Molecular Ecology (2007) **16**, 1605–1624 doi: 10.1111/j.1365-294X.2006.03168.x

Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: an interplay of behaviour and geography

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Abstract

Analysis of population genetic relationships reveals the signatures of current processes such as spawning behaviour and migration, as well as those of historical events including vicariance and climate change. This study examines these signatures through testing broad- to fine-scale genetic patterns among smallmouth bass *Micropterus dolomieu* spawning populations across their native Great Lakes range and outgroup areas, with fine-scale concentration in Lake Erie. Our primary hypotheses include whether genetic patterns result from behavioural and/or geographical isolation, specifically: (i) Are spawning groups in interconnected waterways genetically separable? (ii) What is the degree of isolation across and among lakes, basins, and tributaries? (iii) Do genetic divergences correspond to geographical distances? and (iv) Are historical colonization patterns from glacial refugia retained? Variation at eight nuclear microsatellite DNA loci are analysed for 666 smallmouth bass from 28 locations, including 425 individuals in Lake Erie; as well as Lakes Superior, Huron, and Ontario, and outgroups from the Mississippi, Ohio, St. Lawrence, and Hudson River drainages. Results reveal marked genetic differences among lake and river populations, as well as surprisingly high divergences among closely spaced riverine sites. Results do not fit an isolation-by-geographical-distance prediction for fine-scale genetic patterns, but show weak correspondence across large geographical scales. Genetic relationships thus are consistent with hypotheses regarding divergent origins through vicariance in glacial refugia, followed by colonization pathways establishing modern-day Great Lakes populations, and maintenance through behavioural site fidelity. Conservation management practices thus should preserve genetic identity and unique characters among smallmouth bass populations.

Keywords: Centrarchidae, glacial refugia, Great Lakes, population genetics, smallmouth bass, spawning site philopatry

Received 1 July 2006; revision accepted 15 September 2006

Introduction

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

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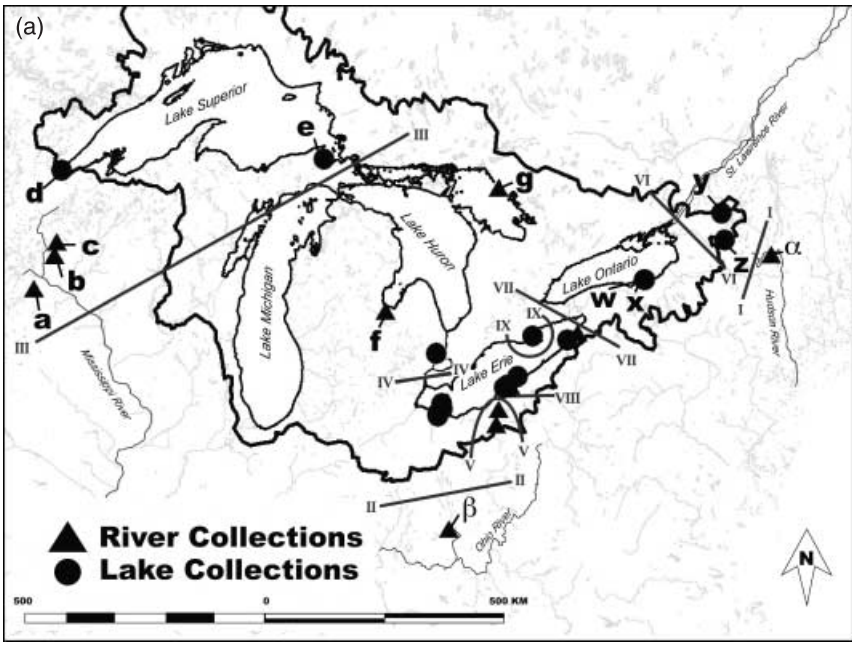

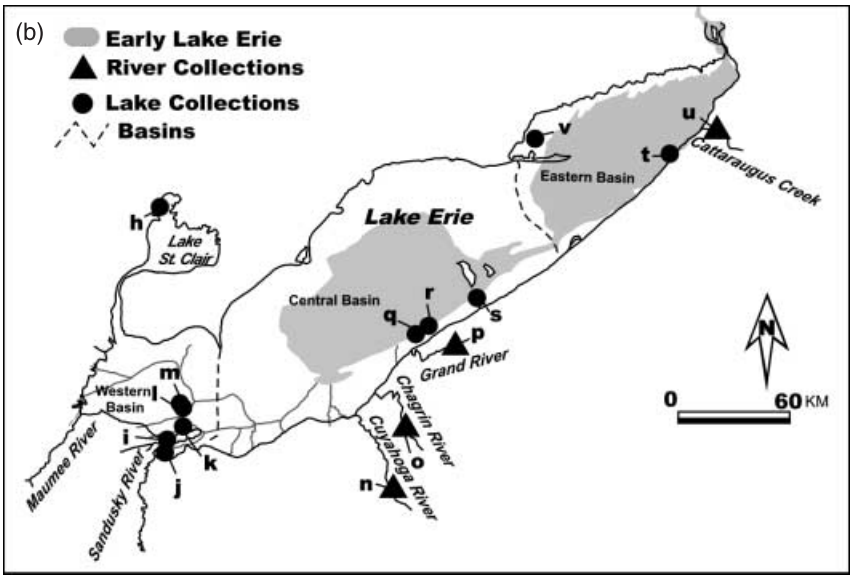

Fig. 1 (a) Map showing locations of collection sites for smallmouth bass spawning groups across the Great Lakes drainage system (enclosed in solid line) and outgroup samples in the Mississippi, Ohio, Hudson, and St. Lawrence River drainages. Lake Erie sites are lettered in Fig. 1b. Latitude and longitude coordinates of these sites are given in Table 1. Triangles denote riverine locations and circles designate lacustrine reef sites. Lines denote primary genetic break divisions among populations using the Manni *et al*. (2004a, b) BARRIER approach, designated with Roman numerals from greatest to less pronounced (I to IX). (b) Spawning sites for smallmouth bass sampled from lacustrine sites in Lakes Erie and St. Clair (circles) and tributaries (triangles). Latitude and longitude coordinates of these sites are given in Table 1. Divisions of Lake Erie into three physiographic basins are indicated by dotted lines. Shaded portion inside modern Lake Erie depicts ancient Lake Erie 7000–10 000 years BP, based on Bolsenga & Herdendorf (1993) and Holcombe *et al*. (2003).

display intermediate levels of migration. Such populations/subpopulations often display some evidence for departure from panmixia, yet retain considerable connectivity (see recent summary by Waples & Gaggiotti 2006). Delineating this fine-scale structure may be critically important for conserving native genetic diversity and divergence patterns, as explored in the present investigation of the population genetic structure of the smallmouth bass *Micropterus dolomieu* (family Centrarchidae).

The range of broad through fine-scale population genetic structure has not been previously analysed for the smallmouth bass, despite its status as one of the most popular sport fishes in the Great Lakes region and beyond. Its original native range was restricted to the fresh waters of eastern central North America, including the Great Lakes (Scott & Crossman 1973), which forms the geographical focus of our investigation (Fig. 1a). Its greatest population sizes and fishing effort are concentrated in Lake Erie, which is an open system of apparent high connectivity with few visible barriers to gene flow (Fig. 1b). Lake Erie thus represents an ideal system in which to test hypotheses of behavioural and reproductive factors that may produce fine-scale population divergences despite few apparent restrictions to gene flow.

One of the primary problems facing conservation scientists and managers today is how to define and maintain both large- and fine-scale population structure in the face of climate change, exploitation, habitat degradation, invasions by exotic species, and range expansions; all of which

are threatening the Great Lakes and its native fishes today. Anthropogenic activities often act to homogenize genetic divergence and diversity patterns, obscuring the historical record of linkages and isolation that have shaped native populations; rendering their baseline genetic analysis critically important before those signatures are lost. For example, the smallmouth bass has been widely introduced to other areas and artificially cultured, and gene flow from those populations may threaten to homogenize the original genetic signature of some native populations (Stark & Echelle 1998). Introductions of spotted bass (*Micropterus punctulatus*) into areas originally occupied by native smallmouth bass resulted in extensive hybridization (determined from mtDNA studies) and changes in species abundance patterns (Avise *et al*. 1997). In addition, the smallmouth bass has been expanding its geographical range northward into lakes across southern Canada and into New England, where it has wrought significant changes to the food webs and affected the abundances of native piscivores (see Vander Zander *et al*. 2004). Those studies illustrate the need for assessing original population genetic characters in smallmouth bass and the importance of the present baseline genetic data as a historical record, while native populations are strong in the Great Lakes. The present investigation is based entirely on indigenous populations, and did not include areas with a known history of stocking. DNA microsatellites, as employed here, offer a high-resolution and low-cost molecular tool whose analysis can be readily applied as a baseline for conserving native genetic diversity and understanding its historical and present-day function in human-regulated ecosystems.

Objectives and hypotheses

This investigation tests for genetic structure and diversity patterns among spawning populations of smallmouth bass across the Great Lakes and outlying areas, in order to discern their relationships to the interplay among historical divergences, contemporary connectivity, and behavioural reproductive isolation factors. Here we analyse allelic variation at eight nuclear microsatellite loci to test the null hypothesis of panmixia among population sites against alternative hypotheses for genetic patterning resulting from behavioural and/or geographical isolation, specifically: (i) Are spawning groups in interconnected waterways genetically separable? (ii) What is the degree of isolation across and among lakes, basins, and tributaries? (iii) Do genetic divergences correspond to geographical distances? and (iv) Are historical colonization patterns from glacial refugia retained? Population genetic relationships for smallmouth bass then are compared to those characterizing other fishes from the Great Lakes and other freshwater systems, to aid interpretation of common influences on genetic divergence and gene flow.

