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ARTICLE

# Genetic Structure of Smallmouth Bass in the Lake Michigan and Upper Mississippi River Drainages Relates to Habitat, Distance, and Drainage Boundaries

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***Abstract***

**Analysis of genetic connectivity helps to define stock boundaries and provides information on interpopulation dynamics, such as migration and spawning site fidelity. We used 16 microsatellite loci to describe the genetic population structure of 1,215 Smallmouth Bass *Micropterus dolomieu* from 32 sites throughout the upper Mississippi River and Lake Michigan watersheds. We found that Smallmouth Bass populations formed two genetically distinct units separated by the Mississippi River–Lake Michigan drainage boundary. Smallmouth Bass from the Lake Michigan drainage could be parsimoniously grouped into two or six genetically distinct units that largely corresponded with either river or lake habitats, while fish from the Mississippi River drainage grouped into two, six, or nine genetic units that were mostly associated with watershed boundaries. In the Lake Michigan and Mississippi River drainages, relative migration was limited between lake and river sites, suggesting that gene flow between neighboring sites with different habitat attributes can be low. Our research provides a higher-resolution assessment of Smallmouth Bass genetic structure in a core portion of the species' range and provides strong evidence that Smallmouth Bass populations are structured at small spatial scales that are potentially associated with habitat type. These results demonstrate the importance of evaluating genetic structure at small spatial scales and adopting management strategies that preserve genetic diversity of black bass populations at both the watershed level and the habitat level.**

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Evaluating the spatial structure and genetic connectivity of fish species helps to identify population boundaries, understand interpopulation migration, estimate spawning site fidelity, and understand the genetic impacts of stocking (Allendorf et al. 1987). Therefore, population genetic studies are the foundation of management and conservation plans for many species (Waples et al. 2008; Dahle et al. 2018). Robust genetic analyses are particularly vital for recreationally important freshwater species, as these species are often stocked and can be transported far from their location of capture before their release by anglers (Wilde 2003; Araki and Schmid 2010). These processes (most notably stocking) can result in the erosion of genetic differences and the potential loss of adaptive diversity when done without knowledge of spatial population structure (Araki and Schmid 2010; Long et al. 2015; Wilson et al. 2016). Genetic information unfortunately still does not exist for many inland sport fishes, and where the data exist, the spatial scales analyzed are too large to accurately inform local management.

Black bass *Micropterus* spp. are among the most common inland sport fishes in North America and play an important role in the ecology of many freshwater systems around the world (Power et al. 1985; Werner and Hall 1988; Long et al. 2015). The popularity of black bass as sport fish has led to widespread introductions throughout much of the remainder of the United States and worldwide (Loppnow et al. 2013; Diedericks et al. 2018). Much of this stocking was done without consideration of genetic stock structure, and many regions inhabited by black bass still lack detailed population genetic information. Presently, there are 19 forms of black bass recognized in North America, 17 of which have been described as unique species (Taylor et al. 2019). However, the conservation of genetic diversity in black bass has only recently become a research priority, and the lack of genetic considerations during stocking has resulted in widespread mixing and hybridization among distinct black bass species and populations (Taylor et al. 2018a, 2019; Hargrove et al. 2019).

Throughout North America, native populations of black bass often have strong spatial genetic structure even across small spatial scales. For example, riverine populations of Smallmouth Bass *M. dolomieu* exhibit strong genetic divergence both between neighboring drainages (Stark and Echelle 2004) and within the same drainage (Coughlin et al. 2005). Even species that are known to migrate long distances (>200 km; e.g., Shoal Bass *M. cataractae*) have considerable genetic structure both within and among river drainages (Sammons 2015; Taylor et al. 2018b). The strong degree of genetic structure in black bass populations can have substantial management implications. For example, the incomplete understanding of genetic differences between Florida Bass *M. salmoides floridanus* and Largemouth Bass *M. salmoides salmoides* has resulted in the widespread introgression of Largemouth Bass with Florida Bass (Barthel et al. 2011). Understanding the causes and consequences of spatial genetic structure in black bass populations can therefore help to guide black bass research and management.

For some species of black bass, strong spatial genetic structure is likely the result of a low dispersal rate and high site fidelity. Age-0 Smallmouth Bass often disperse less than 1 km from where they hatch (Ridgway et al. 2002), and adult males may return to within 20 m of their previous year's nest site when spawning (Ridgway et al. 1991). Even in large, open systems, such as the Laurentian Great Lakes, black bass have small home ranges and are generally observed to remain within 5 km of where they are tagged (Savitz and Treat 2007; Kaemingk et al. 2011). Movements greater than 10 km are rare but have been observed more frequently in river populations (Langhurst and Schoenike 1990; Bunt et al. 2002). Given that many species of fish in both the Great Lakes and large river systems consistently move hundreds or thousands of kilometers over the course of their lifetime (Auer 1999; Adlerstein et al. 2008), even the long-distance movements of black bass are short in comparison.

The Smallmouth Bass is one of the most common species of black bass in North America. The native range of the Smallmouth Bass is centered around the Laurentian Great Lakes but extends as far south as Alabama. Across their range, Smallmouth Bass form several genetically distinct lineages associated with postglacial recolonization (Stepien et al. 2007, 2017). However, Smallmouth Bass populations are also structured at much smaller scales related to environmental factors, such as geographic distance (isolation by distance [IBD]) or barriers to movement (Taylor et al. 2018a), and possibly form discrete genetic populations associated with nesting site fidelity and habitat preference for rivers versus lakes (Barthel et al. 2008; Borden 2008). Unfortunately, both previous large population genetic studies of Smallmouth Bass in the Upper Midwest lacked samples from a large portion of the species' native range surrounding Lake Michigan and northern Mississippi River tributaries (Stepien et al. 2007, 2017). These studies also focused on large spatial patterns and populations and therefore had broad site definitions, such as the main stem of the Mississippi and Hudson rivers, and often included a single site from each major population. Therefore, almost nothing is known about the genetic substructuring of Smallmouth Bass in a large portion of their native range or at spatial scales smaller than hundreds to thousands of kilometers.

To resolve this gap in the understanding of Smallmouth Bass population genetics, we focused our study on the poorly described region of the species' native range in the upper Mississippi River and Great Lakes drainages.

Because Smallmouth Bass often move only short distances, we evaluated Smallmouth Bass populations in close proximity to each other to resolve patterns of genetic structure at a scale of tens to hundreds of kilometers. We first described the broad-scale patterns of genetic structure in Smallmouth Bass sampled throughout the study region; we then investigated genetic patterns at smaller scales within the upper Mississippi River and Lake Michigan drainages. We identified multiple discrete genetic clusters of Smallmouth Bass and hypothesized that nesting site fidelity and differences in habitat preference for rivers versus lakes may be driving population differentiation at small spatial scales. Our study has broad implications for both research and management by indicating that the small home ranges and nesting site fidelity of Smallmouth Bass and similar black bass species, can lead to strong genetic discontinuity in otherwise physically unfragmented black bass populations.

