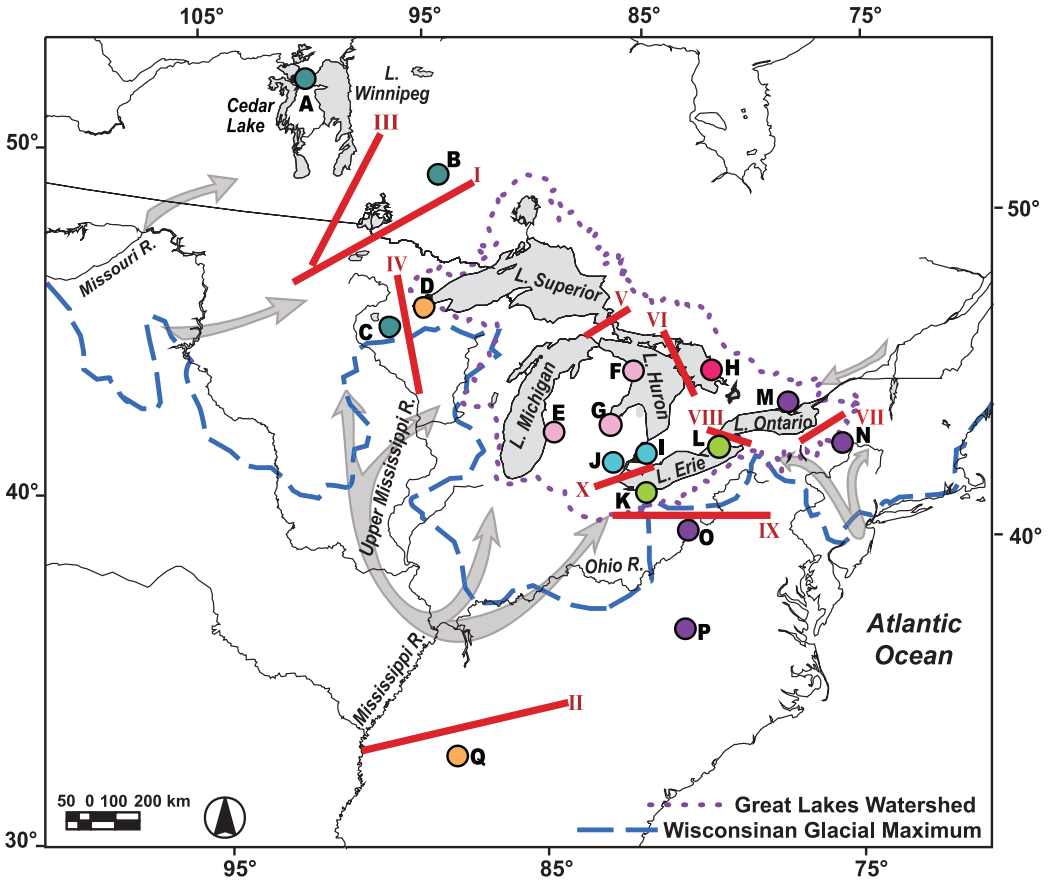

Fig. 1 Map showing locations of collection sites for walleye, with primary sampling sites lettered (see Table 1). Dotted line encircles the Great Lakes watershed, dashed line designates maximal extent of the Wisconsin glaciations and arrows denote hypothesized colonization pathways from glacial refugia (adapted from Murdoch & Hebert 1997; Mandrak & Crossman 1992). Roman numerals show the 10 primary genetic divisions among populations based on our results using the Manni *et al.* (2004a,b) BARRIER approach, designated with Roman numerals ranked from the greatest to less pronounced (I to X; % Bootstrap support and number of loci supporting each barrier are given in Results). Colours of dots correspond to primary population group membership representation according to Bayesian STRUCTURE analysis (Pritchard *et al.* 2000; Pritchard & Wen 2004) detailed in Fig. 3.

et al. (1999) (*Svi* L6, L7 and L8) and Eldridge *et al.* (2002) (*Svi* 2 and 7). PCR reactions contained 50 mm KCl, 1.5 mm $MgCl_2$, 10 mm Tris–HCl, 50 μm of each deoxy-nucleotide, 0.5 μm of both forward and reverse primers, 5–30 ng DNA template and 0.6–1.2 units of Taq polymerase per 10 μL of reaction volume, topped by an oil overlay. For each locus, the 'forward primer' was synthesized with a 5′ fluorescent label and four different dye labels were used, facilitating multiplexing and poolplexing. The program on a MJR DYAD thermalcycler (Bio-Rad Laboratories) was 2 min at 94 °C for initial denaturation; followed by 35 cycles of denaturation (94 °C, 30 s), primer annealing (1 min) at 54 °C for *Svi*L6, L7, L8 and L7 or 60 °C for *Svi*2, 4, 6, 7, 18 and 33, and polymerase extension (72 °C, 30 s), succeeded by a final extension at 72 °C for 5 min.

Amplification products were diluted 1:50 with dH_2O, of which 1 μL was added to 13 μL of a solution containing formamide and ABI (Applied Biosystems) Gene Scan 500 size standard and then loaded onto a 96-well plate. Microsatellite products were denatured for 2 min at 95 °C and analysed on an ABI 3130 Genetic Analyzer with GeneMapper 3.7 (ABI) software. We also manually checked all output profiles to confirm allelic size variants.

Data analyses

Population samples were tested for conformance to Hardy–Weinberg (H–W) equilibrium expectations at each locus, and the Markov chain Monte Carlo (MCMC) method and 1000 randomization procedures were used to estimate significance following the procedure described by Guo & Thompson (1992), as implemented in GENEPOP (v4.0, Rousset 2008). H–W deviations were tested for heterozygosity deficiency or excess, and for

Table 1 Walleye sample locations and summary genetic statistics based on microsatellite loci