Factors leading to genetic divergence patterns are explored — including historical (e.g. differences arising in southerly glacial refugia during the Pleistocene ice ages and colonization patterns of the Great Lakes *c*. 13 000 years ago), geographical (patterns corresponding to isolation by spatial distance among interconnected waterways and vicariant biogeographical barriers formed by drainage systems and lake basins), and behavioural (tendency to remain to spawn in natal localities from generation to generation). Fine-scale patterns are tested at the centre of the smallmouth bass' native distribution and abundance in Lake Erie, comparing populations from connected lacustrine basins vs. riverine sites for divergence and cohesion. A pilot study of smallmouth bass variation in Lake Erie by Borden & Stepien (2006) using mtDNA sequences and some preliminary microsatellite data found slight genetic differences across some spawning sites, but was markedly hampered in resolution by small sample sizes in comparison with the present investigation. The present analysis develops a high-resolution, low cost, and widely applicable DNA database for use by researchers and conservation managers to delineate essential areas for preserving native genetic diversity.

Life history and behaviour features that may lead to genetic divergence

Adult smallmouth bass are found in moderately shallow rocky and sandy areas of lakes and rivers (Scott & Crossman 1973). Tagging studies have shown that movements of individuals usually are limited to a few kilometres and demonstrate appreciable homing tendency (Ridgway & Shuter 1996; Hodgson *et al*. 1998; Lyons & Kanehl 2002), indicating potential for significant population genetic structure. Acquiring and defending a home range and successfully foraging in social groups appear prerequisite for reproductive success (Ridgway *et al*. 2002), and may lead to fine-scale genetic differentiation. The smallmouth bass is a specialist in spawning habitat, with adults congregating on spawning grounds in clean riffles of tributaries or shallow areas of lakes in late spring and early summer over a period of 6–10 days (Scott & Crossman 1973). The degree of genetic mixing among spawning groups is presently unknown, and is examined here.

Reproductive and parental care specificity may promote genetic divergence among spawning groups of smallmouth bass. Nests are patchily distributed and their locations remain stable from year-to-year (Rejwan *et al*. 1999; Ridgway *et al*. 2002). Studies have found that females may spawn with more than one male and the largest individuals of both sexes are the most reproductively successful, due to high mate selectivity (Wiegmann & Baylis 1995). Parental care-associated territorial behaviour of the males significantly influences the spacing among nests (Scott *et al*. 1997), and

represents a high energetic and individual fitness investment (Gillooly & Baylis 1999). The male defends the nest, fans the eggs, and continues to guard the young for five to seven days after hatching (Scott *et al*. 1997). Mortality totals 94% from egg through juvenile stages, and is significantly less for larger males (Knotek & Orth 1998). A genetic study of spawning males and surviving offspring suggested that only 5.4% of all spawning males produced 55% of the total number of fall young-of-the-year recaptured (Gross & Kapuscinski 1997), indicating that an assessment of genetic variability among spawning areas as undertaken here will provide important information useful to conservation managers. Thus, territoriality and nest-guarding behaviour of smallmouth bass suggests high potential for fine-scale genetic structure among spawning groups through philopatry, which is analysed in our study.

Hypothesized relationships in the Great Lakes region

The primary phylogeographical determinant that shaped modern-day populations in the North American Great Lakes region was their geographical isolation in southerly refugia during the Pleistocene ice ages, and subsequent colonization of the newly formed Great Lakes about 13 000 years ago (Scott & Crossman 1973; Bolsenga & Herdendorf 1993; Bernatchez & Wilson 1998). Populations of fishes from the Great Lakes today are believed to comprise a genetic admixture originating from several areas — including the Missouri, Mississippi and Atlantic-glacial refugia (Ward *et al*. 1989; Billington *et al*. 1992; Murdoch & Hebert 1997; Stepien & Faber 1998). The historical 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 in close proximity. These alternative scenarios are explored in this study.

Present-day Lake Erie appears relatively geographically isolated from 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 Detroit River that drains from Lake St. Clair (see Fig. 1b). The Detroit River presumably constrains gene flow between Lake Erie and the upper Great Lakes, forcing any migrating fish to pass through this narrow connection — which may form a geographical and genetic bottleneck. Lake Erie is classified as divided in three physiographic basins (Fig. 1b) with distinctive depths and separations by reefs (Bolsenga & Herdendorf 1993), whose connectivity for smallmouth bass populations are tested here. Smallmouth bass populations in Lake Erie declined significantly from the 1950s through the 1980s, presumably due to pollution, exploitation, and habitat loss (Trautman 1981). Smallmouth bass populations rebounded during the 1990s and there were particularly strong year classes in 1994, 1995, and 1996; however, they have declined in the past few years (Ohio Division of Wildlife 1999, 2005, 2006). Increased angling effort directed towards smallmouth bass has caused concern over the continued stability of the smallmouth bass population (Ohio Division of Wildlife 2006), indicating the importance of our baseline genetic analyses for the future.

Materials and methods

Sample locations and preparation

Collections focused on lacustrine and river spawning sites for Lake Erie smallmouth bass, as well as population samples in the other Great Lakes (Lakes Superior, Huron, and Ontario) and river drainages (the Mississippi, Ohio, St. Lawrence, and Hudson Rivers), encompassing fine- to broad-scale geographical scales (see Fig. 1a and Table 1). Geographical distribution of sampling sites is shown on Fig. 1 and Table 1, and totaled 666 individuals among 28 locations. These included 14 spawning sites and 425 individuals in Lake Erie, encompassing its three physiographic lake basins (Fig. 1a). Tributary samples in Lake Erie included three adjacent rivers in the central basin; the Cuyahoga River, OH; Chagrin River, OH, and Grand River, OH; and an eastern basin site at Cattaraugus Creek, NY. In addition, we compared allelic variation between smallmouth bass and its sister species, the spotted bass *Micropterus punctulatus* (Near *et al*. 2004), in order to evaluate possible (shared) ancestral characters at given loci and to provide a comparison for phylogenetic trees. Eight spotted bass individuals were analysed here, including samples from Big Darby Creek, a tributary of the Ohio River, OH; Sipsey Fork, AL; the Pecos River, TX; and the Chattahoochee River, Apalachicola, FL.

Most of the sampling was conducted by fishery agencies (see Acknowledgements). A small portion of a pectoral fin about 1–2 cm^2 was clipped from adult fish at known spawning sites during the spawning season. The fish were measured, often tagged, and then released. The clip was placed directly in 95% ethanol and archived at room temperature.

Microsatellite loci procedure

Genomic DNA was extracted and purified from the ethanol-fixed tissues with a DNeasy Tissue (QIAGEN, Inc.), then frozen and archived. The polymerase chain reaction (PCR) was used to amplify allelic length variants from eight microsatellite loci developed for smallmouth bass and other centrarchid species (e.g. DeWoody & Avise 2000; Malloy *et al*. 2000; Neff *et al*. 2000; Coughlin *et al*. 2003). PCRs consisted of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 μM of each deoxy-nucleotide, 0.5 μM each of the forward and reverse primers, 5–30 ng DNA template,

Table 1 Summary statistics by spawning population site (lettered — also see Fig. 1 map) using eight microsatellite loci in smallmouth bass

Locality	Lat °N	Long °W	N	H_O	H_E	F_{IS}	N_A	N_{PA}
Upper Mississippi River Drainage			28	0.540	0.628	0.139*	45	4
a. Cannon River, MN	44.52	92.88	12	0.573	0.565	–0.014	31	1
b. Apple River, WI	45.38	92.44	9	0.486	0.508	0.043	27	1
c. St. Croix River, MN	45.13	92.44	7	0.571	0.612	0.081	26	2
Lake Superior			9	0.500	0.561	0.108	27	0
d. St. Louis Bay, MN	46.72	92.16	6	0.583	0.554	–0.053	24	0
e. Whitefish Bay, MI	46.52	85.01	3	0.333	0.344	0.030	14	0
Lake Huron			42	0.411	0.421	0.025	31	0
f. Saginaw Bay, MI	43.55	83.90	7	0.571	0.539	–0.061	23	0
g. Georgian Bay, ON	44.59	80.94	35	0.379	0.394	0.040	29	0
Lake St. Clair			31					
h. Anchor Bay, MI	42.63	82.77	31	0.488	0.575	0.152	32	1
Lake Erie			425	0.469	0.513	0.085*	56	3
Lake Erie, Western Basin			132	0.484	0.507	0.046*	46	0
i. Port Clinton, OH	41.51	82.94	31	0.435	0.467	0.068	33	0
j. Sandusky Bay, OH	41.45	82.96	20	0.552	0.529	–0.044	34	0
k. Gem Beach, OH	41.57	82.82	30	0.426	0.514	0.170	30	0
l. South Bass Island, OH	41.66	82.82	34	0.454	0.478	0.050	35	0
m. Middle Bass Island, OH	41.67	82.96	17	0.537	0.544	0.012	30	0
Lake Erie, Central Basin			147	0.474	0.516	0.083*	42	2
n. Cuyahoga River, OH	41.13	81.54	10	0.450	0.576	0.218	33	0
o. Chagrin River, OH	41.41	81.41	11	0.557	0.508	–0.096	25	2
p. Grand River, OH	41.75	80.99	26	0.512	0.508	–0.009	29	0
q. Fairport Harbor, OH	41.79	81.20	43	0.477	0.487	0.020	31	0
r. Perry, OH	41.82	81.11	35	0.454	0.477	0.049	30	0
s. Conneaut, OH	41.96	80.81	22	0.423	0.513	0.176	28	0
Lake Erie, Eastern Basin			146	0.452	0.506	0.108*	41	1
t. Van Buren Bay, NY	42.47	79.40	68	0.493	0.543	0.097	38	1
u. Cattaraugus Creek, NY	42.57	79.11	30	0.471	0.483	0.026	28	0
v. Long Point Bay, ON	42.66	80.26	48	0.376	0.387	0.027	31	0
Lake Ontario			39	0.381	0.500	0.237*	33	0
w. Rochester, NY	43.33	77.62	19	0.382	0.426	0.104	23	0
x. Pultneyville, NY	43.30	77.18	20	0.381	0.513	0.257	25	0
St. Lawrence River Drainage			59	0.265	0.448	0.409*	26	0
y. Cranberry Lake, NY	44.21	74.84	29	0.384	0.411	0.067	25	0
z. Little Moose Lake, NY	43.69	74.92	30	0.150	0.247	0.392	16	0
Hudson River Drainage			16					
α. Hudson River, NY	43.24	73.83	16	0.313	0.361	0.135	21	0
Ohio River Drainage			17					
β. Paint Creek, OH	39.32	83.08	17	0.588	0.611	0.037	38	1
All sites in the Great Lakes region (d-z)	—	—	605	0.522	0.651	0.199*	60	4
All sites in the study (a-β)	—	—	666	0.442	0.564	0.218*	66	9