## METHODS

We sampled 1,223 bass from 32 sites throughout the upper Mississippi River and Lake Michigan drainages following Michigan Department of Natural Resources and Wisconsin Department of Natural Resources animal use protocols (Table 1; Figure 1). Fin clips were collected from adult fish that were captured using electrofishing or trapnetting between 2014 and 2018, primarily during the spring (April–June) but also during the summer and fall (Table 1). Sample sites in the Mississippi River drainage were chosen to maximize the number of sampled watersheds, while sample sites in the Lake Michigan drainage were chosen to maximize habitat diversity (i.e., a combination of shallow bays and tributaries). In both drainages, some sites were selected that were within close proximity to best evaluate fine-scale genetic structure (Figure 1). Fish were sampled from both riverine and lake habitats; all lake sites were naturally formed lakes other than Lake Wisconsin (site APS1), which is an impoundment. All samples were preserved in an ethanol solution greater than 95% until DNA extraction with a Promega Wizard Genomic DNA purification kit (Promega Corp., Madison, Wisconsin).

Purified genomic DNA was normalized to a final concentration of 20 ng/μL for PCR amplification of 16 microsatellite loci previously developed for Smallmouth Bass (Malloy et al. 2000), Largemouth Bass (Seyoum et al. 2013), or Bluegill *Lepomis macrochirus* (Colbourne et al. 2011). All loci that were developed for species other than Smallmouth Bass were first tested to ensure cross amplification in Smallmouth Bass before being included in the panel. Amplification of the 16-microsatellite panel was conducted using a suite of multiplex reactions with the same annealing temperatures. The PCR amplicons were separated and sized using an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, California) at the Molecular Conservation Genetics Laboratory, University of Wisconsin–Stevens Point. Genotypes for each fish were scored using GeneMapper version 4.0 and were confirmed by at least one additional scorer. To prevent bias associated with missing data, any individual that was missing genotypes at eight or more loci was re-genotyped; if the individual was still missing at least 30% of its genotypes, it was removed from further analysis.

To ensure that genotypes were unbiased by null alleles or genotyping errors, loci were evaluated for conformance to Hardy–Weinberg equilibrium (HWE) expectations within each site using exact testing implemented with the basicStats() function of the package diveRsity version 1.9.90 in R version 3.3.3 (Keenan et al. 2013; R Core Team 2015). All *P*-values were adjusted using a Bonferroni correction, and any significant deviations from HWE were investigated further to check for signs that the significant deviation was the result of scoring errors or null alleles. One major sign that a marker is being mis-scored is a consistent deviation from HWE expectations at all (or many) sampled sites. Therefore, any loci that were significantly out of HWE at more than 50% of sites were removed from analysis. Loci that deviated from HWE expectations at less than 50% of sites were re-assessed in GeneMapper to confirm that genotypes were called correctly and that there were no signs of scoring issues. If no evidence of scoring errors was identified (i.e., low peak height, excessive stutter, etc.), the genotype data for that locus were included in analysis. Genetic diversity at each locus and for each site was also estimated in the diveRsity package. To summarize genetic diversity, we calculated observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), the inbreeding coefficient ($F_{IS}$), and allelic richness ($A_R$) scaled to the smallest population, with rarefaction used to account for differences in sample size. Next, we compared average $A_R$ and $H_O$ among populations by using a two-way ANOVA with drainage and habitat type (lake versus river) as principal factors. Relatedness among individuals was estimated using the Wang relatedness estimator deployed in the R package "related" (Pew et al. 2015). However, all individuals were retained in downstream analysis regardless of relationship status, since removing individuals when the criteria for a random sample have been met (as we believe they have in our case) can result in an increase rather than a decrease in bias (Waples and Anderson 2017). Differences between average within- and among-site diversity were evaluated with a one-way ANOVA using within- versus among-site comparison designation as the principal factor.

To evaluate both broad- and fine-scale genetic structure within our study region, we chose to evaluate population structure hierarchically. First, all sites were evaluated

TABLE 1. Summary of sampling information and diversity estimates for Smallmouth Bass at 32 sites in the Lake Michigan and upper Mississippi River drainages ($N$ = individuals genotyped; $A_R$ = allelic richness estimated with rarefaction; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; $F_{IS}$ = inbreeding coefficient; $P_A$ = number of private alleles).