Locality	Lat °N	Long °W	N	H_O	H_E	F_{IS}	N_A	N_{PA}	P_{PA}
Lake Winnipeg Drainage									
A Cedar Lake, MB	53.33	100.10	16	0.587	0.622	0.055	48	0	0
Papaonga River Drainage									
B McKim Lake, ON	50.82	92.50	20	0.512	0.526	−0.027	39	0	0
Upper Mississippi River Drainage									
C Mille Lacs, MN	46.37	93.19	39	0.590	0.614	0.038	55	2	0.036
Lake Superior									
D St. Louis River, MN	46.73	92.13	28	0.686	0.751	0.087	72	2	0.028
Lake Michigan									
E Muskegon River, MI	43.48	85.83	50	0.720	0.766	0.060	71	0	0
Lake Huron			**125**	**0.709**	**0.750**	**0.056**	**96**	**2**	**0.021**
F Alpena, MI	45.02	83.43	40	0.701	0.719	0.024	72	1	0.013
Saginaw Bay									
G Flint River, MI	43.33	84.05	50	0.729	0.748	0.024	79	0	0
Georgian Bay									
H Moon & Musquash Rivers, ON	44.84	79.80	35	0.687	0.721	0.047	67	0	0
Lake St. Clair			**78**	**0.735**	**0.749**	**0.018**	**98**	**3**	**0.031**
I Thames River, ON	42.32	82.45	38	0.738	0.732	0.007	80	0	0
J Detroit River, MI	43.24	82.79	40	0.737	0.749	0.016	70	3	0.043
Lake Erie			**450**	**0.711**	**0.771**	**0.077**	**119**	**6**	**0.050**
K Western & Central Basins			**268**	**0.696**	**0.755**	**0.078**	**113**	**3**	**0.027**
Huron River, MI	42.09	83.29	20	0.756	0.748	−0.012	74	0	0
Maumee River, OH	41.56	83.65	76	0.699	0.744	0.061	90	2	0.022
West Reefs, OH	41.63	83.02	20	0.689	0.667	−0.032	61	0	0
Hen Island Reef, ON	41.81	82.79	82	0.672	0.743	0.096	88	0	0
Chickenolee Reef, ON	41.72	82.61	20	0.651	0.688	0.053	59	0	0
Sandusky River, OH	41.46	82.89	20	0.778	0.775	−0.004	70	0	0
Grand River, OH	41.78	81.25	30	0.693	0.760	0.089	74	0	0
L Eastern Basin			**182**	**0.733**	**0.788**	**0.069**	**99**	**1**	**0.010**
Van Buren Bay, NY	42.46	79.41	77	0.742	0.761	0.025	90	0	0
Cattaraugus Creek, NY	42.57	79.13	50	0.729	0.747	0.024	79	0	0
Smokes Creek, NY	42.81	78.86	20	0.783	0.777	−0.008	73	0	0
Grand River, ON	42.86	79.58	35	0.692	0.767	0.097	67	1	0.015
Lake Ontario Watershed			**70**	**0.690**	**0.754**	**0.086**	**98**	**0**	**0**
M Bay of Quinte, ON	44.16	77.37	50	0.698	0.742	0.059	86	0	0
N Oneida Lake, NY	43.28	75.44	20	0.669	0.731	0.085	57	0	0
Ohio River Drainage			**39**	**0.682**	**0.786**	**0.133**	**85**	**1**	**0.012**
O Ohio River									
Hannibal Dam, OH	39.67	80.87	4	0.611	0.861	0.290	41	0	0
P Upper New River									
Foster Falls, VA	36.83	80.67	35	0.690	0.778	0.113	82	0	0
Tombigbee River Drainage									
Q North River, AL	33.26	87.51	6	0.537	0.712	0.247	35	8	0.229
All sites in Great Lakes Region (D–N)	—	—	**801**	**0.711**	**0.782**	**0.090**	**129**	**33**	**0.256**
All sites (A–Q)	—	—	**921**	**0.698**	**0.789**	**0.116**	**139**		

N, sample size (no. individual fish); H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS} (as measured by θ_{IS}), deviation from Hardy–Weinberg (H–W) proportions, with positive values indicating heterozygote deficiency and negative values denoting heterozygote excess (Weir and Cockerham 1984); N_A, no. alleles; N_{PA}, no. private alleles; P_{PA}, proportion of private alleles (those for bodies of water are sometimes greater than that for the individual sites when private alleles are shared among two or more component sites). Bold type denotes totals and means for regions.

Locus *Svi* L8 was dropped from these and all other population genetic analyses due to deviation from H–W expectations in several sampling sites, many of which persisted following Bonferroni correction. Without that locus, all sampling sites conformed to H–W equilibrium.

the presence of null alleles with MICRO-CHECKER v2.23 (van Oosterhout *et al.* 2004, 2006). In addition, each locus was tested for linkage disequilibrium (LD). Levels of significance for H–W and LD tests were adjusted using nonsequential Bonferroni corrections (Sokal & Rohlf 1995).

We tested question (1) 'Whether reproductive groups of walleye in interconnected waterways are genetically separable?' using unbiased θ estimates of *F*-statistics (Weir & Cockerham 1984), with their associated levels of significance to quantify genetic heterogeneity at different scales in the program FSTAT v2.9.3.2 (Goudet 1995, 2002). Additional pairwise tests of allelic frequency heterogeneity were conducted according to the procedure described by Goudet *et al.* (1996) based on an exact nonparametric procedure with probabilities estimated using MCMC simulations in GENEPOP, which is not affected by sample size or dependent on a normal distribution (Raymond & Rousset 1995; Rousset 2008). Probability values of tests employing multiple post-hoc comparisons were then adjusted with the sequential Bonferroni method (Rice 1989) to minimize type I errors.

Hierarchical partitioning of genetic variation (% variance) among groups of populations, population samples within groups and variation within sampling sites was evaluated with AMOVA (Excoffier *et al.* 1992) in ARLEQUIN (Excoffier *et al.* 2005; v3.11 by Excoffier 2007). We tested which population groupings and divisions best explained the patterns of genetic variation for (a) broad-scale relationships across all sites and (b) finer scale relationships within the Great Lakes.

Question (2), 'Whether genetic divergences among sampling sites corresponded to an isolation of geographical distance hypothesis?' was tested using the program ISOLDE (in GENEPOP), which compared genetic similarity estimated by $\theta_{ST}/(1-\theta_{ST})$ with the natural logarithm of geographical distance (measured as the shortest waterway distances between pairs of spawning sites, in cases where connectivity or possible connectivity occurred). This relationship was expected to be linear under an isolation by distance hypothesis (Rousset 1997). Regression significance was tested using Mantel's (1967) procedure with 1000 MCMC permutations. Three separate runs examined isolation with geographical distance across various scales, including: (a) overall, containing all sites (A–Q, plus all individual spawning sites in Lake Erie listed in Table 1), (b) sites in the Great Lakes watershed (D–N) and (c) those at fine-scale within Lake Erie (see Table 1 for sites grouped in K and L, and Strange & Stepien (2007)).

We tested question (3), 'What is the degree of population delineation across and among watersheds and lakes?' using phylogenetic, landscape genetic and Bayesian approaches. Cavalli-Sforza & Edwards (1967) chord distances (Dc) were calculated from the allelic frequency data with the GENDIST program and used to construct a neighbour-joining tree (Saitou & Nei 1987) in PHYLIP v3.68 (Felsenstein 2008). Relative support values for the nodes were estimated using 2000 bootstrap pseudoreplicates (Felsenstein 1985) in PHYLIP.