N, sample size (number of individual fish); H_O, = observed heterozygosity; H_E, expected heterozygosity; F_{IS} (as measured by θ_{IS}) = deviation from Hardy–Weinberg proportions, with positive values indicating heterozygote deficiency and negative values denoting heterozygote excess (Weir & Cockerham 1984; *significant deviation after Bonferroni correction); N_A, number of alleles, and N_{PA}, number of private alleles.

and 0.6–1.2 U of *Taq* polymerase per 10 μL of reaction volume. For each locus, the 'forward primer' was synthesized with a 5′ fluorescent label to allow visualization on an ABI (Applied Biosystems Inc.) 3130 Genetic Analyser, and four different dye labels were used in order to facilitate multi- and pool-plexing of loci. An oil overlay was added to ensure that the reaction volume remained constant throughout the PCRs. 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 (1:00 min) at a primer-specific temperature (below), and polymerase extension (72 °C, 30 s). Following this series, a final extension at 72 °C for 5 min was included to minimize partial strands. Annealing temperatures for multiplexed primer pairs were 48 °C (MS19 and RB7), 52 °C (Mdo 2, Mdo8, and Mdo11), and 54 °C (Mdo3, Mdo5, and Mdo9).

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 GeneScan-500 size standard and then loaded onto a 96-well plate. Microsatellite products were denatured for 2 min at 95 °C and analysed on the ABI 3130 Genetic Analyser with GENEMAPPER 3.7 software. We also manually checked all output profiles to confirm allelic size variants. Representative alleles of all loci were sequenced to verify that length polymorphisms were attributable to variation in copy number of single repeat motifs.

Data analyses

Population samples were tested for conformance to Hardy–Weinberg equilibrium expectations at each locus, and the Markov chain Monte Carlo method and 1000 randomization procedures were used to estimate significance following Guo & Thompson (1992), as implemented in GENEPOP (Raymond & Rousset 1995, 2004). Deviations were tested for heterozygosity deficiency or excess. In addition, each locus was tested for linkage disequilibrium. Level of significance for Hardy–Weinberg and linkage disequilibrium tests were adjusted using nonsequential Bonferroni corrections (Sokal & Rohlf 1995).

Unbiased θ estimates of *F*-statistics (Weir & Cockerham 1984) and their associated levels of significance were used to quantify genetic heterogeneity at different scales using the program FSTAT (Goudet 2002) in GENEPOP. There is ongoing debate as to which equivalents of F_{ST} are best suited for analysing divergences based on microsatellite data (see Hedrick 1999; Balloux & 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 & Cockerham 1984; see Balloux & Lugon-Moulin 2002), that method was adopted here. Values of θ_{ST} that differed significantly from zero were interpreted as evidence rejecting the null hypothesis of panmixia between sites.

In addition, comparative pairwise tests of allelic frequency heterogeneity were conducted using the method of Goudet *et al*. (1996) based on an exact nonparametric procedure with probabilities estimated using a Markov chain Monte Carlo method in GENEPOP. Probability values of tests employing multiple *post hoc* comparisons then were adjusted using the sequential Bonferroni method (Rice 1989) to minimize type I errors, in both types of pairwise tests. Number of migrants (N_m) among groups were estimated using Slatkin's (1985) private allele method as implemented by ARLEQUIN 3.0 (Excoffier *et al*. 2005). Hierarchical partitioning of genetic variation (% variance) among groups of populations, population samples within groups, and variation within sampling sites was evaluated using AMOVA (analysis of molecular variance; Excoffier *et al*. 1992) in ARLEQUIN.

The program ISOLDE (in GENEPOP) was employed to analyse the relationship of 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, kilometres). This relationship is expected to be linear under an isolation-by-distance hypothesis (Rousset 1997). Regression significance was tested using Mantel's (1967) procedure with 1000 permutations in GENEPOP. To further examine the relationship among population sites, Nei's (1972) pairwise genetic distances (D_S) and Cavalli-Sforza & Edwards' (1967) chord distances (D_c) were calculated from the allelic frequency data with the GENDIST program and used to construct neighbour-joining trees (Saitou & Nei 1987) in PHYLIP 3.63 (Felsenstein 2005). Relative support values for the nodes of the trees were estimated using 1000 bootstrap pseudoreplicates (Felsenstein 1985) in PHYLIP.

The relative magnitude of genetic structure among smallmouth bass populations was further investigated using an analytical approach based on computational geometry by Manni *et al*. (2004a, b). BARRIER 2.2 (Manni *et al*. 2004a, b) was employed to identify geographically continuous and discontinuous assemblages of sampling sites, independent from a priori knowledge of geographical population structure (e.g. lakes or river drainages). Pairwise estimates of θ_{ST} were mapped onto a matrix of their geographical coordinates (latitude and longitude). The spatial organization of subpopulations was modelled by Voronoi tessellation, and a Monmonier (1973) maximum-difference algorithm identified which of the borders between neighbouring populations exhibited the highest levels of genetic differences (Manni *et al*. 2004a, b). Initial estimates of genetic discontinuities were made with a multilocus θ_{ST} matrix, followed by a second analysis that incorporated single-locus θ_{ST} values. This procedure ranked each identified barrier in relative magnitude, according to respective support from individual loci θ_{ST} values.

In order to further evaluate distinctive populations, we employed a Bayesian-based clustering algorithm, which is independent of assumptions about mutation process, as implemented in the program STRUCTURE (version 2.2., Pritchard *et al*. 2000; Pritchard & Wen 2004). The analysis was used to identify groups having distinctive allelic frequencies, with and without prior knowledge of their true spawning population identity. We analysed correspondence to spawning populations by specifying number of groups (*K*) in independent runs of the algorithm, ranging from $K = 1$ (thus testing the null hypothesis of panmixia) to $K = 28$ (the total *N* of spawning sites sampled). The program assigned individuals to one or more groups, with their relative frequency of predicted membership in groups totaling 1.00. We used 10 independent runs for each *K*, with burn-ins of 100 000 and 200 000 replicates. We then examined the consistency among runs, the comparative

Table 2 Summary statistics for allelic variation at each microsatellite locus for smallmouth bass across the entire data set (see Table 1 and text for details)

Locus	N_A	Allele size range	Frequency (and length) of most abundant alleles			F_{IS}	F_{IT}	F_{ST}
			First	Second	Third			
Mdo2	5	195–203	0.73 (197)	0.20(201)	0.06(199)	0.202	0.296	0.118
Mdo3	6	120–130	0.59(120)	0.33(122)	0.06(126)	0.063	0.110	0.050
Mdo5	4	195–203	0.89 (199)	0.07(197)	0.03(203)	0.155	0.246	0.109
Mdo8	11	209–229	0.44(217)	0.19(213)	0.12(211)	0.065	0.122	0.061
Mdo9	8	122–136	0.59(130)	0.31(126)	0.04(132)	0.040	0.205	0.172
Mdo11	5	170–178	0.54(172)	0.41(176)	0.04(174)	0.228	0.417	0.244
RB7	15	111–141	0.41(131)	0.24(135)	0.12(119)	–0.011	0.208	0.217
MS19	12	99–127	0.47(101)	0.29(105)	0.09(111)	0.051	0.198	0.155
Total	66	—	—	—	—	0.085	0.218	0.147

N_A, number of alleles. Allelic frequencies are given for the first, second, and third most abundant alleles. F_{IS}, genetic variation within subsamples; F_{IT}, genetic variation in the total sample; F_{ST}, genetic divergence among spawning populations, as measured by θ (Weir & Cockerham 1984).

probabilities of individuals assigning to one or more groups, the log-likelihood and posterior probability values from each run, and their respective grouping patterns. Results of the STRUCTURE analyses then were compared with population relationships derived from the BARRIER, genetic divergence, and AMOVA analyses.

Results

Overall and within-site variation

Genotypic data were analysed for 666 smallmouth bass from 14 spawning locations within Lake Erie, sites in other Great Lakes, and 7 outlying areas (Table 1). Sampling sites conformed to Hardy–Weinberg expectations (Table 1). Overall, when artificially grouped together as single populations, several combined sites differed significantly from overall Hardy–Weinberg equilibrium proportions (Table 1), supporting the alternative hypothesis of genetic structure among lakes, basins, and tributaries. This artificial combination of multiple biological populations thus revealed Wahlund effects as evidenced by heterozygote deficiencies (see Table 1) due to mixing of individuals from separable spawning groups having different allelic frequencies (see Balloux & Lugon-Moulin 2002; Hedrick 2005). Loci were unlinked.

Observed heterozygosities per sampling site were similar across the Great Lakes, averaging 0.522 for the Great Lakes as a whole (Table 1). Sites in the east (Hudson and St. Lawrence River drainages and Lake Ontario) had overall lower heterozygosities than did other locations. A total of 66 alleles were identified among the eight loci, of which 60 (91%) were found in the Great Lakes. The average number of alleles per sampling location was 27%, and 25% (7/28) of the sampling sites housed one to two private alleles (Table 1). Lake Erie populations contained 56 alleles, totaling 93% of those identified across the Great Lakes and three of which were private alleles (5.3%).

All loci had moderate to high levels of polymorphism, with variation ranging from 4 (Mdo5) to 15 (RB7) alleles per locus (Table 2). One to three predominant alleles characterized most loci, with additional alleles present at low frequencies. Among the eight loci for smallmouth bass, the total amount of genetic variation ranged from $F_{IT} = 0.110$ (Mdo3) to 0.417 (Mdo11), averaging 0.218 (Table 2). The greatest levels of genetic divergence among sampling locations occurred for the Mdo11 and RB7 loci. Overall mean genetic divergence among sites was $\theta_{ST} = 0.147$.