| Site | Region | Habitat | Subdrainage | Sample month(s) | Sample year(s) | $N$ | $A_R$ | $H_O$ | $H_E$ | $F_{IS}$ | $P_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lake Michigan drainage** | | | | | | | | | | | |
| LBDN | Little Bay De Noc | Lake | Fishdam–Sturgeon rivers | Sep | 2017 | 11 | 3.62 | 0.46 | 0.46 | −0.002 | 0 |
| BBDN | Big Bay De Noc | Lake | Fishdam–Sturgeon rivers | Sep | 2017 | 30 | 3.23 | 0.43 | 0.42 | −0.012 | 0 |
| BOAR | Boardman River | River | Boardman–Charlevoix rivers | May, Jul | 2017, 2018 | 35 | 3.11 | 0.36 | 0.37 | 0.032 | 2 |
| CEDR | Cedar River | River | Cedar River | Apr, May | 2018 | 57 | 3.35 | 0.45 | 0.45 | 0.002 | 3 |
| EPFC | Ephraim | Lake | Door–Kewaunee rivers | Apr, May | 2017 | 40 | 3.21 | 0.44 | 0.45 | 0.014 | 0 |
| FOXR | Fox River | River | Lower Fox River | May | 2017 | 29 | 3.07 | 0.43 | 0.41 | −0.037 | 0 |
| LSTB | Sturgeon Bay | Lake | Door–Kewaunee rivers | Apr, May | 2017 | 30 | 3.49 | 0.47 | 0.48 | 0.017 | 0 |
| MANI | Manistique | Lake | Fishdam–Sturgeon rivers | Sep | 2018 | 13 | 3.06 | 0.47 | 0.42 | −0.103 | 1 |
| MENA | Upper Menominee River | River | Menominee River | Jun | 2016 | 50 | 3.91 | 0.52 | 0.53 | 0.034 | 3 |
| MENB | Lower Menominee River | River | Menominee River | May | 2017 | 50 | 3.86 | 0.49 | 0.50 | 0.049 | 1 |
| MILR | Milwaukee River | River | Milwaukee River | Mar, Apr | 2018 | 31 | 4.04 | 0.49 | 0.53 | 0.081 | 3 |
| NBLM | North Bay | Lake | Door–Kewaunee rivers | May | 2016 | 36 | 2.89 | 0.37 | 0.39 | 0.093 | 0 |
| OCON | Oconto River | River | Oconto River | Oct | 2017 | 20 | 3.88 | 0.57 | 0.53 | −0.062 | 0 |
| PESH | Peshtigo River | River | Peshtigo River | Jul, Aug, Oct | 2017 | 17 | 3.92 | 0.53 | 0.54 | 0.033 | 0 |
| ROWB | Rowleys Bay | Lake | Door–Kewaunee rivers | May | 2016 | 50 | 2.86 | 0.40 | 0.40 | 0.006 | 0 |
| SAWH | Sawyer Harbor | Lake | Door–Kewaunee rivers | Apr, May | 2017 | 29 | 3.44 | 0.47 | 0.47 | 0.006 | 1 |
| SHEB | Sheboygan River | River | Manitowoc–Sheboygan rivers | Aug | 2018 | 38 | 4.42 | 0.55 | 0.55 | 0.009 | 2 |
| WASH | Washington Island | Lake | Door–Kewaunee rivers | Jun | 2014 | 50 | 2.96 | 0.41 | 0.42 | 0.008 | 0 |
| WTWI | West Twin River | River | Manitowoc–Sheboygan rivers | Jul | 2018 | 20 | 3.22 | 0.37 | 0.43 | 0.178 | 3 |
| **Upper Mississippi River drainage** | | | | | | | | | | | |
| APS1 | Lake Wisconsin | Lake | Castle Rock | Sep | 2015 | 50 | 3.56 | 0.49 | 0.48 | 0.018 | 4 |
| APS2 | Lower Wisconsin River | River | Wisconsin River | Aug, Sep | 2016 | 50 | 3.48 | 0.47 | 0.48 | 0.014 | 0 |
| BPS1 | Lower Wisconsin River | River | Lower Wisconsin River | Oct | 2015 | 50 | 3.76 | 0.47 | 0.49 | 0.035 | 3 |
| BPS2 | Lower Wisconsin River | River | Lower Wisconsin River | Oct | 2015 | 49 | 3.71 | 0.44 | 0.47 | 0.079 | 0 |
| CHCF | Chippewa River | River | Lower Chippewa River | Apr, May | 2017 | 50 | 3.30 | 0.47 | 0.47 | −0.004 | 1 |
| GALR | Galena River | River | Apple–Plum rivers | Aug | 2017 | 49 | 3.37 | 0.47 | 0.48 | −0.003 | 0 |
| LKEG | Lake Kegonsa | Lake | Lower Rock River | May | 2017 | 31 | 2.54 | 0.31 | 0.32 | 0.033 | 1 |
| LWAU | Lake Waubesa | Lake | Lower Rock River | Apr | 2017 | 19 | 2.84 | 0.36 | 0.36 | −0.023 | 0 |
| LWIR | Lower Wisconsin River | River | Lower Wisconsin River | Aug | 2017 | 47 | 3.90 | 0.47 | 0.47 | −0.002 | 1 |
| MINE | Pecatonica River | River | Pecatonica River | Aug | 2017 | 46 | 3.09 | 0.41 | 0.42 | 0.037 | 2 |
| NEBL | Nebish Lake | Lake | Flambeau River | May | 2016 | 38 | 2.50 | 0.36 | 0.37 | 0.016 | 1 |
| PALL | Pallette Lake | Lake | Flambeau River | May | 2017 | 50 | 1.69 | 0.16 | 0.16 | 0.012 | 0 |
| USHF | Illinois–Fox River | River | Upper Fox River | Aug | 2018 | 50 | 3.23 | 0.40 | 0.40 | −0.018 | 0 |

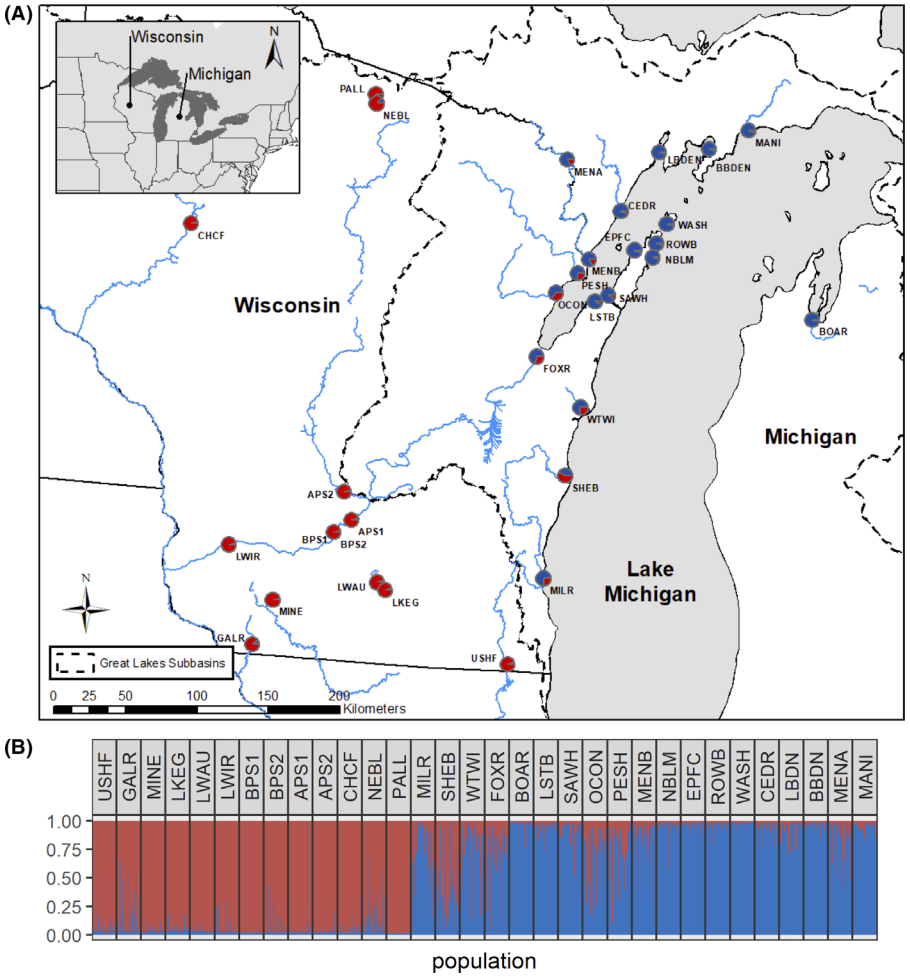


FIGURE 1. **(A)** Locations where Smallmouth Bass samples were collected throughout the Wisconsin region of the upper Mississippi River and Lake Michigan drainages (site codes are defined in Table 1) and **(B)** results of the most likely number of unique genetic clusters ($K = 2$ clusters) identified using Bayesian STRUCTURE analysis. Sample sites are marked with pie charts representing the average cluster membership determined in STRUCTURE for all individuals at that site based on $K = 2$, and colors correspond to the bar plot in panel B. The colors in the STRUCTURE bar plot represent the proportion of cluster membership (admixture) for each individual based on $K = 2$. Each bar represents a single individual, with the dominant color representing the most likely cluster ancestry for that individual. [Color figure can be viewed at afsjournals.org.]