Genetic barriers among walleye populations were investigated using BARRIER v2.2 (Manni *et al.* 2004a,b), an analytical approach based on computational geometry that identifies genetic barriers between samples, independent from a priori knowledge of their geographical relationships. Pairwise estimates of θ_{ST} were mapped onto a matrix of site geographical coordinates (latitude and longitude), their spatial organization was modelled by Voronoi tessellation (in this study based on connectivity among water bodies, where applicable) and a Monmonier (1973) maximum-difference algorithm identified which of the borders between neighbouring sites exhibited the highest levels of genetic differences and ranked them accordingly (Manni *et al.* 2004a,b). Genetic barriers designated sites with greater θ_{ST} values than would be expected from their geographical connectivity, which were determined using a multilocus matrix, followed by a second analysis that evaluated how many single-locus values supported each barrier. Lastly, to further evaluate relative support and rankings for the barriers, we performed a bootstrap analysis of the multilocus θ_{ST} matrix with 2000 iterations using the program GENELAND v2.3.41 (Guillot *et al.* 2005a,b, 2008), based on the R statistical analysis software suite v2.8.1 (R Development Core Team 2008). Barriers with bootstrap values higher than 50% are reported in this study.

Bayesian-based clustering algorithms were used to delineate distinctive population groups in the program STRUCTURE v2.2.3 (Pritchard *et al.* 2000; Pritchard & Wen 2004), with and without prior knowledge of their true reproductive population identity, and without other prior assumptions. We tested for the number of true population groups (*K*) in independent runs of the algorithm, ranging from $K = 1$ (panmixia) to $K = 17$ (the total number of independent sites sampled). The program assigns individual fish to one or more groups, with their relative frequency of predicted membership totalling 1.00. We used 10 independent runs for each *K*, with burn-ins of 100 000 and 500 000 replicates. We then evaluated the consistency among runs, the comparative probabilities of individuals assigned to one or more groups, the log likelihood and posterior probability values from each run, and their respective grouping patterns. STRUCTURE analysis results are presented based on the 'best' *K* value(s) selected using the posterior probability procedure of Pritchard *et al.* (2000) vs. the

ΔK method of Evanno *et al.* (2005), the latter of which is based on the rate of change in the log probability values between successive *K*. We graphed the magnitude of ΔK vs. *K* for the mean of 10 replicate runs for each *K*, whose peak values designated the most probable *K*.

Results of the STRUCTURE analysis were then compared with population relationships derived from the BARRIER, genetic divergence and AMOVA analyses to address question (4), 'Are historic colonization patterns from glacial refugia retained?'.

Results

Overall and within-site genetic composition

Loci are unlinked and samples at all sites conform to H–W expectations following Bonferroni correction (Table 1); except for locus *Svi*L8, which was removed from further analysis due to indication of null alleles by MICRO-CHECKER analyses (see Appendix S1 and Chapuis & Estoup 2007). Summary statistics per locus are given in Appendix S1, with loci *Svi* 17 and 33 being the most informative for discerning variation among samples. Overall, when combinations of spawning sample sites are artificially grouped together (as in the lake and watershed summaries for multiple sampling sites in Table 1), several differ from H–W equilibrium proportions, supporting the alternative hypothesis of significant genetic structure among lakes, basins and tributaries. This artificial combination of multiple biological populations thus reveals some apparent Wahlund effects as evidenced by heterozygote deficiencies (see Table 1). This is due to mixing of individuals from separable reproductive groups having different allelic frequencies (see Balloux & Lugon-Moulin 2002; Hedrick 2005), thereby supporting the hypothesis of significant differences among reproductive groups. No significant differences are found in the genetic composition of males vs. females at any sampling sites, thus data from both sexes are combined. Male and female spawning walleye exhibit congruent biogeographical and genetic patterns.

Genetic diversity (=heterozygosity) values are relatively consistent among sampling sites, averaging 0.698 among all sites and 0.711 in the Great Lakes region (Table 1). These values appear highest in the Great Lakes, reaching 0.783 at the Smokes Creek site in the eastern basin of Lake Erie (located in L; Table 1 & Fig. 1). Genetic diversity is lower in the peripheral range locations, especially in the North River/Tombigbee River relict population in the Gulf Coast drainage (site Q; 0.537) and McKim Lake/Papaonga River in Ontario (site B; 0.512). The greatest proportion of private alleles occurs in the relict North River/Gulf Coast (Q; 0.230), which is more than five times greater than next-highest values found in Lake St. Clair's Detroit River (J; 0.043) and Mille Lacs of the upper Mississippi River drainage (C; 0.036). The overall proportion of walleye microsatellite alleles that exclusively occur in the Great Lakes is 0.256, and the proportion found only in Lake Erie is 0.050 (Table 1).

Genetic relationships among walleye spawning sites (question 1): fine to broad-scale trends

Genetic divergences between walleye spawning groups (summarized in Table 2) reveal significant differences among most locations – including within lakes and their basins, as well as among geographically distant sites. Pairwise tests of genetic differentiation using the F_{ST} analogue θ_{ST} (Weir & Cockerham 1984) and χ^2 genetic divergence (Goudet *et al.* 1996) yield congruent results (Table 2), supporting that most walleye spawning groups comprise separable biological populations. These include walleye reproducing within the Lake Huron system (including site F in the lake proper, site G in Saginaw Bay and H in Georgian Bay; mean θ_{ST} = 0.039, range = 0.010–0.067), Lake St. Clair (site I in the Thames River and J in the Detroit River; θ_{ST} = 0.012) and those within the eastern basin of Lake Erie (4 spawning areas, grouped in this study as L; mean θ_{ST} = 0.036, range = 0.034–0.058). Some of the 7 spawning location samples in western-central Lake Erie (designated as group K) genetically diverge, whereas others along the southern shore exchange genes [mean θ_{ST} = 0.048, range = 0.001–0.065; see Strange & Stepien (2007) for further analysis within Lake Erie]. The genetic compositions of walleye from the Ohio River and the upper New River are similar (θ_{ST} = 0.002), and thus are grouped in our analyses. Our overall study results thus reveal considerable fine-to broad-scale population genetic structure within and among watersheds, lakes and their basins.