Broad- to fine-scale genetic trends

Genetic divergence comparisons within lake and river systems and their component locations are summarized in Table 3, for which most tests were significant. These results reveal considerable broad- to fine-scale genetic structure among and within systems. The greatest values occurring among sites within river or lake systems were those separating the two closely spaced sites in the St. Lawrence River drainage (a geographical distance of a mere 50 km) and that separating western vs. eastern Lake Superior (a geographical distance of 411 km). For example, Fig. 2a illustrates marked differences at the Mdo8 locus among three closely spaced sites in the Upper Mississippi River system that are geographically separated. Overall levels of genetic divergence separating sampling locations did not appear to closely mirror the geographical distances of connected waterways (Table 3). However, the regression of pairwise $\theta_{ST}/(\theta_{ST} - 1)$ against the natural logarithm of geographical distance between all collection localities within the Great Lakes system (including river and lake sites) showed a positive association (Fig. 3a).

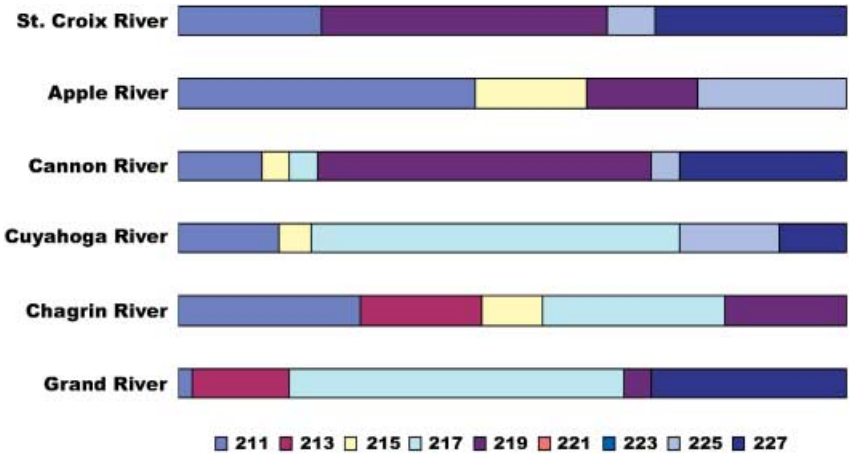

Fig. 2 Examples of allelic frequencies at the Mdo8 locus for smallmouth bass among tributary spawning sites locations in the upper Mississippi River drainage system (upper three) and the central basin of Lake Erie (lower three). Note: alleles 221 and 223 do not appear in these particular rivers.

Table 3 Summary of population divergence calculated using θ_{ST} (Weir & Cockerham 1984) and nonparametric genetic differentiation tests (Goudet *et al*. 1996) for smallmouth bass by Great Lakes basin or river drainage for systems with multiple sampling locations and high potential connectivity among the sites

System	Tested subdivision	θ_{ST}	Genetic differentiation (X^2)
Upper Mississippi River drainage	3 closely spaced sites	0.162**	Inf**
Lake Superior	West vs. east	0.233**	34.88**
Lake Huron	West vs. northeast	0.021	19.39
Lake Erie	3 basins and rivers	0.040**	Inf**
	Western basin	0.011**	46.57**
	Central basin (including lake and river sites)	0.042**	Inf**
	Central basin lake sites (without rivers)	0.008	22.28
	Central basin rivers only (without lake reefs)	0.091**	Inf**
	Eastern basin	0.079**	Inf**
Lake Ontario	2 adjacent sites	0.109**	53.94**
St. Lawrence River drainage	2 closely spaced sites	0.446**	Inf**
Great Lakes Region overall	All of the above except Mississippi River	0.122**	Inf**
All sites	All of the above	0.147**	Inf**

Inf, X^2 denoted as infinite by GENEPOP; *significant at 0.05 level; **also significant following sequential Bonferroni correction for multiple *post hoc* tests.

Almost all θ_{ST} and X^2 (Goudet *et al*. 1996) pairwise comparisons among smallmouth bass population sites from lakes and river drainages were highly significant (Table 4), denoting pronounced genetic structure. Results of our study thus indicate that there is little to no gene flow among smallmouth bass populations in separate lakes or river drainages. The largest θ_{ST} pairwise divergence values (Table 4A) occurred between the Hudson River population vs. other locations, and was relatively closer in genetic relationship to (yet significantly divergent from) samples in the eastern Great Lakes region (e.g. Lake Ontario and Lake Erie). The St. Lawrence River vs. the Upper Mississippi River system and Lake St. Clair populations showed the next highest pairwise levels of divergences, falling outside the other Great Lakes locations. Within the Great Lakes themselves, the greatest differences occurred between populations from Lake St. Clair vs. the other lakes, including Lake Superior, Lake Huron, Lake Ontario, and Lake Erie (Table 4A).

The outlying populations sampled from tributaries of the Ohio and upper Mississippi Rivers were more closely related to each other than to other population areas, albeit being highly divergent (Table 4A). The Ohio River sample also showed some genetic affinity to the samples from Lake Erie, reflecting its rough proximity to the Lake (due south). Smallmouth bass populations from the Upper Mississippi River and Lake Superior locations were closer in genetic relationship, roughly corresponding to their

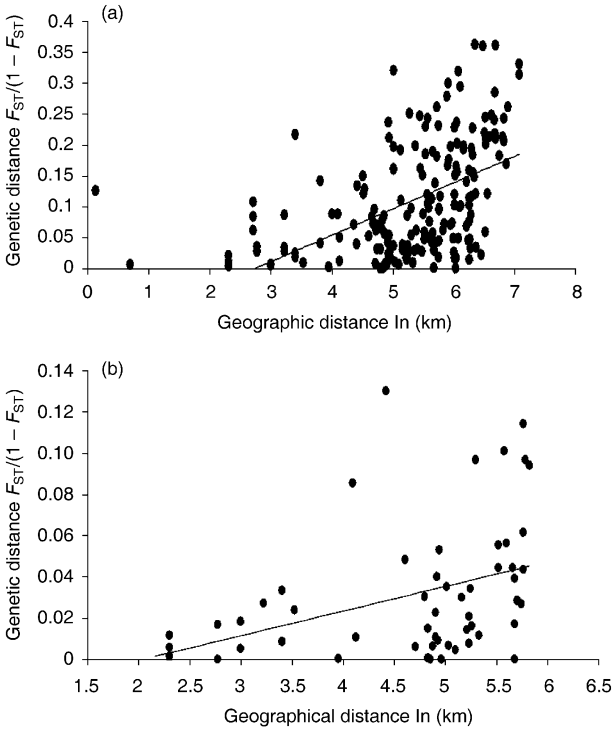

Fig. 3 Pairwise relationship between genetic distance ($\theta_{ST}/1 - \theta_{ST}$) vs. the natural logarithm of geographical distance (km). (a), Across Great Lakes region. Test of isolation by distance: $P = 0.002$**, $R^2 = 0.201$, equation is 10.1148 + 0.1425 (ln km). (b), Across Lake Erie, without river sites. Test of isolation by distance: $P = 0.002$**, $R^2 = 0.043$, equation is –0.0244 + 0.0119 (ln km).

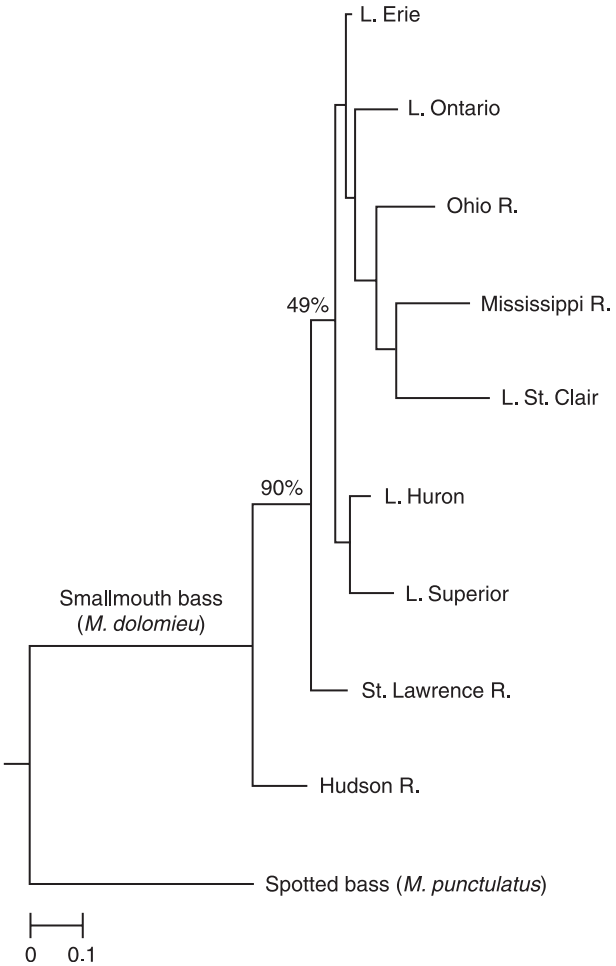

Fig. 4 Neighbour-joining tree (Saitou & Nei 1987; constructed in PHYLIP) showing relationships among major population areas for smallmouth bass based on Nei (1972) genetic distances. The tree calculated from (Cavalli-Sforza & Edwards 1967) chord distances was similar in topology and resolution, and is thus not shown. Tree is 'rooted' with its sister species, the spotted bass. Values at nodes denote relative support from 1000 bootstrap iterations.

geography. In turn, samples from Lake St. Clair appeared more closely related to those from western Lake Erie than to other sites, and those from eastern Lake Erie were more similar to those in Lake Ontario (Table 4A).

Trees based on Nei (1972) and Cavalli-Sforza & Edwards (1967) chord distances were similar in resolution and topology. The neighbour-joining tree shown in Fig. 4 depicts the relationship among overall broad-scale population sites for smallmouth bass (e.g. the sites given in Table 4A), in comparison to its sister species the spotted bass *Micropterus punctulatus*. We identified 38 alleles among the eight loci for the spotted bass tested. Fifteen (40%) of the alleles in spotted bass appeared unique and were not identified in our smallmouth bass samples. The neighbour-joining trees showed Hudson River population of smallmouth bass as basal to the Great Lakes and other river systems, indicating a long-term separation. The remaining populations clustered together and were supported by 90% of the 1000 bootstrap pseudoreplications. The St. Lawrence River population group was basal to the remaining sites, located outside the Great Lakes populations. Other relationships on the trees largely reflected geographical proximity but were not supported by bootstrapping and thus are highly uncertain (Fig. 4).