together to estimate the global pattern of population structure. Next, population structure was evaluated separately for the Mississippi River and Lake Michigan drainages, which were highly differentiated during global analysis (see Results). Pairwise genetic distances ($F_{ST}$) among all sites were estimated using the fastDiv() function in the diveRsity package (Weir and Cockerham 1984). Significance was determined with bootstrapped 95% CIs whereby a pairwise comparison was considered significant if the 95% CI did not include zero. There is currently no feasible connection between the Mississippi River and Lake Michigan drainages that could be used by Smallmouth Bass; therefore, we do not report global signatures of IBD. However, IBD was evaluated separately for the Mississippi River and Lake Michigan drainages using Mantel tests conducted in the R package ADEGENET (Jombart 2008). Pairwise $F_{ST}$ was used as the estimate of genetic distance, and the shortest distance by water was used as the estimate of geographic distance. To test whether genetic variance was significantly partitioned by drainage and habitat type, we used analysis of molecular variance (AMOVA) implemented in Arlequin (Excoffier and Lischer 2010). We first tested how variance was distributed among drainages by grouping sites into either the Mississippi River drainage or Lake Michigan drainage and evaluating the amount of variation explained by drainage. We then hierarchically analyzed the effect of habitat on genetic variance by grouping sites based on whether they were in a river or in a lake. The variance explained by habitat was estimated by conducting three separate AMOVAs: first for the total data set (14 lake sites and 18 river sites) and subsequently for sites in the Mississippi River

drainage (5 lake sites and 8 river sites) and Lake Michigan drainage (9 lake sites and 10 river sites) separately.

To identify genetic structure without a priori assumptions of connectivity, we used the program STRUCTURE (Pritchard et al. 2000) deployed through Structure_threader to enable parallel evaluation of different values of $K$ (number of genetic clusters; Pina-Martins et al. 2017). STRUCTURE was run hierarchically, first on the global data set and then again for sites within each major drainage. Running STRUCTURE hierarchically is an important step in the analysis because population structure may be hierarchical, with subtle subdivisions within diverged groups (Lawson et al. 2018). Each data set was examined separately through five replicate runs, each with a burn-in of 50,000 cycles followed by 200,000 replicates and a $K$-value of 1 to $N-1$, where $N$ is the number of sample sites. All STRUCTURE runs primarily used default settings, which, in short, (1) did not use location as a prior, (2) used an admixture model and assumed that alleles were correlated within sites, and (3) assumed no linkage disequilibrium. Replicate runs of each $K$ were combined using CLUMPAK (Kopelman et al. 2015) and were visualized in R. The optimal number of clusters was chosen using the $\Delta K$ method of Evanno et al. (2005) implemented in Structure_threader, which in turn uses STRUCTURE Harvester to estimate and calculate average $\Delta K$ across all replicates of each tested $K$-value (Earl and vonHoldt 2012). Both methods use dips or peaks in the test statistic to indicate a value or multiple values of $K$ that best describe the variation in allele frequencies while accounting for model overfitting. The assumption that there is a single "true" value of $K$ is incorrect and can be misleading; therefore, multiple values of $K$ associated with $\Delta K$ peaks are considered and discussed (Lawson et al. 2018).

Although black bass intermix throughout the year, nesting site fidelity may limit the amount of migration (defined as movement to a novel site and successfully spawning) between sites. To determine whether Smallmouth Bass, at certain sites, displayed more genetic connectivity than those at other sites, relative migration between all site pairs and evidence of asymmetrical migration were measured with the divMigrate() function in the diveRsity package within R. Jost's $D$ (Jost et al. 2018) was used as the genetic distance estimate, as it is a better estimator of allelic differentiation for fast-mutating markers like microsatellites. A full description of how migration is estimated is provided by Keenan et al. (2013) and Sundqvist et al. (2016). In short, this method works by generating a hypothetical pool of migrants for a given pair of sites and then estimates a measure of genetic differentiation between each site and the hypothetical pool. The estimated directional genetic differentiation can then be used to estimate relative levels of migration, or a proportion of migrants, where 1 indicates complete connectivity (high migration) between two sites and 0 indicates no connectivity (no migration). By estimating relative migration, we were able to determine whether Smallmouth Bass in certain regions or habitats exhibited higher rates of migration than others and investigate source–sink population dynamics.

## RESULTS

### Genetic Diversity

After re-genotyping, eight individuals did not meet our genotyping rate threshold and were removed. The resulting data set contained 1,215 individuals, with an overall successful genotype amplification and scoring rate of 98% (i.e., only 2% of genotype calls were missing across all individuals and loci). No locus deviated from HWE expectations at more than 4 of 32 sites ($\alpha = 0.05$) or more than 2 of 32 sites after Bonferroni correction (Table S1 available in the Supplement in the online version of this article). Upon re-evaluation of genotype scores, we found no evidence of misidentified genotypes. We concluded that genotype calls were accurate and that those sites deviated from HWE expectations for reasons other than laboratory error; therefore, the loci were retained for further analysis. A single site (APS1) deviated from HWE at 9 of 12 loci prior to Bonferroni correction ($\alpha = 0.05$); however, this number dropped to just two loci after correction for multiple comparisons. Genotype scores were again assessed for accuracy, and all evidence suggested that the observed divergence from HWE was likely due to reasons other than laboratory error; thus, all loci and all individuals were included in the analysis. All loci had moderate to high levels of polymorphism, ranging from 3 to 55 alleles, and $H_O$ ranged between 0.07 and 0.69 (Table S1). Average $H_O$ was generally similar among 30 of the 32 sites (range = 0.33–0.55; mean = 0.43) but was substantially lower in Pallette Lake (PALL; average $H_O = 0.17$; Table 1). Allelic richness and $H_O$ were slightly lower in the Mississippi River drainage (mean $H_O = 0.41$; mean $A_R = 3.2$) than in the Lake Michigan drainage (mean $H_O = 0.46$; mean $A_R = 3.5$; $H_O$: $F_{1,\ 28} = 3.7$, $P = 0.066$; $A_R$: $F_{1,\ 28} = 4.5$, $P = 0.042$). Values of $A_R$ and $H_O$ were significantly higher at river sites (mean $H_O = 0.46$; mean $A_R = 3.7$) than at lake sites (mean $H_O = 0.40$; mean $A_R = 3.1$; $H_O$: $F_{1,\ 28} = 18.9$, $P = 0.0002$; $A_R$: $F_{1,\ 28} = 8.7$, $P = 0.006$). Individuals within each site showed moderate levels of relatedness (mean = 0.14), and mean relatedness was generally higher within sites than between sites ($F_{1,\ 526} = 106.4$, $P < 0.0001$).