The largest θ_{ST} pairwise divergence values (Table 2) distinguish walleye from outside the Great Lakes drainage as the most differentiated, including populations from the North River in the Tombigbee River/Mobile Bay/Gulf Coast drainage (site Q; mean θ_{ST} from all other individual sampling sites = 0.120), Cedar Lake in the Lake Winnipeg drainage (A; 0.135), McKim Lake in the Papaonga River drainage (B; 0.223) and Mille Lacs Lake in the upper Mississippi River drainage (C; 0.160). The largest pairwise θ_{ST} divergence values within the Great Lakes differentiate the walleye population in the St. Louis River of western Lake Superior (D; mean θ_{ST} from other Great Lakes sites = 0.061). Populations in the other lakes are separated from other Great Lakes sites by intermediate mean divergence values, including

Table 2 Pairwise θ_{ST} (below diagonal) and genetic differentiation χ^2 (above diagonal) comparisons between walleye populations in lakes, basins and river drainages (sites lettered according to Table 1 & Fig. 1)

Sampling sites	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O–P	Q
A Lake Winnipeg Drainage, Cedar Lake	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
B Papaonga River Drainage, McKim Lake	0.131**	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
C Upper Mississippi River Drainage, Mille Lacs	0.126**	0.200**	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
D Lake Superior, St. Louis River	0.083**	0.160**	0.110**	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	105.8**	inf**	129.5**
E Lake Michigan, Muskegon River	0.123**	0.217**	0.162**	0.050**	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
F Lake Huron, Alpena	0.119**	0.253**	0.169**	0.069**	0.028**	—	5.84**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
G Lake Huron, Saginaw Bay,Flint River	0.132**	0.231**	0.168**	0.058**	0.010*	0.010*	—	inf**	74.8**	102.9**	inf**	inf**	inf**	90.0**	96.1**	inf**
H Lake Huron, Georgian Bay	0.188**	0.273**	0.214**	0.087**	0.048**	0.067**	0.046**	—	inf**	inf**	inf**	inf**	inf**	inf**	inf**	inf**
I Lake St. Clair, Thames River	0.138**	0.242**	0.145**	0.051**	0.032**	0.025**	0.013**	0.039**	—	70.5**	inf**	inf**	inf**	76.5**	69.7**	inf**
J Lake St. Clair, Detroit River	0.134**	0.252**	0.161**	0.062**	0.033**	0.022**	0.017**	0.052**	0.013**	—	inf**	inf**	inf**	inf**	inf**	131.8**
K Lake Erie, Western & Central Basins	0.157**	0.228**	0.166**	0.067**	0.055**	0.060**	0.039**	0.072**	0.039**	0.042**	—	inf**	inf**	inf**	inf**	inf**
L Lake Erie, Eastern Basin	0.131**	0.193**	0.133**	0.044**	0.040**	0.048**	0.027**	0.057**	0.024**	0.034**	0.008**	—	inf**	inf**	inf**	inf**
M Lake Ontario, Bay of Quinte	0.126**	0.232**	0.138**	0.061**	0.039**	0.039**	0.028**	0.069**	0.026**	0.038**	0.059**	0.043**	—	97.6**	inf**	inf**
N Oneida Lake	0.137**	0.228**	0.163**	0.056**	0.037**	0.064**	0.029**	0.060**	0.035**	0.062**	0.060**	0.040**	0.049**	—	45.6*	inf**
O–P Ohio & New Rivers	0.122**	0.216**	0.138**	0.050**	0.027**	0.038**	0.019**	0.050**	0.010*	0.028**	0.043**	0.023**	0.032**	0.018*	—	inf**
Q Tombigbee River Drainage, North River	0.171**	0.295**	0.206**	0.102**	0.067**	0.081**	0.068**	0.146**	0.114**	0.096**	0.096**	0.069**	0.085**	0.122**	0.074**	—

inf, χ^2 denoted as infinite by GENEPOP ($P < 0.00001$**, with the 'infinite' designation occurring when values exceed the maximum specified by the program; see VanDeHey *et al.* 2009), **significant following sequential Bonferroni correction for multiple post-hoc tests, *significant at 0.05 level before Bonferroni correction.

Lake Michigan (E; 0.039), Lake Huron (F–H; 0.039), Lake St. Clair (I–J; 0.032), western/central Lake Erie (K; 0.048), eastern Lake Erie (L; 0.036) and Lake Ontario (M; 0.045).

Relationship between genetic divergence and geographical distance (question 2)

The Mantel test for all collection localities (A–Q), which analyses pairwise genetic distances $\theta_{ST}/1-\theta_{ST}$ against the natural logarithm of geographical distance (km; using nearest waterway connections among physically linked sites), shows a positive association (Fig. 2), indicating overall broad-scale correspondence to a genetic isolation by geographical distance hypothesis ($P < 0.0001$, $R^2 = 0.263$ (Pearson's coefficient of regression), $r = 0.513$ (correlation coefficient; Sokal & Rohlf 1995), $y = 0.043x + 0.180$). Sites having the greatest genetic distances compared with geographical distances include McKim Lake (B) in the Papaonga River drainage (outliers above the regression line to the right; Fig. 2) and those in Lake Erie (K&L; outliers above the regression line to the left). This isolation with genetic distance trend also is significant, but weaker, when the Great Lakes sites (D–M) alone are analysed ($P = 0.003$, $R^2 = 0.111$, $r = 0.317$, $y = 0.008x + 0.008$), indicating that isolation by genetic distance does not alone explain the divergence patterns among spawning groups.

By contrast, the Mantel test is not significant in a fine-scale test using individual Lake Erie spawning sites (i.e. individual sites within the western-central basin (K) and the eastern basin (L) given in Table 1; $P = 0.816$, $R^2 = 0.016$, $r = 0.127$, $y = -0.003x + 0.006$), which was detailed by Strange & Stepien (2007). In Lake Erie, some closely spaced spawning groups are distinguished by large genetic separations. These results indicate that genetic divergence patterns are complex and often pro-

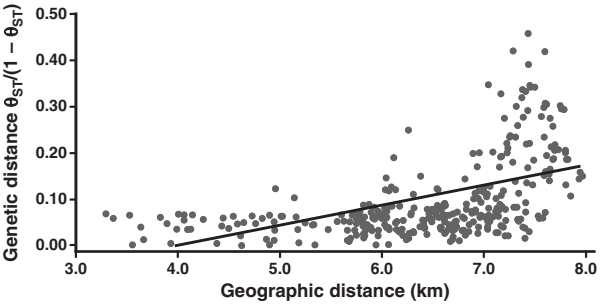

Fig. 2 Pairwise relationship between genetic distance ($\theta_{ST}/1-\theta_{ST}$) vs. the natural logarithm of geographical distance (km) across all population samples (A–Q) $P < 0.0001$**, $R^2 = 0.263$, $r = 0.513$, $y = 0.043x + 0.180$.

nounced within a single system – and therefore cannot be explained by the genetic isolation by geographical distance hypothesis.

Patterns of population delineation and relationships (questions 3 & 4)

The neighbour-joining tree based on Cavalli-Sforza & Edwards (1967) D_cs depicts the relict population site from the North River in the Tombigbee River/Mobile Bay/Gulf Coast drainage (Q) as the sister group to all other walleye population samples (Appendix S2). Remaining samples form two primary population groups, one comprising the northwest sites (A–C) and the other containing those in the Great Lakes (A–M), Oneida Lake (N), and the Ohio and New River drainages (O–P). Walleye from spawning populations Lake Huron proper and its Saginaw Bay cluster together on the tree, as do those from Lake Erie (Appendix S2).