Analyses of primary genetic barriers using the BARRIER approach of Manni *et al*. (2004a, b) were congruent with the neighbour-joining trees (Fig. 4) in placing the major division between the Hudson River sample and all other populations of smallmouth bass (Fig. 1a, designated I), which was supported independently by five loci (Mdo 2, 9, 11, MS19, and RB7). The next strongest barriers separated the Ohio River (barrier II) and the upper Mississippi River and Lake Superior sampling sites (III) from the other Great Lakes sites. These were supported by five and seven individual loci, respectively, with the difference due to relative strength of support by specific loci (Fig. 1a; see relative θ_{ST} values in Table 4). In AMOVA (Excoffier *et al*. 1992) analyses, variance partitioning among the groups designated by barriers I through III contained 9.07% of the overall variation

Table 4A Pairwise θ_{ST} (below diagonal) and genetic differentiation X^2 (above diagonal) comparisons based on eight microsatellite loci between smallmouth bass populations in lakes, basins, and river drainages

	Upper Mississippi River	Lake Superior	Lake Huron	Lake St. Clair	Western + Central Lake Erie	Eastern Lake Erie	Lake Ontario	St. Lawrence River Drainage	Hudson River Drainage	Ohio River Drainage
Upper Mississippi River Drainage	—	65.67**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**
Lake Superior	0.139**	—	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**
Lake Huron	0.212**	0.112**	—	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**
Lake St. Clair	0.155**	0.228**	0.231**	—	Inf**	Inf**	Inf**	Inf**	Inf**	Inf**
Western + Central Lake Erie	0.157**	0.105**	0.061**	0.175**	—	Inf**	Inf **	Inf**	Inf**	Inf**
Eastern Lake Erie	0.169**	0.085**	0.064**	0.207**	0.011**	—	80.88**	Inf**	Inf**	Inf**
Lake Ontario	0.198**	0.163**	0.195**	0.208**	0.084**	0.067**	—	Inf**	Inf**	Inf**
St. Lawrence River Drainage	0.291**	0.190**	0.224**	0.276**	0.123**	0.122**	0.148**	—	Inf**	Inf**
Hudson River Drainage	0.312**	0.267**	0.351**	0.354**	0.246**	0.248**	0.294**	0.300**	—	Inf**
Ohio River Drainage	0.115**	0.160**	0.190**	0.180**	0.111**	0.143**	0.181**	0.226**	0.262**	—

Inf, X^2 denoted as infinite by GENEPOP; **significant following sequential Bonferroni correction for multiple *post hoc* tests; *significant at 0.05 level before Bonferroni correction; NS, not significant.

Table 4B Pairwise θ_{ST} (below diagonal) and genetic differentiation X^2 (above diagonal) comparisons based on eight microsatellite loci between smallmouth bass spawning groups from 14 locations in the Lake Erie drainage. Inf, X^2 denoted as infinite by GENEPOP. Probability values are: **significant after sequential Bonferroni correction for multiple *post hoc* tests; *significant at $P < 0.05$ level (prior to Bonferroni correction)

	Port Clinton	Gem Beach	Sandusky Bay	South Bass Island	Middle Bass Island	Cuyahoga River	Chagrin River	Grand River	Fairport Harbor	Perry	Conneaut	Van Buren Bay	Cattaraugus Creek	Long Point Bay
Port Clinton, OH	—	**10.01**	**28.09***	**33.24***	**31.31***	**77.97****	**Inf****	**54.45****	**18.14**	**21.78**	**22.66**	8.73	**Inf****	**54.88****
Gem Beach, Sandusky, OH	0.000	—	**25.03**	**31.07***	**22.05**	**85.04****	**Inf****	**52.40****	**13.12**	**15.26**	**22.48**	**49.90****	**Inf****	**56.52****
Sandusky Bay, OH	0.025*	0.018*	—	**27.06***	**28.85***	**42.85****	**43.55****	**19.36**	**39.74****	**24.64**	**28.48***	**28.86***	**43.26****	**Inf****
South Bass Island, OH	0.006	0.006	0.009	—	**37.35***	**82.78****	**50.48****	**47.61****	**52.44****	**36.80***	**36.51***	**59.70****	**Inf****	**Inf****
Middle Bass Island, OH	0.021*	0.004	0.034*	0.027*	—	**66.70****	**33.65***	**51.20****	**25.97**	**27.95***	**26.11**	**47.90****	**Inf****	**40.26****
Cuyahoga River, OH	0.114**	0.108**	0.038*	0.077**	0.123**	—	**69.90****	**69.68****	**Inf****	**76.63****	**Inf****	**Inf****	**72.70****	**Inf****
Chagrin River, OH	0.067**	0.058**	0.068**	0.054**	0.030	0.178**	—	**57.29****	**Inf****	**Inf****	**40.96****	**60.50****	**Inf****	**Inf****
Grand River, OH	0.091**	0.080**	0.027**	0.052**	0.085**	0.064**	0.114**	—	**Inf****	**32.71***	**44.02****	**51.99****	**48.09****	**Inf****
Fairport Harbor, OH	0.000	0.000	0.030**	0.015**	0.003	0.124**	0.058**	0.092**	—	**18.42**	**18.72**	**44.67****	**Inf****	**44.17****
Perry, OH	0.007	0.009	0.008	0.011*	0.023*	0.081**	0.079**	0.054**	0.011	—	**16.93**	**23.90**	**49.01****	**Inf****
Conneaut, OH	0.014	0.018*	0.017*	0.007	0.026*	0.087**	0.066**	0.072**	0.012	0.003	—	**29.45***	**46.08****	**48.51****
Van Buren Bay, NY	0.028**	0.029**	0.001	0.018*	0.040**	0.058**	0.078**	0.033**	0.030**	0.006	0.008	—	**50.97****	**Inf****
Cattaraugus Creek, NY	0.087**	0.089**	0.042**	0.058**	0.104**	0.080**	0.165**	0.074**	0.089**	0.036**	0.040**	0.020**	—	Inf**
Long Pt. Bay, ON	0.045**	0.056**	0.095**	0.054**	0.048**	0.201**	0.100**	0.169**	0.036**	0.051**	0.050**	0.081**	0.118**	—

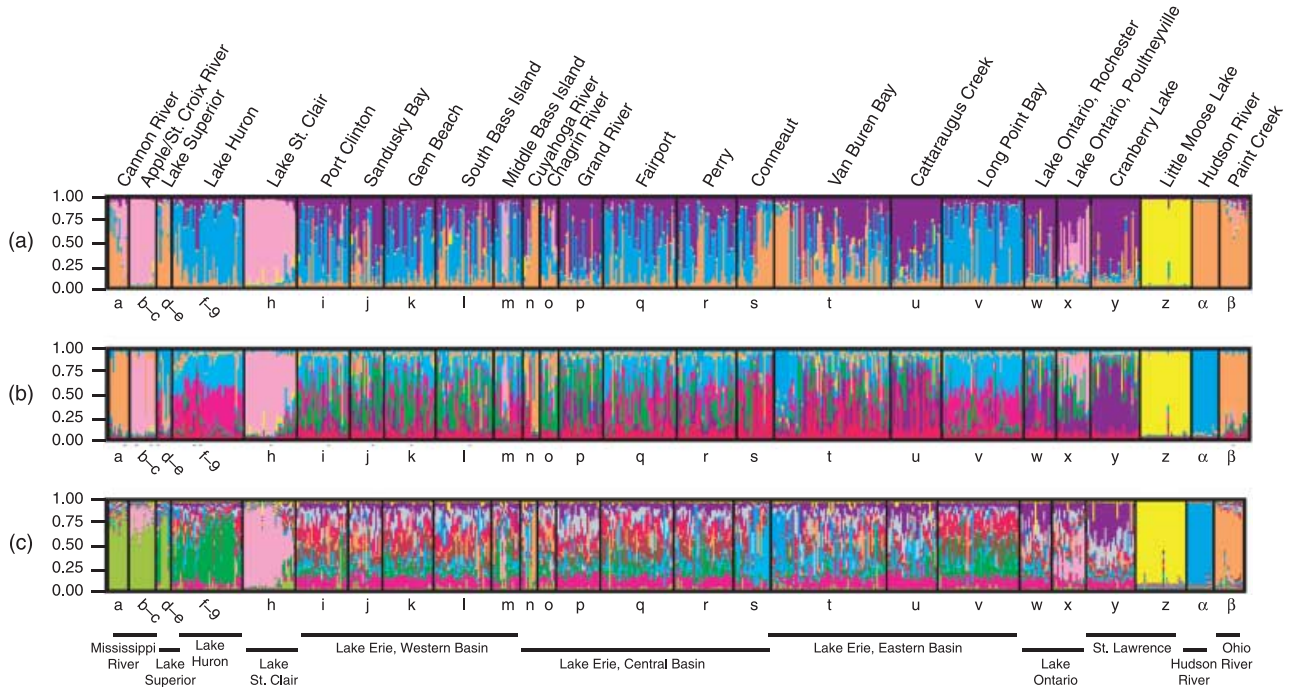

Fig. 5 Estimated population structure for smallmouth bass from STRUCTURE analysis for $K = 5$(a), 9(b), and 12(c) groups (with the latter number having the greatest mean likelihood and posterior probability values). Each individual is represented by a thin vertical line, which is partitioned into K coloured segments that represent the individual's estimated membership fractions. Black lines separate individuals from different spawning sites, which are labelled below the figure. Ten STRUCTURE runs at each K produced nearly identical individual membership coefficients, having pairwise similarity coefficients above 0.95, and the figure illustrating a given K is based on the highest probability run at that K.

among all 28 populations, with $F_{CT} = 0.91$ ($P < 0.00001$). Variation among populations within the groups comprised 11.39%, with $F_{SC} = 0.125$ ($P < 0.00001$).