### Global Structure

The pattern of global structure across the entire sample range and all 32 sites appeared to be explained primarily by the Lake Michigan–Mississippi River drainage divide (Figure 1). Although almost all pairwise estimates of $F_{ST}$ were significantly greater than zero (Table 2), many of the

largest estimates of pairwise $F_{ST}$ were between sites in the Lake Michigan and Mississippi River drainages (Figure 2). Additionally, when sites were grouped by drainage, drainage explained 11% of the variation (AMOVA: $F_{RT}$ [regional to total] = 0.103, $P = 0.001$; Table 3). Bayesian STRUCTURE analysis of all 32 sites also suggested that the most probable number of genetic clusters in the global study area was 2, and each cluster aligned almost exactly with the Mississippi River and Lake Michigan drainages. However, a small amount of potential admixture or genetic similarity was observed in some Lake Michigan sites (e.g., Sheboygan River [SHEB] and Fox River [FOXR]) and Mississippi River drainage populations (Figure 1).

### Structure in the Lake Michigan Drainage

When only Lake Michigan sites were evaluated, there was significant evidence of IBD (Mantel test: $P = 0.001$; Figure 2). Although geographic distance did explain some of the variation, there was substantial population substructuring among sites that did not appear to be directly related to geographic distance. The average pairwise $F_{ST}$ among Lake Michigan sites was 0.074 and ranged from 0.004 to 0.194. STRUCTURE indicated that the sites we sampled in Lake Michigan represented at least two but possibly as many six genetic clusters, as indicated by the Evanno $\Delta K$ analysis, which showed clear peaks in likelihood at $K$-values of 2 and 6 (Supplemental Materials available separately online). Both of these $K$-values appeared to primarily partition samples collected in rivers from those collected in the lake (Figure 3). Although most sites contained a high degree of admixture, habitat explained 2% of the variance between river and lake sites ($F_{RT} = 0.016$, $P = 0.001$; Table 4).

Estimates of relative migration confirmed the spatial structure identified with $F_{ST}$ and STRUCTURE analysis. Relative migration was high and mostly symmetrical among sites in close proximity to one another, but it was much lower between sites on the Door Peninsula and sites in rivers less than 30 km across Green Bay (Figure 3C). There was also evidence of differential migration among sites on the Door Peninsula. Relative migration was higher among sites inside Green Bay (Sawyer Harbor [SAWH], Ephraim [EPFC], and Sturgeon Bay [LSTB]) than between sites that were of equal distance apart but on opposites sides of the Door Peninsula (Rowleys Bay [ROWB] and North Bay [NBLM]) or close to the mouth of the bay (Washington Island [WASH]; Figure 3C). Most migration appeared to be symmetrical; when significant asymmetrical migration was identified, it appeared to indicate directional migration from sites of low $A_R$ to sites of high $A_R$, which can be an artifact of the technique used to estimate migration (Figure S2 available in the Supplement in the online version of this article).

### Structure in the Mississippi River Drainage

There was also evidence of IBD among sites in the Mississippi drainage (Mantel test: $P = 0.001$; Figure 2). The relationship between genetic distance and geographic distance was upwardly biased due to the high pairwise $F_{ST}$ values between PALL and other Mississippi River drainage sites ($F_{ST} = 0.300$–$0.479$), including Nebish Lake (NEBL), which is less than 1 km from PALL. When pairwise comparisons with PALL were removed, the IBD relationship was still significant (Mantel test: $P = 0.001$). In addition to geographic distance, much of the genetic variation among sites corresponded to watershed or sample site habitat ($F_{ST}$: mean = 0.136; range = 0.001–0.479). Estimates of pairwise $F_{ST}$ were generally low for sites within the same drainage. For example, sites in the Wisconsin River (APS1; lower Wisconsin River [APS2, BPS1, BPS2, and LWIR]) had relatively low pairwise $F_{ST}$ values compared to those calculated between Wisconsin River sites and the Chippewa River (CHCF), PALL, and NEBL in the north or nearby sites to the south (Pecatonica River [MINE], Galena River [GALR], Lake Waubesa [LWAU], Lake Kegonsa [LKEG], and Illinois–Fox River [USHF]) that are in different watersheds (Table 2). Clustering by watershed was further supported by STRUCTURE and relative migration estimates (Figure 4). STRUCTURE indicated that $K$-values of 2, 6, and 9 were the most likely numbers of clusters for the Mississippi River drainage. When a $K$-value of 2 was evaluated, most of the diversity was partitioned between (1) PALL and NEBL in the north and (2) all other sites. When a $K$-value of 6 or 9 was used, diversity was partitioned by watershed, with each river or drainage containing a unique genetic cluster. Although genetic structure was more strongly associated with watershed, habitat type did still explain 2% of the variance in allele frequency ($F_{RT} = 0.018$, $P = 0.001$; Table 4). The strong influence of watershed on genetic structure was corroborated by low estimates of relative migration among sites that were not directly connected by the same river (Figure 4). Migration rates were high and symmetric throughout the Wisconsin River, and some evidence of recent gene flow was present between the GALR and MINE sites. Relative migration was below 0.2 among all other sites. Significant asymmetrical migration was primarily from sites of low $A_R$ to sites of high $A_R$, therefore indicating that most sites had low but significant asymmetrical migration from PALL (Figure 2).

## DISCUSSION

We detected multiple, discrete genetic subpopulations of Smallmouth Bass isolated broadly by geographic distance and the Mississippi River–Lake Michigan drainage divide and more finely by subwatershed and habitat type (river versus lake). Smallmouth Bass had substantial

TABLE 2. Pairwise genetic differentiation index $F_{ST}$ (Weir and Cockerham 1984) between all sites for Smallmouth Bass sampled in the upper Mississippi River and Lake Michigan drainages. Bold italics denote $F_{ST}$ values with bootstrapped 95% confidence intervals that did not overlap with zero (i.e., the $F_{ST}$ values were considered significant). Site codes are defined in Table 1.