Higher-order relationships among population groups are revealed by AMOVA analyses, indicating that the most significant hierarchy nests walleye samples in three primary groups, comprising (i) the northwest sampling sites A–C, (ii) sites D–N – including all of the Great Lakes plus Lake Oneida and (iii) the Atlantic and Gulf coastal samples O–Q. Partitioning of variation among these three groups accounts for 6.93% of the variation ($\phi_{CT} = 0.069$, $P < 0.0001$), and variation among populations within these groups totals an additional 5.04% ($\phi_{SC} = 0.054$, $P < 0.0001$), thus explaining 11.97% of the overall genetic variation. When Lake Oneida is alternatively grouped with the Atlantic-Gulf coastal sites O–Q, less of the variation is explained (6.44% among groups, $P < 0.0001$; 4.97% within groups $P < 0.0001$; totalling 11.41%).

When genetic variation is analysed across the Great Lakes sites alone, hierarchical partitioning is best explained by higher level groupings comprising: (a) sites D&H (Lake Superior and Lake Huron's Georgian Bay), (b) E–G & I–J (Lakes Huron, Michigan and St. Clair) and (c) K–M (the lower Great Lakes). Variation among these three groups comprises 1.92% ($\phi_{CT} = 0.019$, $P < 0.0001$) and that among their component populations is 3.74% ($\phi_{SC} = 0.038$, $P = 0.008$), totalling 5.6%.

Results from BARRIER analysis (Manni *et al.* 2004a,b; shown in Fig. 1) identify 10 genetic barriers, having 50% or more bootstrap support and with majority support from separate analyses based on individual loci (6/9 loci or greater). The four highest-ranked barriers distinguish populations outside the Great Lakes watershed, delineating walleye from the northwest (sites A–C; Barriers I, III & IV) and the Gulf coast (site Q, Barrier II) from all samples located in the Great Lakes and along the Atlantic-Gulf coasts (D–P). Northwest populations of walleye are defined from McKim Lake (site B; Barrier I, 75% bootstrap support, 9/9 loci), Cedar Lake (site A; Barrier III, 68%, 8/9 loci) and Mille Lacs (C; IV, 66%, 9/9 loci). Barrier II isolates the Gulf coastal walleye population in the Tombigbee River/Mobile Bay drainage (site Q) from all other populations (58%, 8/9 loci). The next most-prominent barriers define walleye populations in upper Great Lakes basins, isolating Lake Superior (D; V; 63%, 8/9 loci) and Georgian Bay of Lake Huron (H; Barrier VI, 62%, 8/9 loci). Barrier VII then separates Great Lakes walleye populations from Lakes Ontario and Oneida (N–M; 51%, 7/9 loci). In the lower Great Lakes, Barrier VIII delineates walleye from Lake Ontario (M) and Lake Erie (K–L; 50%, 6/9 loci). Barrier IX then differentiates the Ohio and New River populations (O–P) from the northern sites (51% and 6/9 loci). The final barrier (X; 50% and 6/9 loci) separates walleye populations in Lake Erie (K–L) from those to the west – including Lakes St. Clair (I–J), Huron (F–G) and Michigan (E). BARRIER results are congruent with those from nested AMOVA hierarchies (above).

Bayesian STRUCTURE analyses (Pritchard *et al.* 2000; Pritchard & Wen 2004) identify $K = 4$ and $K = 6$ walleye population groups using the ΔK criterion (Evanno *et al.* 2005) and $K = 9$ as having the greatest posterior probability (0.998; Fig. 3 & Appendix S3). Northwest samples (sites A–C) group together in all three K scenarios (with memberships in the blue-green group ranging from 74% to 97%). The $K = 4$ analysis (Fig. 3a, Appendix S3a) largely differentiates walleye in Lakes Michigan and Huron (sites E–G; pink, 83% for E in Lake Michigan and 59% for F–G in Lake Huron), as well as those in Lake Erie (sites K–L; light green, 62%), with many of the remaining sites being blue. In the $K = 6$ scenario (Fig. 3b, Appendix S3b), walleye from the Gulf Coastal site are distinguished (site Q; 78%, dark blue), as well as those from Lake Huron's Georgian Bay (H; red, 77%), followed by those in Lake Ontario (M; red, 50%). At $K = 9$ (Fig. 3c, Appendix S3c), the Gulf coastal population (Q) has membership almost exclusively in the orange group (74%), whereas sites from Lake Huron's Georgian Bay (H; red, 80%) and Lake Ontario (M; purple, 55%) show the next highest degree of unique population group assignment. Walleye from Lake Michigan (E; pink, 57%), Lake Huron proper (F; pink, 51%) and Lake Huron's Saginaw Bay (G; pink, 41%) are linked. The lower Great Lakes area samples from Lakes Ontario (M) and Oneida (N) share predominant membership in the purple cluster, which also characterizes the Ohio and New River sites (O–P). Eastern Lake Erie (L) composition is predominantly blue green, shared by western Lake Erie (K), with the latter having