Using Bayesian analyses in STRUCTURE (Pritchard *et al*. 2000; Pritchard & Wen 2004), the greatest mean log-likelihood and posterior probability values occurred for $K = 12$ groups, which had a posterior probability of 0.998 (Fig. 5). Figure 5 illustrates a comparison of the results for $K = 5$, $K = 9$, and $K = 12$ groups. These analyses most strongly assigned the Hudson River population (0.96 membership assignment for $K = 5$, 0.93 in $K = 9$, and 0.90 for $K = 12$ groups), samples from the upper Mississippi River system (0.94, 0.81, and 0.80), the Ohio River (0.87, 0.81, and 0.78), Lake Superior (0.93, 0.85, and 0.80), Lake St. Clair (0.86, 0.77, and 0.71), and Little Moose Lake from the St. Lawrence River drainage (0.95, 0.93, and 0.90) to single population groups. These results thus were similar to those obtained with the BARRIER approach.

Likewise, the next primary barrier within the Great Lakes system designated by the Manni *et al*. (2004a, b) BARRIER analysis occurred between Lakes St. Clair and Erie, differentiating the Upper and the Lower Great Lakes (designated as IV on Fig. 1a, and supported by four loci). In AMOVA analyses, this barrier corresponded to 3.71% of the overall variation within the Great Lakes system, with $F_{CT} = 0.037$ ($P < 0.0411$). Variation among spawning populations in the Great Lakes comprised 8.28%, with $F_{SC} = 0.086$ ($P < 0.00001$). Other demarcations designated by BARRIER analysis separated the central Lake Erie basin Cuyahoga and Chagrin Rivers that are connected to the Lake (ranked V), the St. Lawrence River system from the other Great Lakes (VI), Lakes Erie and Ontario at Niagara Falls (ranked VII), the Grand River in the central Lake Erie basin (VIII) and the Long Point Bay site in northern Lake Erie (IX). These results were similar to those from AMOVA analyses. For example, the difference between Lakes Erie and Ontario smallmouth bass (which did not include the river sites) accounted for 6.97% of the variation and $F_{CT} = 0.070$ ($P < 0.0147$) using AMOVA. Variation among spawning locations within Lakes Erie and Ontario then was 3.26%, with $F_{SC} = 0.035$ ($P < 0.00001$).

Differences in rankings of the barriers between the STRUCTURE and the BARRIER analyses likely were due to the latter's weighting of geographically proximate sites as comprising stronger barriers, relative to given genetic divergences; whereas the former method evaluated allelic frequencies independent from geographical separation. In summary, results for major population divisions were congruent among the different approaches used in our study.

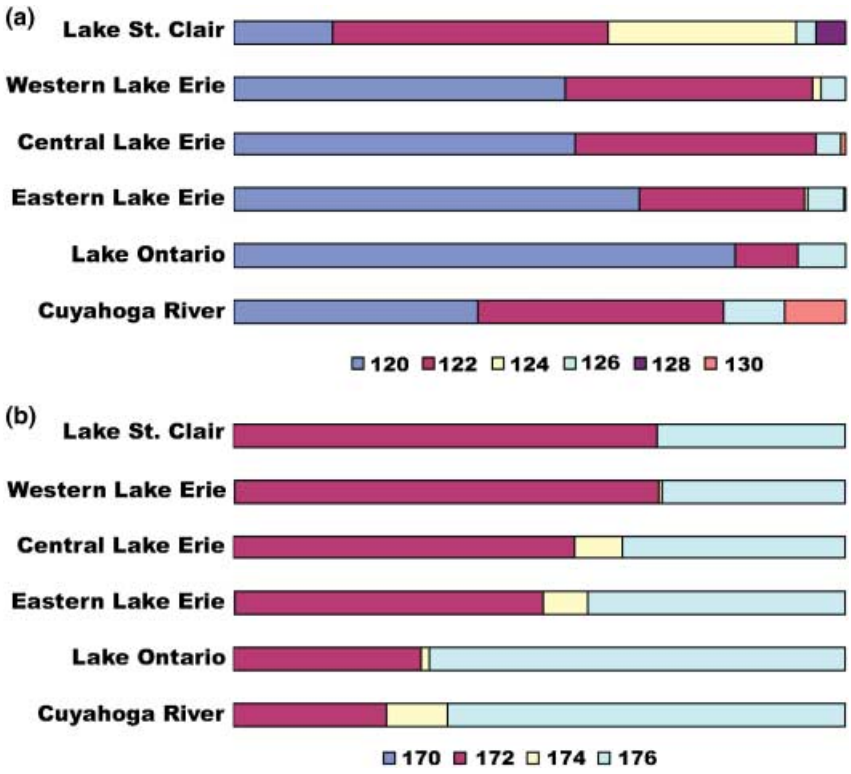

Fig. 6 Examples of allelic frequency patterning for smallmouth bass among spawning sites in Lake St. Clair, Lake Erie (western, central, and eastern basins), Lake Ontario (combined sites), and the Cuyahoga River (located off central basin of Lake Erie). Upper = Mdo3 locus, Lower = Mdo11 locus.

Fine-scale genetic patterns in Lake Erie

Overall numbers of alleles characterizing smallmouth bass from the three Lake Erie basins were similar, totalling 46 in the western basin, 42 in the central basin, and 41 in the eastern basin; based on similar sample sizes per basin (Table 1). Of these, two private alleles were identified in samples from the central basin (in the Chagrin River, OH), and a single private allele appeared restricted to the eastern basin (at Van Buren Bay, NY; Table 1). In addition, three alleles were exclusively found in smallmouth bass from Lakes St. Clair and Erie, including allele 195 bp at Mdo2, 124 bp at Mdo3, and 121 bp at RB7. The 124-bp allele of the Mdo3 locus was relatively common in the Lake St. Clair sample (comprising 31%), rare in smallmouth bass from western Lake Erie, and absent from Lake Ontario (see Fig. 6a). Allelic variation at the Mdo3 and Mdo11 loci revealed a west to east pattern of clinal variation (Fig. 6).

Pairwise θ_{ST} and X^2 (Goudet *et al.* 1996) tests among smallmouth bass spawning population sites within Lake Erie revealed similar relationships among sites (Table 4B). Significant allelic frequency differences ($P < 0.05$) were determined for 69 (76%) of the 91 pairwise θ_{ST} comparisons among smallmouth bass from the 14 Lake Erie sampling sites (Table 4B). Of those estimates, 58 (64%) remained significant following sequential Bonferroni correction. Smallmouth bass from spawning locations in the eastern basin of Lake Erie were significantly divergent from one other, as well as from those reproducing in the central and western basins (Tables 3 and 4B). Overall, the greatest differences in genetic compositions among smallmouth bass spawning populations in Lake Erie occurred in the eastern basin, whereas western basin spawning groups displayed less structure (Table 3). Reef spawning groups in the western and central basins were less divergent from one another than were riverine spawning populations (Table 4 and Fig. 6a). Thus, the riverine populations of smallmouth bass exhibited far greater genetic structuring, as well as little gene flow to and from the Lake Erie sites (Tables 4B and 5).

The largest overall population divergences in the Lake Erie system involved pairwise comparisons of smallmouth bass from tributary locations (e.g. the Cuyahoga, Chagrin, and Grand Rivers in the central basin and Cattaraugus Creek in the eastern basin; see Table 4B and Fig. 6). The greatest differences among closely spaced populations occurred between the Cuyahoga and Chagrin Rivers, indicating their long-term separation despite their close geography (Fig. 1b). Figure 2 illustrates the marked differences at the Mdo8 locus differentiating smallmouth bass from the Cuyahoga, Chagrin, and Grand Rivers. Large divergences also were found between smallmouth bass populations from the riverine sites vs. most lake spawning locations, showing their fine-scale distinctiveness and little or no gene flow (Table 4B).

Table 5 Analyses of the distribution of genetic variability among basins and sites in the Lake Erie system. NS, not significant; *significant at $P < 0.05$ level; **also significant following sequential Bonferroni correction. (A) Calculations of percentage of genetic variation using analysis of molecular variance (AMOVA; Excoffier *et al*. 1992, 2005) Left, including Lake reef and river spawning sites; Right, including only spawning sites within the Lake. (B) Pairwise θ_{ST} comparisons between basins. Above diagonal, includes only spawning sites within the Lake; Below diagonal, includes both river and lake spawning sites

A

Source of variation	Percentage variation in lake and rivers	Percentage variation in lake only
Among 3 lake basins	0.001%	0.001%
Among spawning sites within basins	4.38%**	2.65%**
Among individuals in spawning sites	4.34%**	4.62%**
Within individuals in spawning sites	91.28%**	92.73%**

B

	Western Basin	Central Basin	Eastern Basin
Western Basin	—	0.003	0.010**
Central Basin	0.002	—	0.001
Eastern Basin	0.016**	0.009**	—

Within Lake Erie, there was a nonsignificant yet slightly positive relationship between genetic distance and geographical distance when both lake reef and river spawning sites were considered. When only lake reef sites (but no river sites) were considered, a significant positive relationship was discerned, showing a weak isolation with geographical distance pattern (Fig. 3b). This relationship was not significant when the rivers were included, likely due to their markedly greater divergences as compared to the lake sites (see Table 4B). In addition, conformance to an isolation-by-spatial-distance pattern was not indicated when comparisons were restricted to either the western basin or the eastern basin of Lake Erie. The genetic isolation by geographical distance pattern thus only was supported for broad-scale distances across lake systems or among lakes and major river drainages.

When spawning sites were grouped together by lake basin, populations of smallmouth bass exhibited moderate, but significant amounts of genetic divergence among sites within the basins and relatively little variation among the basins (Table 5A). The greatest pairwise θ_{ST} value between basins was found between the east vs. the west, followed by the central and eastern basins (Table 5B). No significant difference was evident overall between samples from the western and central basins. These results were more pronounced when river population sites were included, reflecting the higher divergences characterizing smallmouth bass found in the tributaries as opposed to the spawning locations within the Lake. The physiographic basins alone thus do not provide appreciable geographical barriers distinguishing smallmouth bass populations.