| Site | APS1 | APS2 | LBDN | BBDN | BOAR | BPS1 | BPS2 | CEDR | CHCF | EPFC | FOXR | GALR | LKEG | LSTB | LWAU | LWIR | MANI | MENA | MENB | MILR | MINE | NBLM | NEBL | OCON | PALL | PESH | ROWB | SAWH | SHEB | USHF | WASH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| APS2 | ***0.04*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LBDN | ***0.17*** | ***0.12*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BBDN | ***0.20*** | ***0.17*** | 0.01 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BOAR | ***0.27*** | ***0.22*** | ***0.08*** | ***0.08*** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BPS1 | ***0.01*** | ***0.03*** | ***0.15*** | ***0.19*** | ***0.25*** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BPS2 | 0.00 | ***0.03*** | ***0.15*** | ***0.19*** | ***0.25*** | 0.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| CEDR | ***0.18*** | ***0.14*** | ***0.03*** | ***0.05*** | ***0.12*** | ***0.17*** | ***0.17*** | | | | | | | | | | | | | | | | | | | | | | | | |
| CHCF | ***0.07*** | ***0.07*** | ***0.17*** | ***0.21*** | ***0.26*** | ***0.06*** | ***0.05*** | ***0.18*** | | | | | | | | | | | | | | | | | | | | | | | |
| EPFC | ***0.19*** | ***0.16*** | ***0.04*** | ***0.02*** | ***0.08*** | ***0.17*** | ***0.17*** | ***0.05*** | ***0.19*** | | | | | | | | | | | | | | | | | | | | | | |
| FOXR | ***0.12*** | ***0.10*** | ***0.10*** | ***0.12*** | ***0.19*** | ***0.10*** | ***0.10*** | ***0.08*** | ***0.13*** | ***0.09*** | | | | | | | | | | | | | | | | | | | | | |
| GALR | ***0.06*** | ***0.04*** | ***0.10*** | ***0.15*** | ***0.22*** | ***0.04*** | ***0.05*** | ***0.13*** | ***0.07*** | ***0.15*** | ***0.07*** | | | | | | | | | | | | | | | | | | | | |
| LKEG | ***0.19*** | ***0.13*** | ***0.23*** | ***0.27*** | ***0.34*** | ***0.13*** | ***0.16*** | ***0.22*** | ***0.17*** | ***0.26*** | ***0.19*** | ***0.14*** | | | | | | | | | | | | | | | | | | | |
| LSTB | ***0.16*** | ***0.12*** | ***0.05*** | ***0.06*** | ***0.09*** | ***0.15*** | ***0.15*** | ***0.06*** | ***0.16*** | ***0.02*** | ***0.07*** | ***0.11*** | ***0.25*** | | | | | | | | | | | | | | | | | | |
| LWAU | ***0.11*** | ***0.09*** | ***0.20*** | ***0.23*** | ***0.30*** | ***0.08*** | ***0.09*** | ***0.21*** | ***0.11*** | ***0.22*** | ***0.14*** | ***0.10*** | ***0.11*** | ***0.20*** | | | | | | | | | | | | | | | | | |
| LWIR | ***0.04*** | ***0.02*** | ***0.13*** | ***0.18*** | ***0.24*** | 0.01 | ***0.02*** | ***0.16*** | ***0.05*** | ***0.17*** | ***0.10*** | ***0.03*** | ***0.10*** | ***0.15*** | ***0.06*** | | | | | | | | | | | | | | | | |
| MANI | ***0.18*** | ***0.13*** | 0.04 | ***0.05*** | ***0.10*** | ***0.16*** | ***0.16*** | ***0.06*** | ***0.16*** | ***0.06*** | ***0.11*** | ***0.11*** | ***0.24*** | ***0.05*** | ***0.20*** | ***0.14*** | | | | | | | | | | | | | | | |
| MENA | ***0.15*** | ***0.11*** | ***0.03*** | ***0.06*** | ***0.07*** | ***0.13*** | ***0.14*** | ***0.07*** | ***0.14*** | ***0.06*** | ***0.11*** | ***0.10*** | ***0.19*** | ***0.05*** | ***0.17*** | ***0.12*** | ***0.04*** | | | | | | | | | | | | | | |
| MENB | ***0.12*** | ***0.11*** | ***0.04*** | ***0.06*** | ***0.11*** | ***0.11*** | ***0.10*** | ***0.05*** | ***0.11*** | ***0.04*** | ***0.06*** | ***0.08*** | ***0.19*** | ***0.06*** | ***0.16*** | ***0.10*** | ***0.06*** | ***0.04*** | | | | | | | | | | | | | |
| MILR | ***0.12*** | ***0.10*** | ***0.07*** | ***0.09*** | ***0.11*** | ***0.11*** | ***0.11*** | ***0.08*** | ***0.12*** | ***0.07*** | ***0.08*** | ***0.10*** | ***0.21*** | ***0.05*** | ***0.16*** | ***0.11*** | ***0.06*** | ***0.06*** | ***0.05*** | | | | | | | | | | | | |
| MINE | ***0.06*** | ***0.04*** | ***0.17*** | ***0.21*** | ***0.29*** | ***0.03*** | ***0.04*** | ***0.20*** | ***0.07*** | ***0.20*** | ***0.12*** | ***0.05*** | ***0.14*** | ***0.17*** | ***0.09*** | ***0.02*** | ***0.17*** | ***0.16*** | ***0.14*** | ***0.14*** | | | | | | | | | | | |
| NBLM | ***0.20*** | ***0.18*** | ***0.05*** | ***0.04*** | ***0.08*** | ***0.20*** | ***0.20*** | ***0.04*** | ***0.22*** | ***0.04*** | ***0.12*** | ***0.18*** | ***0.29*** | ***0.07*** | ***0.25*** | ***0.20*** | ***0.06*** | ***0.07*** | ***0.06*** | ***0.09*** | ***0.23*** | | | | | | | | | | |
| NEBL | ***0.13*** | ***0.14*** | ***0.25*** | ***0.28*** | ***0.35*** | ***0.11*** | ***0.12*** | ***0.26*** | ***0.12*** | ***0.25*** | ***0.19*** | ***0.14*** | ***0.24*** | ***0.23*** | ***0.20*** | ***0.13*** | ***0.26*** | ***0.21*** | ***0.19*** | ***0.15*** | ***0.16*** | ***0.30*** | | | | | | | | |
| OCON | ***0.07*** | ***0.07*** | ***0.06*** | ***0.08*** | ***0.14*** | ***0.06*** | ***0.05*** | ***0.07*** | ***0.11*** | ***0.06*** | ***0.07*** | ***0.07*** | ***0.19*** | ***0.08*** | ***0.14*** | ***0.07*** | ***0.08*** | ***0.07*** | ***0.03*** | ***0.06*** | ***0.10*** | ***0.09*** | ***0.18*** | | | | | | | |
| PALL | ***0.36*** | ***0.33*** | ***0.50*** | ***0.49*** | ***0.55*** | ***0.35*** | ***0.36*** | ***0.43*** | ***0.34*** | ***0.47*** | ***0.42*** | ***0.30*** | ***0.48*** | ***0.44*** | ***0.45*** | ***0.36*** | ***0.51*** | ***0.36*** | ***0.40*** | ***0.40*** | ***0.37*** | ***0.52*** | ***0.34*** | ***0.46*** | | | | | | |
| PESH | ***0.09*** | ***0.09*** | ***0.06*** | ***0.09*** | ***0.15*** | ***0.08*** | ***0.08*** | ***0.08*** | ***0.13*** | ***0.06*** | ***0.05*** | ***0.07*** | ***0.22*** | ***0.07*** | ***0.17*** | ***0.09*** | ***0.10*** | ***0.06*** | 0.02 | ***0.05*** | ***0.12*** | ***0.10*** | ***0.18*** | 0.01 | ***0.45*** | | | | | |
| ROWB | ***0.19*** | ***0.17*** | ***0.05*** | ***0.03*** | ***0.09*** | ***0.19*** | ***0.19*** | ***0.05*** | ***0.22*** | ***0.03*** | ***0.11*** | ***0.17*** | ***0.28*** | ***0.07*** | ***0.24*** | ***0.19*** | ***0.07*** | ***0.09*** | ***0.07*** | ***0.10*** | ***0.22*** | 0.00 | ***0.29*** | ***0.08*** | ***0.49*** | ***0.09*** | | | | |
| SAWH | ***0.14*** | ***0.12*** | ***0.03*** | ***0.04*** | ***0.09*** | ***0.13*** | ***0.13*** | ***0.04*** | ***0.16*** | 0.01 | ***0.06*** | ***0.11*** | ***0.23*** | 0.01 | ***0.19*** | ***0.13*** | ***0.05*** | ***0.06*** | ***0.03*** | ***0.05*** | ***0.17*** | ***0.04*** | ***0.21*** | ***0.04*** | ***0.44*** | ***0.05*** | ***0.03*** | | | |
| SHEB | ***0.10*** | ***0.07*** | ***0.06*** | ***0.10*** | ***0.13*** | ***0.07*** | ***0.08*** | ***0.10*** | ***0.11*** | ***0.09*** | ***0.07*** | ***0.07*** | ***0.17*** | ***0.07*** | ***0.14*** | ***0.07*** | ***0.08*** | ***0.06*** | ***0.06*** | ***0.05*** | ***0.11*** | ***0.11*** | ***0.17*** | ***0.03*** | ***0.37*** | ***0.04*** | ***0.12*** | ***0.06*** | | |
| USHF | ***0.10*** | ***0.09*** | ***0.18*** | ***0.21*** | ***0.27*** | ***0.08*** | ***0.08*** | ***0.19*** | ***0.09*** | ***0.19*** | ***0.12*** | ***0.08*** | ***0.16*** | ***0.17*** | ***0.09*** | ***0.06*** | ***0.17*** | ***0.16*** | ***0.12*** | ***0.14*** | ***0.07*** | ***0.23*** | ***0.19*** | ***0.12*** | ***0.42*** | ***0.15*** | ***0.22*** | ***0.17*** | ***0.11*** | |
| WASH | ***0.23*** | ***0.20*** | ***0.04*** | ***0.02*** | ***0.07*** | ***0.21*** | ***0.22*** | ***0.07*** | ***0.24*** | ***0.03*** | ***0.13*** | ***0.18*** | ***0.29*** | ***0.07*** | ***0.26*** | ***0.21*** | ***0.08*** | ***0.08*** | ***0.08*** | ***0.10*** | ***0.24*** | 0.02 | ***0.30*** | ***0.10*** | ***0.48*** | ***0.09*** | ***0.03*** | ***0.05*** | ***0.11*** | ***0.24*** | |
| WTWI | ***0.15*** | ***0.15*** | ***0.16*** | ***0.19*** | ***0.19*** | ***0.14*** | ***0.14*** | ***0.17*** | ***0.14*** | ***0.14*** | ***0.15*** | ***0.14*** | ***0.28*** | ***0.12*** | ***0.21*** | ***0.15*** | ***0.14*** | ***0.12*** | ***0.12*** | ***0.09*** | ***0.19*** | ***0.19*** | ***0.21*** | ***0.11*** | ***0.52*** | ***0.12*** | ***0.18*** | ***0.12*** | ***0.08*** | ***0.18*** | ***0.19*** |