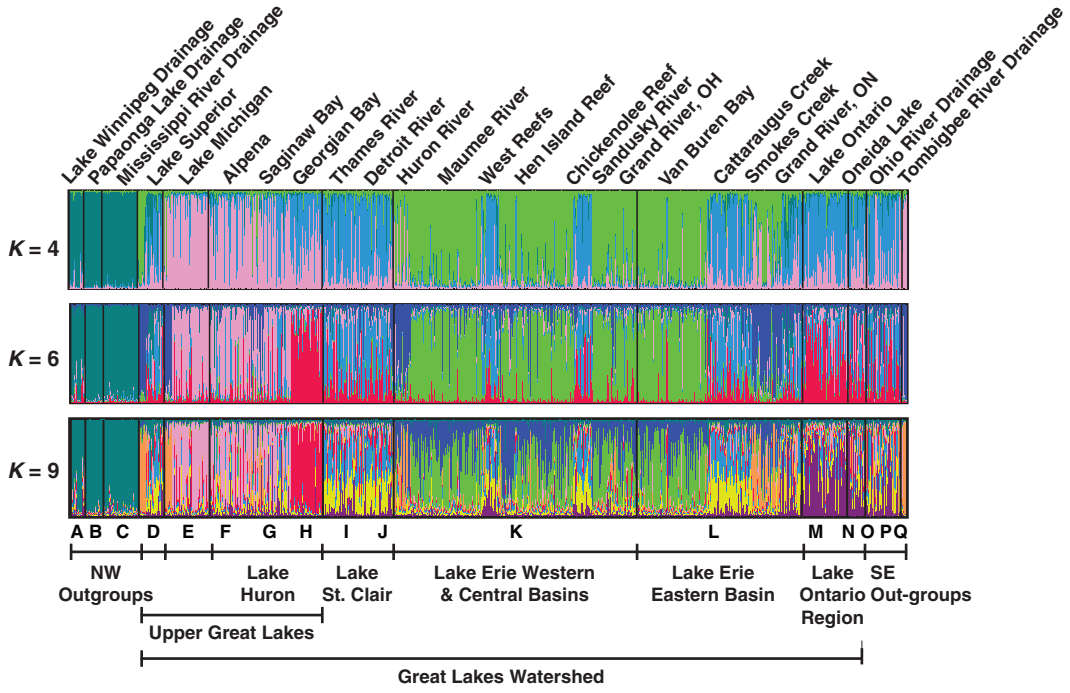

Fig. 3 Estimated population structure of walleye from Bayesian STRUCTURE analysis (Pritchard *et al*. 2000; Pritchard & Wen 2004) for $K = 4$, 6 and 9 population groups. $K = 4$ and 6 had the highest ΔK vs. K peak height (Evanno *et al*. 2005), and $K = 9$ had the highest posterior probability value (0.998; Pritchard *et al.* 2000). Each individual is represented by a thin vertical line, which is partitioned into K coloured segments that represent its estimated population group membership fractions. Black lines separate individuals from spawning site locations (labelled above), whose geographical regions are labelled below (following Table 1 & Fig. 1).

additional primary membership in dark blue. Lake St. Clair walleye (I–J) have unique membership, primarily in yellow and blue. Results from STRUCTURE reveal population groupings similar to those discerned with AMOVA nested hierarchies and BARRIER analysis. A difference is that the northwest peripheral populations appear more similar to each other in STRUCTURE – indicating their common refugium origins – yet the other methods more clearly show their pronounced divergence within this grouping.

Further marked and fine-scale divergences among some proximate walleye spawning groups within lakes are indicated in the STRUCTURE analyses (Fig. 3b,c). For example, colour group differences distinguish walleye reproducing in the Huron River, western reefs and Chickenolee Reef in Lake Erie's western basin, which are discussed by Strange & Stepien (2007). These colour patterns thus further illustrate that fine-scale relationships among walleye reproductive groups do not correspond to isolation by genetic distance or simple clinal variations, as found in the Mantel tests and further discussed below. In conclusion, results from the various analysis approaches are largely congruent, indicating both broad- and fine-scale patterns among walleye spawning groups.

Discussion

Broad to fine-scale genetic patterns

The data of our study support the following answers to its underlying questions, as discussed below:

1 Are reproductive groups of walleye in interconnected waterways genetically separable? – Yes, in most cases; however, there is some gene flow among interconnected spawning locations along the southern shore of western-central Lake Erie.

2 Do genetic divergences correspond to geographical distances? – Yes for broad-scale patterns alone and no for fine-scale relationships, with some closely spaced reproductive groups within lakes distinguished by relatively high genetic divergences.

3 What is the degree of population delineation across and among watersheds and lakes? – There are 9–10 primary population groups among walleye sampled across North America, with the greatest isolation and divergence found in the northwest and the southeast.

4 Are historic colonization patterns from glacial refugia retained? – The general patterns correspond to the biogeographical history of isolation of northerly pop-

ulations in refugia and dispersal from them. Genetic barriers in our study distinguish walleye populations from the northwest and the Gulf coastal region, differentiating these from those in the Great Lakes system. There is more recent genetic exchange, yet pronounced differentiation, between samples in the Great Lakes watershed and the Atlantic coastal region. These genetic barriers reflect long-term divergence of walleye in watersheds and lakes, whose patterns are detailed below.

Isolation of northwest walleye populations

The most genetically isolated walleye population groups, of those analysed, are found in the northwest: in the watersheds of Lake Winnipeg (site A, Cedar Lake), Papaonga River (site B, McKim Lake) and the upper Mississippi River (C, Mille Lacs). These walleye populations appear to be historically linked (as shown in the STRUCTURE results), yet are very divergent from each other, as they are delineated by three of our strongest genetic barriers and high pairwise divergences. These populations appear to have been relatively long isolated from those in the Great Lakes watershed, including relatively nearby Lake Superior.

Northwest walleye groups were postulated by Ward *et al.* (1989) and Billington *et al.* (1992) to descend largely from a Missourian glacial refugium, based on allozyme and mtDNA RFLP data (see arrows in Fig. 1). Walleye populations A–C probably co-existed in glacial Lake Agassiz, which formed ~13 kya and drained south, with ice blocking its connection to former glacial Lake Duluth (now western Lake Superior, including our site D; Bailey & Smith 1981; Rempel & Smith 1998). Northward glacial retreat then changed the drainage of Lake Agassiz to flow north into the Hudson Bay ~8.5–7.5 kya, isolating it from its former connection to the Mississippi River system. Lake Agassiz subsequently emptied, isolating lakes A–C for the past 7 ky (Rempel & Smith 1998), as reflected in their pronounced divergences in our study.

Our results show that divergences of these northwestern populations (A–C) from walleye in the St. Louis River of Lake Superior (site D) are more pronounced than were detected using earlier genetic techniques (Ward *et al.* 1989; Billington *et al.* 1992; Stepien & Faber 1998). This supports the hypothesis that walleye colonizing Lake Superior (as glacial Lake Duluth) largely originated from the Mississippian refugium. Fishes in the Lake Superior basin and the rest of the Great Lakes are not derived from a western source in the Lake Agassiz basin or an exchange via their former connectives, according to Underhill (1986), which is supported by our results. Likewise, smallmouth bass (Stepien *et al.* 2007) and yellow perch (Sepulveda-Villet *et al.* 2009) populations from the upper Mississippi River watershed show long-term genetic isolation from those in the Great Lakes.

Gulf coastal relict population

The walleye population in the southern relict population area of the Tombigbee River/Mobile Bay/Gulf Coast drainage appears as the sister group of other walleye populations in our tree (Appendix S3). This Gulf coastal walleye population is small but persistent and is believed to represent a long-isolated unique historic strain (Boschung & Mayden 2004). Its distinctiveness was described by Hackney & Holbrook (1978) based on life history characters, faster growth and spawning at higher temperatures. Ours is the first analysis of its DNA microsatellite variability.

Our genetic data – as well as earlier investigations using allozymes (Murphy 1990; Billington & Maceina 1997) and mtDNA restriction haplotypes (Billington *et al.* 1992; Billington & Strange 1995; Billington & Maceina 1997) – indicate population divergence of walleye from the Tombigbee River/Mobile Bay/Gulf Coast drainage from other native population areas. As in our microsatellite data, previous genetic studies using other (less variable) techniques described lower overall levels of genetic diversity in these Gulf Coast walleye, along with unique characters and genetic distinctiveness. Notably, Billington *et al.* (1992) suggested that these southern walleye diverged ~1.17 (± 0.31) mya, becoming isolated from northern walleye during the pre-Pleistocene breakup of the Appalachian River (Mayden 1987). Congruent with this pronounced isolation, we discern 20% private alleles in this Gulf Coastal walleye group, more than that in any other location we sampled. Boschung & Mayden (2004) noted that this unique walleye strain is in danger of potential introgression with introduced northern strains of walleye entering from the Tennessee River drainage via the Tennessee–Tombigbee Waterway. Our study thus provides an important baseline for its further and future assessment.