West to east clinal trends extending from Lake St. Clair, across the three Lake Erie basins and through Lake Ontario, were evident in the general distribution of allelic variation at several loci (two of which are illustrated in Fig. 5). Affinity between smallmouth bass inhabiting eastern Lake Erie and Lake Ontario also is shown by their closer genetic relationship (Table 4 A). The pronounced divergence of bass from the central Lake Erie basin reef sites vs. the nearby Cuyahoga River site also is illustrated for both the Mdo3 and Mdo11 loci (Fig. 5).

Genetic barriers between riverine and reef spawning groups of smallmouth bass from central Lake Erie are depicted in the barrier analysis in Fig. 1a, which used the Manni *et al*. (2004a) approach. This divergence also is clearly shown by the magnitudes of genetic differences distinguishing the riverine groups from each other as well as from sites in Lake Erie (Tables 3 and 4B; Figs 2 and 5). Another genetic break in the population structure of Lake Erie smallmouth bass shown by BARRIER analysis occurred at the Long Point Bay, ON site along the northeast shore (Fig. 1a), which also is supported by the appreciable levels of genetic divergences (Table 4B).

Bayesian analyses using STRUCTURE (Pritchard *et al*. 2000; Pritchard & Wen 2004) for Lake Erie spawning groups revealed greatest mean log-likelihood and posterior probability values at $K = 4$ population groups, assigning most individuals to a single separable group (in decreasing relative order of support) for the Cuyahoga River, the Chagrin River, Long Point Bay, and Cattaraugus Creek. The program also assigned the Grand River sample to the same group as the Chagrin River, with relatively high support. Relationships among population groups thus approximated results from BARRIER and genetic differentiation analyses.

Discussion

Phylogeography of broad-scale patterns

Broad-scale phylogeographical relationships among populations of smallmouth bass in the Great Lakes reveal moderate to very great levels of genetic divergences (according to Wright's 1978 values; also see Balloux & Lugon-Moulin 2002), which appear to reflect the pattern of colonization of the newly formed Great Lakes about 13 000 years ago (Scott & Crossman 1973; Bolsenga & Herdendorf 1993; Bernatchez & Wilson 1998). Contemporary

populations of smallmouth bass (this study) and other fishes (Ward *et al.* 1989; Billington *et al.* 1992; Murdoch & Hebert 1997; Stepien & Faber 1998) in the Great Lakes comprise a genetic admixture originating from a number of pathways from glacial refugia, whose genetic signatures remain discernable today. The historical signatures of these refugia have been reinforced by geographical barriers among lakes and their basins that, together with behavioural philopatry, acted to constrain gene flow. Similar genetic patterning among lakes and river systems in the Great Lakes system to those discerned here are observed in other fishes, including walleye (Stepien & Faber 1998; Stepien *et al.* 2004), brown bullhead (Murdoch & Hebert 1997), and yellow perch (Ford & Stepien 2004). Congruent population patterns among these fishes across a broad geographical range reflect common vicariance and dispersal scenarios.

Relative divergence levels among separate drainage systems and the Great Lakes sites sampled for smallmouth bass approximate values obtained for other freshwater fishes using a similar number of microsatellite loci. For example, populations of the cyprinid *Squalius aradensis* were distinguished by a mean $F_{ST} = 0.297$ (ranging from 0.008 to 0.456), showing significant divergence among small drainages in southern Portugal that are separated by reaches of brackish water and periodic drying up of habitats, across a geographical scale of only ~100 km (Mesquita *et al*. 2005). The mean divergence levels for *S. aradensis* thus average about twice those found here among smallmouth bass populations, which are in separate drainages across a range of ~1540 km (Table 4A). In comparison, our smallmouth bass samples revealed a mean genetic divergence of $F_{ST} = 0.147$ among locations, ranging to 0.354 separating the Hudson River sample from the Lake St. Clair population (Tables 3 and 4A). Some of this difference is attributable to the unglaciated history of *S. aradensis* in that study vs. the glaciation history of smallmouth bass in our study.

An analysis of divergence among northern pike *Esox lucius* populations across a broad-scale European distribution by Jacobsen *et al*. (2005) using microsatellites discerned higher values than those found in our study, averaging $F_{ST} = 0.497$. Those results included previously glaciated and nonglaciated areas, as well as a greater geographical range than used here for smallmouth bass. In contrast, microsatellite analyses of *E. lucius* sampled along the coastal Baltic Sea by Laikre *et al*. (2005) for a geographical range of ~1300 km with high potential connectivity, displayed a range of $F_{ST} = 0.001–0.108$ among sites; which is less than the divergences of smallmouth bass across the Great Lakes drainage. Results for northern pike populations fit an isolation-by-geographical-distance pattern along the Baltic Sea, as well as behavioural patterns indicating strong homing to spawning sites (Laikre *et al*. 2005).

Our results thus suggest that smallmouth bass populations in the Great Lakes exhibit an intermediate level of genetic divergence pattern between these reference examples of freshwater fish species. Smallmouth bass fall in between examples of population divergence patterns shaped by considerable geographical barriers to gene flow (i.e. *Squalius aradensis;* Mesquita *et al*. 2005 and *Esox lucius* across separated European drainages; Jacobsen *et al*. 2005) vs. that showing apparent habitat connectivity and behavioural restrictions due to spawning site philopatry (*E. lucius* in the Baltic Sea area; Laikre *et al*. 2005). Our findings thus reveal appreciable genetic structure and spatial patterning among smallmouth bass populations despite relatively high potential for migration and connectivity and recent postglacial history, indicating behavioural spawning site specificity.

Rankings of genetic separation barriers in our study of smallmouth bass populations correspond to known geographical barriers. The primary division in our genetic data set for smallmouth bass occurred between the Hudson River (Atlantic coastal group) vs. all other sites (including the St. Lawrence River system, entire Great Lakes, and outgroups from the upper Mississippi and Ohio River systems; designated as barrier I on Fig. 1, and see Fig. 5). This ancient divergence break has been documented in other fishes such as the rainbow smelt *Osmerus mordax* (Bernatchez 1997), brown bullhead *Ameiurus nebulosus* (Murdoch & Hebert 1997), and lake cisco *Coregonus artedi* (Turgeon & Bernatchez 2001); apparently reflecting early separation of fish lineages that were isolated in an Atlantic coastal plain glacial refugium (and are now descendent in the Hudson River drainage). Our data indicate that smallmouth bass populations have retained that early separation between the Atlantic coastal refugium descendents vs. all other population sites sampled to the west and the north. These results show that lineages of smallmouth bass occupying the Hudson River drainage are quite genetically distinct from those in the Great Lakes.

Primary genetic breaks in our data set also separate smallmouth bass populations from the Great Lakes vs. the Ohio River and Upper Mississippi River/Lake Superior region (Fig. 1a, designated as II and III, respectively). Similar long-term divergence of native Ohio River walleye from those in the Great Lakes was discerned by Stepien & Faber (1998) from mtDNA sequences and dated at about 1.5 million years using a molecular-clock calibration. Some geological evidence suggests that the upper portion of the Ohio River drainage and its predecessor (the Teays River drainage) have been geographically isolated from the Great Lakes for at least 1 million years, predating the Pleistocene ice ages (Flint 1971), however, there may have been some subsequent short-lived connections across the drainage divide from meltwater runoff that fish may have used (T. Fisher, personal communication). Smallmouth bass

from the Ohio River and Upper Mississippi River drainages show relatively closer relationship, which may trace to their former connectivity through the ancient Teays River System that once drained west into the Mississippi River (Bolsenga & Herdendorf 1993). Preglacially, much of the Great Lakes region drained northeast through Lake Erie and along the ancestral St. Lawrence River valley. This ancient division of the preglacial Great Lakes/St. Lawrence River system from the Mississippi/Teays River system thus appears to correspond to some of the oldest divergences evident in our microsatellite data set.

Smallmouth bass from the Upper Mississippi River drainage appear next most closely genetically related to those from Lake Superior, congruent with patterns documented for brown bullhead from these regions by Murdoch & Hebert (1997). This is likely due to a western postglacial colonization linkage to what is now Duluth in western Lake Superior via the upper Mississippi River about 11 500 years ago (Bailey & Smith 1981; Crossman & McAllister 1986). This former connection subsequently gradually diverged into a primary divide separating all of the Great Lakes from the upper Mississippi Basin, which also is evident by the high levels of genetic divergences among those smallmouth bass populations in our data despite the remnant signature of that former connectivity (Burr & Page 1986).

Lakes Superior and Huron smallmouth bass populations appeared genetically close on the neighbour-joining tree (Fig. 4), although their genetic distances were significantly different, which reflects the partial barriers that separated their basins ~10 000–8000 years ago (Bailey & Smith 1981). The Lake Huron samples placed intermediate in genetic divergence values between the upper and lower Great Lakes populations, corresponding to their geography, as well as their historical traces of linkage and separation. Notably, the early Lake Huron and Erie basins were connected to the Wabash-Ohio-Mississippi River drainage about 14 000 years ago; and subsequently the basins gradually separated 11 000–6000 years ago (Bailey & Smith 1981). Our data reveal a pronounced genetic break between upper and lower Great Lakes populations of smallmouth bass below Lake St. Clair, reflecting historical isolation patterns (Bolsenga & Herdendorf 1993), as well as a very divergent composition likely due to Lake St. Clair's small size, severe modern-day anthropogenic habitat disturbance, and genetic drift.

Most broad-scale genetic relationships among the Great Lakes and their connected tributaries followed a rough isolation by geographical distance pattern. Smallmouth bass from St. Lawrence River drainage were genetically less distant from those in the lower Great Lakes, as would be expected by their geographical proximity. Populations from Lakes Erie and Ontario shared closest genetic affinity, presumably reflecting common ancestral contribution from an easterly refugium pathway (Underhill 1986). Walleye showed similar clinal variation in mtDNA across Lakes St. Clair, Erie, and Ontario (Stepien & Faber 1998), suggesting common ancestral colonization patterns shared with smallmouth bass populations.