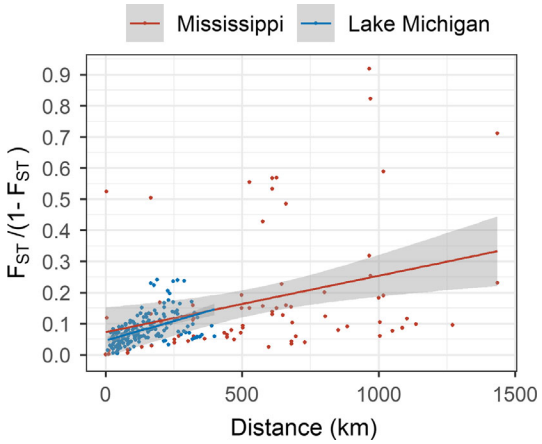


FIGURE 2. Linear regression of Smallmouth Bass genetic distances and waterway geographic distances among all sample sites in the upper Mississippi River drainage (red) and the Lake Michigan drainage (blue). Point colors indicate pairwise comparison type (legend); lines indicate the linear regression, and the gray shaded area represents the 95% CI. [Color figure can be viewed at afsjournals.org.]

genetic structure across the Lake Michigan drainage and at an even smaller scale within Green Bay between the Door Peninsula and Green Bay tributaries. Within the Mississippi River drainage, sites were distinguishable by watershed but may also differ between rivers and lakes (see Ruzich et al. 2019). Our results indicated that there was a large degree of genetic variation among Smallmouth Bass subpopulations and therefore an increased potential for local adaptation.

When all sites were considered, we found a strong genetic boundary associated with the Mississippi River and Great Lakes drainages. Smallmouth Bass collected from the upper Mississippi River were previously found to represent a genetically distinct lineage from the Smallmouth Bass in the Great Lakes (Stepien et al. 2007, 2017). However, those studies collected samples from the main stem of the Mississippi River but did not include samples from tributaries close to the actual watershed boundary. Near the watershed boundary, fish could have easily been transported across watershed lines by anglers, resulting in introgression. Some introgression between the Great Lakes and Mississippi River lineages has likely occurred over the long stocking history of Smallmouth Bass, but the divide between the Great Lakes and Mississippi River drainages is still clearly a strong genetic boundary for Smallmouth Bass and other species (e.g., Wilson and Hebert 2011; Sepulveda-Villet and Stepien 2012).