In comparison with walleye, a recent study of yellow perch *Perca flavescens* (Sepulveda-Villet *et al.* 2009) shows closer relationship of its relict Gulf coastal populations to those from the Atlantic seaboard. Thus patterns of population relationships among geographical areas appear to differ among fishes, even among percid relatives. A similarity between the studies is that genetic diversity of the Gulf coastal yellow perch populations was lower than that of historically unglaciated populations to the north along the Atlantic slope, as

found in walleye. Additional sampling and analysis of walleye from this relict population would be valuable.

Linkage of Atlantic coastal group

Also, located outside the Great Lakes watershed, the walleye population spawning in the New River (Virginia) inhabits the southeastern edge of the natural distribution of the species. Findings by Palmer *et al.* (2005) indicated that the upper New River contains a distinctive walleye stock that spawns at Fosters Falls, and probably is native to this system. In contrast, nearby Claytor Lake walleye have been stocked, including supplementation with Great Lakes walleye. Our results suggest little or no genetic contribution from Great Lakes walleye to the New River population, pointing to the latter's long-term divergence and likelihood that stocking has not influenced its composition.

Microsatellite data suggest genetic linkage of walleye from the New River with those from the Ohio River – unglaciated areas that were influenced by meltwaters and fluvioglacial outwashes (Hocutt *et al.* 1986). The Ohio River walleye that we included in this study have unique distinctive mtDNA sequences (Faber & Stepien 1997; Stepien & Faber 1998), and we excluded samples that may have descended from hatchery stockings of Lake Erie lineages into the Ohio River. Genetic divergence of the unique mtDNA haplotype found in the Ohio River suggested its long-term separation from walleye in the Great Lakes (~1.5 myr; Stepien & Faber 1998). Geological evidence concurrently indicates that this 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 (Flint 1971). The New River is a remnant of the earlier upper Teays River channel (Hocutt *et al.* 1986; Robison 1986), and our results in this study illustrate close genetic relationship between populations from the Ohio and New River today, reflecting this former connectivity.

These patterns also suggest some common – but distant – genetic relationship of walleye spawning in the Ohio and New Rivers with those in Oneida Lake. Oneida Lake's walleye have been supplemented with Oneida Lake broodstock, and our results suggest they have maintained a distinctive genetic signature. Although walleye in Oneida Lake share a common history with the Lake Ontario population, the two significantly diverge. Oneida Lake is situated in a depression of the glacially created former Lake Iroquois in the Ontario Basin, a system that once drained to the Hudson River (Bailey & Smith 1981). The outlet of Oneida Lake today is the Oneida River, which flows to the Oswego River and eventually makes its way to Lake Ontario (Mills *et al.* 1978), as reflected by our results that show its grouping with the Great Lakes sites.

Genetic linkage reflects a common Atlantic coastal ancestry for these groups, with walleye in Lakes Ontario and Oneida being descendent from origins in an easterly glacial refugium (see arrows on Fig. 1). Todd & Hatcher (1993) hypothesized such a scenario for yellow perch, with the Susquehanna River drainage postulated as a portal for colonization of Lake Ontario from the Atlantic coastal glacial refugium. Turgeon & Bernatchez (2001) likewise cited Atlantic refugium inputs into the eastern Great Lakes via the Susquehanna, Mohawk and Hudson River outlets to explain genetic linkages of lake cisco *Coregonus artedi*. Populations of other Lake Ontario fishes have been linked to this origin (summarized by Mandrak & Crossman 1992), including a recent analysis of the rainbow darter *Etheostoma caeruleum* (Haponski *et al.* 2009).

Great Lakes population groups: glacial origins and separations

Billington & Hebert (1988) used mtDNA RFLPs to differentiate two primary genetic groups of central Great Lakes walleye that diverged in Mississippian and Atlantic glacial refugia ~230 ± 100 kya, during the Sangamon interglacial period prior to the Wisconsinan glacial advance. Those dates were congruent with estimates from mtDNA control region sequences for Great Lakes walleye (Faber & Stepien 1997, 1998; Stepien & Faber 1998). The present nuclear DNA microsatellite data reveal greater genetic diversity and differentiation among walleye reproductive groups than was found in the earlier studies. Our data also depict more complicated relationships among walleye population sites across the Great Lakes, which also differentiate among those spawning in individual lake basins and many closely spaced locations.

According to present findings, the primary population barriers in the Great Lakes separate walleye reproductive groups in Lake Superior, Georgian Bay of Lake Huron, Lake Erie and Lake Ontario. Populations in the upper Great Lakes through western Lake Erie were founded by waves of colonizations stemming from the Mississippian refugium, entering through its various outlets. Walleye populations in Lake Ontario and eastern Lake Erie additionally were colonized by descendents from the Atlantic coastal refugium, and retain some similarities with Atlantic coastal populations today. Populations in the glacial lake basins periodically differentiated during periods of isolation, interrupted by gene flow during periods of connectivity (see Bailey & Smith 1981; Mandrak & Crossman 1992).

The Lake Superior region was long covered in ice, except for glacial Lake Duluth in the west, until ~9–8.5 kya, thus isolating its walleye gene pool. Common genetic history of walleye spawning in Lake Michigan with those in Lake Huron proper (including Saginaw Bay) reflects their former connection in glacial Lake Algonquin ~12–10.6 kya, which drained west to the Mississippi River system (Bailey & Smith 1981). Lake Huron walleye populations diverged ~11.5 kya when Georgian Bay (the former glacial Lake Hough) was isolated from the main basin population (the former glacial Lake Stanley; Lewis *et al.* 1994). Lake Erie's formation dates to glacial Lake Maumee ~14 kya, which then flowed west via the Ohio River to the Mississippi, changing its flow direction during several lake stages, to its current flow east to Lake Ontario ~10 kya (Underhill 1986). Its genetic composition today largely descends from the Mississippian glacial refugium, with some Atlantic glacial refugium contribution from the east via Lake Ontario (whose basin dates to glacial Lake Iroquois ~12 kya). Today, Lake Erie walleye appear relatively geographically isolated and genetically differentiated from those spawning in the other Great Lakes; from Lake Ontario to the east by Niagara Falls, and from the upper Great Lakes to the west by the narrow and short Detroit River that drains from Lake St. Clair.