Genetic variation and fine-scale patterns: an interplay of geography and behaviour

A recent study by our laboratory (Strange & Stepien 2007) of Lake Erie walleye *Sander vitreus* used 10 microsatellite loci to test divergences among all 10 major spawning site locations; finding mean heterozygosity levels of 0.704 for 474 individuals. This value is considerably higher than the 0.464 value discerned here for smallmouth bass in Lake Erie (Table 1). Comparison of divergence levels between Lake St. Clair and Lake Erie overall for both species shows considerably greater divergence levels for smallmouth bass ($\theta_{ST} = 0.184$, $P < 0.000001$; this paper) than for walleye ($\theta_{ST} = 0.036$, ranging from 0.006 to 0.051; $P < 0.0001$). As in smallmouth bass, most eastern basin spawning locations for walleye were markedly divergent from both nearby spawning groups, as well as those located across the lake. Unlike smallmouth bass, genetic relationships among spawning groups of walleye did not correspond to an isolation-by-geographical-distance pattern (Strange & Stepien 2007). In contrast to smallmouth bass, walleye return to their natal river or reef sites to spawn, and then mix in lake populations during the remainder of the year (Jennings *et al*. 1996; Stepien & Faber 1998). By comparison, smallmouth bass display high site fidelity throughout the year as well as throughout their lives (Scott & Crossman 1973; Rejwan *et al*. 1997).

Fine-scale divergence levels between pairs of smallmouth bass spawning population samples across Lake Erie are similar to those obtained with microsatellites in naturally occurring anadromous brown trout (*Salmo trutta*) population samples among five rivers across Denmark at a roughly similar spatial scale (average $F_{ST} = 0.037$, and ranging from 0.010 to 0.071 over geographical distances of about 150 km; Hansen *et al*. 2002). In contrast to that study, we found that smallmouth bass populations markedly diverge among rivers separated only by a few kilometres, demonstrating strong isolation and little migration. Similar to the pronounced divergences we documented between river and lake populations of smallmouth bass, Reusch *et al*. (2001) described great divergence between proximate lake and river populations of the stickleback *Gasterosteus aculeatus* in Germany. This pattern of greater differentiation in riverine vs. lacustrine populations merits further investigation in smallmouth bass, as well as in other fishes.

Present-day Lake Erie is isolated from the other Great Lakes, as shown by the pronounced genetic differences among fish from these lakes in the present analysis. Similar long-term divergence has been determined in walleye from Lake St. Clair vs. Lake Erie (Todd & Haas 1993; Stepien

& Faber 1998; Strange & Stepien 2007). This may be due to the preponderance of alleles descendent from a more westerly Mississippi glacial refugium pathway predominant in Lake St. Clair and western Lake Erie, vs. those descendent from the east in Lake Ontario and eastern Lake Erie (see Stepien & Faber 1998). It is possible that this easterly post-glacial colonization pathway may have been along the Susquehanna River to Lake Ontario (see Bailey & Smith 1981; Burr & Page 1986; Underhill 1986). Clinal variation in allelic frequencies as shown in Fig. 4 can result from gene flow between partially isolated populations that have diverged via drift or from admixture between two or more genetically differentiated founding populations (see Storz 2002), as likely occurred across Lake Erie. Similar west-east gradients in allelic frequencies across Lake Erie have been documented for brown bullhead (Murdoch & Hebert 1997) and yellow perch (Ford & Stepien 2004) populations.

Lake Erie today commonly is regarded as being divided into three physiographic basins (Fig. 1b); however, genetic divergence patterns in smallmouth bass support appreciable separation of only the eastern basin from the central and western basins. This pattern for smallmouth bass appears similar to findings for walleye (Stepien & Faber 1998; Strange & Stepien 2007) and yellow perch (Ford & Stepien 2004; Sepulveda-Villet, Ford, & Stepien, in preparation). Historically, the eastern basin of Lake Erie is older, at which time (~10 000–7000 years ago) the west was a series of rivers (Fig. 1b; Bolsenga & Herdendorf 1993; Holcombe *et al.* 2003). At that time, the eastern Lake Erie basin was connected by a narrow river to the central basin, restricting genetic exchange. The signature of this genetic restriction apparently has persisted to the present day, and likely is behaviourally maintained by site fidelity. Smallmouth bass evolved in rivers and probably retained philopatric behavioural specificity to areas even as the lake basins deepened over the past several thousand years (Fig. 1b). Greater genetic divergence among spawning groups in the eastern basin of Lake Erie may be due to a longer evolutionary history at those particular spawning localities, as well as more pronounced philopatry. The genetic divergence of the Long Point Bay, ON spawning population along the northeastern shore was enhanced by a paucity of suitable spawning habitat to the west and south. During the transition of western Lake Erie from a riverine to a lacustrine system about 5000 years ago, population areas likely exchanged genes, resulting in the low or absent genetic differentiation observed in some adjacent sites in western and central Lake Erie.

River locations for smallmouth bass display greater genetic distinctiveness both from other tributary sites as well as from lake samples, denoting high site-specific fidelity and little migration. Based on smaller sample sizes than used here, preliminary mtDNA cytochrome *b* sequence data also show much greater divergences of spawning groups in rivers in the central Lake Erie region than in Lake Erie proper (Borden & Stepien, unpublished). Smallmouth bass groups thus likely maintain high site fidelity from generation to generation and seldom reproduce with individuals from other locations.

Genetic patterns in relation to behaviour and conservation

Tagging data for smallmouth bass have implicated homing (Ridgway & Shuter 1996; Hodgson *et al*. 1998; Lyons & Kanehl 2002) and nest locations remain stable from year-to-year (Rejwan *et al*. 1997), suggesting high potential for genetic structure of spawning populations, which is supported by our results. When adults return to the close proximity of natal sites to spawn, the genetic differentiation among closely spaced populations will increase over time thereby producing fine-scale divergences as observed in this study. Fine-scale differences did not show significant correspondence to isolation by geographical distance, and were often great in magnitude between closely spaced locations, especially among rivers. This indicates the importance of behavioural reproductive barriers — even in the absence of present-day vicariant geographical ones. In order to conserve the genetic diversity of smallmouth bass, it is especially important to prohibit exploitation during the spawning season, as has been enacted in Ohio (Ohio Division of Wildlife 2006). This appears particularly essential in riverine habitats, which do not mix with larger lake populations. In Lake Erie, lacustrine sites in the eastern basin house unique populations, which also is characteristic of other species (Stepien & Faber 1998) and is likely a historical factor related to the greater age of the system (Fig. 1b).

In summary, we discerned considerable geographical population structure across the formerly glaciated northern range of the smallmouth bass, whose broad-scale relationships reflect historical patterns stemming from colonization pathways from glacial refugia and drainage divides. Fine-scale differences among closely spaced sites appear to have resulted from low migration and a tendency for behavioural site fidelity throughout the life history. Despite connectivity and migration opportunity, geographical proximate sites often showed marked divergences, especially among riverine populations. Smallmouth bass spawning within lakes tended to have somewhat higher migration among sites, and their genetic differences more closely corresponded to geographical distances. Further studies of fine-scale patterning within riverine systems and among closely spaced lacustrine nesting locations thus would be especially useful for coupling behavioural effects with population genetic relationships. In conclusion, the present study provides a high-resolution, low cost, and widely applicable comparative DNA database useful for delineating essential areas for the maintenance of genetic diversity and divergence patterns in smallmouth bass, as well as for evaluating range expansions and invasive populations.

Acknowledgements

This work originated with a request to us from the Lake Erie Committee of the Great Lakes Fishery Commission to investigate the molecular genetics basis of stock structure in smallmouth bass. The project was supported by grants from NOAA Ohio Sea Grant R/LR-5 and R/LR-9 PD, NSF #DBI-0243878 (river field collections with Research Experiences for Undergraduate students), and the USEPA CR-83281401. The Ohio Division of Wildlife of the Department of Natural Resources (especially Roger Knight, Jeffrey Tyson, Gene Emond, Kevin Kayle, John Deller, Carey Knight, Timothy Bader, John Gellar, Mike Greenlee, Christopher Vandergoot, and Ann Marie Gorman), Ohio Environmental Protection Agency (William Zawiski and Steven Tuckerman), New York State Department of Environmental Conservation (Donald Einhouse, Webster Pearsall, and Matthew Sanderson), the Lake Erie Management Unit of the Ontario Ministry of Natural Resources (Lawrence Witzel, Andrew Cook, Elizabeth Wright, and David Gonder), Michigan Department of Natural Resources (Mike Thomas and Robert Haas), Indiana State University (Ryan Butryn and Rusty Gonser), Ohio State University (Roy Stein and Geoffrey Steinhart), the US Geological Survey (James Williams, David Neeley, and Robert Robins), Minnesota Department of Natural Resources (Konrad Schmidt and John Lindgren), and Kleinschmidt USA (Brandon Kulik) generously provided samples. Data collection for a preliminary pilot study was aided by Great Lakes Genetics Laboratory technician Clifford Taylor and graduate student W. Calvin Borden. We thank James Coss of the Lake Erie Center for G.I.S. support and the maps, Patricia Uzmann for grant accounting and ordering, and Matthew Neilson and Rachel Lohner for reference formatting, data analysis assistance, and proofreading. The manuscript benefited substantially from discussions with Great Lakes glacial geologist Timothy Fisher (University of Toledo) and with population geneticist Christopher Wilson (University of Trent) about the program STRUCTURE. Smallmouth bass behavioural biologists Geoffrey Steinhart (Cornell University) and Daniel Wiegmann (Bowling Green State University) addressed the question of whether mating is assortative (the evidence suggests yes), raised by a reviewer.

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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 native Great Lakes fishes and understanding nonindigenous species invasions. Douglas Murphy is the laboratory's DNA technician. Rex Strange was a postdoctoral research associate in the laboratory during this study, and began an assistant professorship at Southern Indiana University in fall 2006 where he studies evolutionary questions in fish populations. The Great Lakes Genetics Laboratory is continuing to investigate the evolutionary genetics of smallmouth bass and other members of the genus *Micropterus* — as well as walleye *Sander vitreus* and yellow perch *Perca flavescens* — using microsatellite loci and mitochondrial DNA sequence variation. Further information and updates are available at http://lakeerie.utoledo.edu. This is publication 2006-12 from the Lake Erie Science Center.