Similar genetic barriers have been discerned among spawning populations of other Great Lakes fishes, including those distinguishing smallmouth bass from Lakes Superior, Erie and Ontario (Stepien *et al.* 2007). Yellow perch microsatellite data congruently reveal marked genetic differences between samples from the upper vs. lower Great Lakes (Grzybowski *et al.* 2009), whose barriers appear less divergent with mtDNA sequences (Sepulveda-Villet *et al.* 2009). These similarities show a common origin of this pattern through re-colonization from glacial refugia, which since have been maintained by reproductive site philopatry from generation through generation.

Fine-scale patterns in lake basins: roles of behaviour and ecology

Dupont *et al.* (2007) investigated the relationships between individual biological characteristics (sex, size, age and population of origin) and dispersal in walleye, showing that population origin of the dispersers was the significant differentiating factor. This finding is supported by our results and previous studies of walleye genetics, indicating substantial divergence among most spawning groups across their native range, including some of those separated by relatively fine geographical scales. The latter includes closely spaced spawning groups that are in the same lake basin; including those we tested in Lake Huron's Georgian Bay, Lake St. Clair and eastern Lake Erie.

Although broad-scale patterns across the walleye's range support the hypothesis of genetic isolation according to geographical distance (measured here by the nearest waterway connections), fine-scale patterns within lakes do not fit. In Lake Erie, several relatively closely spaced walleye spawning group sites are genetically distinct, especially in the eastern basin and some isolated reefs in the western basin. Levels of fine-scale divergences among walleye spawning groups within water bodies in this study (see Table 2) are similar in magnitude to those detected in a landscape genetic analysis of yellow perch in the St. Lawrence River system using microsatellite loci and similar analysis methods (mean $\theta_{ST} = 0.039$; LeClerc *et al.* 2008). Analogous lack of fine-scale correspondence to an isolation by distance hypothesis among fish spawning groups across Lake Erie is found in smallmouth bass (Stepien *et al.* 2007) and yellow perch (Ford & Stepien 2004; Sepulveda-Villet *et al.* 2009), both of which fit such a model in broad-scale analyses across their North American distributions. This cross-specific pattern may reflect relative ages of the lake basins, with fish reproducing in eastern Lake Erie displaying greater site fidelity as its habitats are older (~10 kya) than those in the west (which were a system of rivers until ~4 kya; see Bolsenga & Herdendorf 1993); thus eastern spawning groups probably experienced longer-term habitat stability, with more time to genetically differentiate (detailed by Strange & Stepien 2007).

Evolution of population genetic patterns in walleye

Broad-scale evolutionary patterns of genetic structure in walleye populations thus are products of extrinsic barriers to gene flow (i.e. vicariance and allopatric isolation) and dispersal patterns originating from ancient (i.e. Pleistocene or earlier) drainage patterns. Barriers to gene flow in walleye appear congruent with those described for other northeastern North American freshwater fishes, including the brown bullhead *Ameiurus nebulosus* (Murdoch & Hebert 1997), smallmouth bass (Stepien *et al.* 2007), greenside darter (Haponski & Stepien 2008) and yellow perch (Todd & Hatcher 1993; Sepulveda-Villet *et al.* 2009), indicating common isolation in glacial refugia, divergences among drainages and postglacial northward re-colonization patterns (Mandrak & Crossman 1992). The strongest broad-scale demarcations occur among isolated systems outside the Great Lakes. However, additional divisions are found within the contiguous Great Lakes, which appear to be maintained by a combination of isolation by genetic

distance and tendency of walleye that once originated from separate glacial refugia to remain separate at spawning time.

We additionally discern genetic discontinuities among proximate spawning groups that do not appear to be the result of environmental barriers to gene flow, but likely are behaviourally based. Such fine-scale divergence in this study is found within Lake St. Clair, Lake Erie and Georgian Bay in Lake Huron. Walleye have high vagility during most of the year, often moving from lake to lake to feed (Scott & Crossman 1973; Colby & Nepszy 1981); yet genetic relationships among these spring spawning groups show relatively high levels of divergence among some – but not all – closely spaced groups. These fine-scale divergences among reproductive groups do not fit an isolation by geographical distance hypothesis, and appear to be reinforced by natal spawning site philopatry (see Stepien & Faber 1998). Fine-scale genetic differentiation was described by VanDeHey *et al*. (2009) between some neighbouring spawning aggregates of lake whitefish *Coregonus clupeaformis* within Lake Michigan, and hypothesized to be related to thermal and depth differences. Ecological characters of spawning habitats may explain some of the fine-scale separations among proximate groups of walleye and other taxa, and should be further investigated.

Reproductive site fidelity thus appears to regulate the fine-scale population structure of walleye, despite habitat connectivity. Other *Sander* species also migrate to specific rivers to spawn (Koed *et al*. 2000, 2002). Notably, Gerlach *et al*. (2001) suggested that Eurasian perch *Perca fluviatilis* recognizes its kin through olfactory cues. Thus, it is possible that walleye and other percids 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 reproductive groups and needs to be tested.

In conclusion, mechanisms driving or constraining gene flow in this example are determined to be both extrinsic (reflecting divisions among former glacial refugia and current watersheds) and intrinsic to the species (due to behavioural patterns of spawning site philopatry). These regulators have operated on both ecological (10–1000 generations) and evolutionary time scales (>1000 generations) to shape the patterns observed among today's populations, which probably additionally have been influenced by human stockings and extirpations during recent decades. Maintaining the ecological opportunity underlying this fine-scale population structure probably is critically important for conserving diversity and divergence patterns in our native fauna.

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Carol Stepien's Great Lakes Genetics Laboratory at the Lake Erie Center of the University of Toledo focuses on conservation genetics of Great Lakes fishes and understanding nonindigenous species invasions. Douglas Murphy is the DNA technician, Rachel Lohner is the education programme manager, Osvaldo J Sepulveda-Villet is a PhD candidate and Amanda Haponski is a PhD student. The Great Lakes Genetics Laboratory also is investigating the evolutionary genetics of yellow perch *Perca flavescens*, smallmouth bass *Micropterus dolomieu*, round goby *Neogobius melanostomus*, tubenose goby *Proterorhinus semilunaris* and the dreissenid mussels *Dreissena polymorpha* and *D bugensis* using microsatellite loci, and mitochondrial and nuclear DNA sequence variation. Additional information and updates are available at http://lakeerie.utoledo.edu.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Summary statistics of allelic variation per microsatellite locus for walleye across the entire data set (see Table 1 and text for details).

Appendix S2 Neighbour-joining tree (Saitou & Nei 1987; constructed in PHYLIP) showing relationships among major population areas for walleye based on Cavalli-Sforza & Edwards (1967) chord distances.

Appendix S3 Summary of membership representation for walleye samples in (a) $K = 4$, (b) $K = 6$ and (c) $K = 9$ population group cluster scenarios shown in Fig. 3 from Bayesian STRUCTURE analyses.

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