TABLE 3. Results of four analyses of molecular variance (based on 16 microsatellite loci) describing Smallmouth Bass genetic variance partitioned between drainages (upper Mississippi River and Lake Michigan). Sources of variation include (1) between drainages (referring to variance explained when individuals were grouped into the Mississippi River and Lake Michigan drainages); (2) among sites (referring to variance explained when individuals were grouped by site); and (3) within sites (referring to the remaining variance among individuals within a given site).

| Source of variation | df | Sum of squares | Percentage of variation |
|---|---|---|---|
| Between drainages | 1 | 576.9 | 11 |
| Among sites | 30 | 906.4 | 8 |
| Within sites | 2,398 | 8,223.0 | 81 |

Within each drainage, Smallmouth Bass formed discrete genetic subpopulations that were partitioned by watershed and habitat type in addition to geographic distance. Despite the lack of physical barriers separating sites in the Lake Michigan drainage, Smallmouth Bass formed two and six genetically distinguishable groups, suggesting that structure was hierarchical. In other words, although the strongest delineation among sites appeared to separate the upper Lake Michigan sites from the Milwaukee River (MILR), SHEB, and West Twin River (WTWI) populations (i.e., $K = 2$), this by no means indicates that the sites within the upper Lake Michigan portion of the study area are panmictic; additional genetic structure was observable at a finer scale when sites were divided into six clusters. Genetic differentiation was significant even among sites within close proximity (10–30 km), which suggests that gene flow among neighboring sites can be low. This finding differs from the results of previous research in Lake Erie (Borden and Stepien 2006), where genetic distance was low among Smallmouth Bass collected along the shoreline, leading the authors to conclude that there was likely at least some gene flow among neighboring sites. Despite the low differentiation between sites, Borden and Stepien (2006) found some evidence of IBD, suggesting that Smallmouth Bass migrated along the shoreline but not long distances. However, that study was conducted independently from our own and included a slightly different set of markers, sampling scheme, and analysis, thus hindering an exact comparison. Nonetheless, we did find evidence for IBD at the drainage level, and we even found low but significant pairwise genetic differentiation among neighboring sites along the Door Peninsula, suggesting that sample sites represented independent populations. Furthermore, mean relatedness was higher within sites than between sites, indicating that within the broad genetic clusters identified using STRUCTURE there remain fine-scale differences in allele frequency among sites, suggestive of site fidelity. Therefore, while IBD and relatedness explain some of the variation in allele frequencies among sites, other factors (e.g., habitat) play a role. Borden and Stepien (2006) sampled only shoreline habitat in Lake Erie, but many of our samples were collected in rivers, which can contain Smallmouth Bass that are genetically distinct from Smallmouth Bass in lakes (Borden

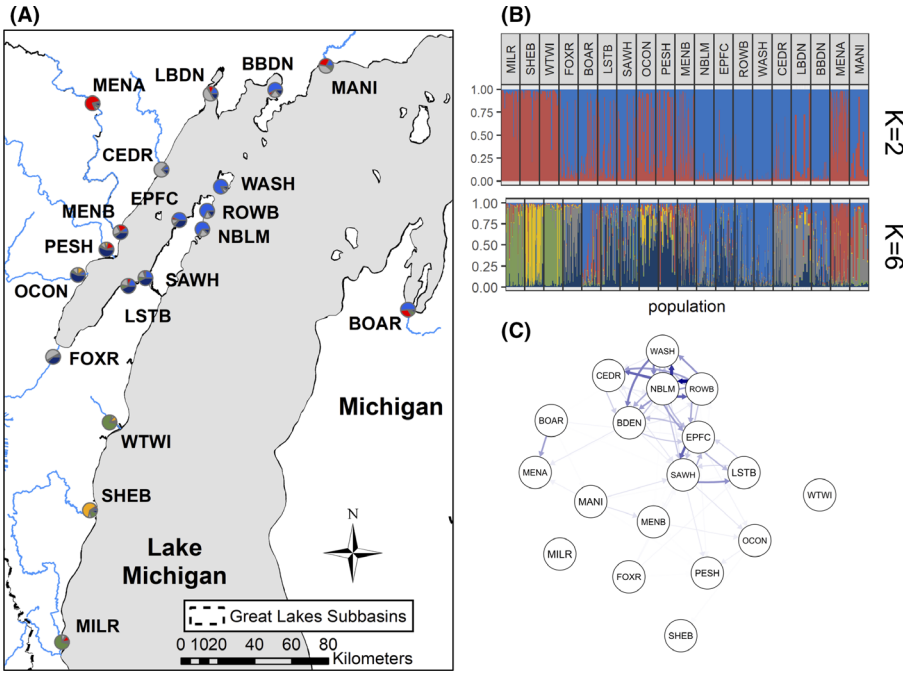


FIGURE 3. Summary of Smallmouth Bass genetic structure identified in the Lake Michigan drainage, including **(A)** a map of Lake Michigan sites (site codes are defined in Table 1); **(B)** bar plots of the most likely number of unique genetic clusters ($K$) identified using Bayesian STRUCTURE analysis; and **(C)** sites with relative migration greater than 0.2, as estimated from Jost's $D$ using methods outlined by Sundqvist et al. (2016). Site locations in panel A are denoted with pie charts of the average cluster membership for fish at each site based on $K = 6$. Each line in the bar plots (panel B) represents the relative admixture from $K$ genetic clusters for a single individual (sample site of origin is indicated at the top of the plot). Relative migration is represented in a network $q$-plot (panel C), with each bubble representing all individuals from a site, and the spatial proximity of each bubble to the other bubbles indicates their relative genetic similarity (i.e., not geographic proximity). Migration between sites is shown as arrows connecting the bubbles; the width and shade of arrows indicate the amount of migration (i.e., darker, thicker arrows indicate more migration). [Color figure can be viewed at afsjournals.org.]

TABLE 4. Results of analyses of molecular variance evaluating the influence of habitat on genetic variance in Smallmouth Bass (SS = sum of squares; PV = percentage of variation). The analysis was run hierarchically—first using all sites and individuals and then within each major drainage—to account for the large amount of variance explained by the Mississippi River–Lake Michigan drainage divide. Sources of variation include (1) between habitats (referring to variance explained when individuals were grouped by lake or river habitat); (2) among sites (referring to variance explained when individuals were grouped by site); and (3) within sites (referring to the remaining variance among individuals within a given site).

| | All sites | | | Lake Michigan | | | Upper Mississippi River | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | SS | PV | df | SS | PV | df | SS | PV |
| Between habitats | 1 | 100.8 | 1 | 1 | 62.3 | 2 | 1 | 68.7 | 2 |
| Among sites | 30 | 1,382.6 | 14 | 16 | 282.7 | 6 | 11 | 406.4 | 11 |
| Within sites | 2,398 | 8,223.1 | 84 | 1,254 | 4,101.3 | 92 | 1,145 | 3,451.7 | 87 |

2008). Distance, site fidelity, and habitat all likely play a role in Smallmouth Bass genetic structure. However, until now it has been difficult to parse out each factor's relative effect on structure. Results from our AMOVAs suggest that (1) distance likely has a slightly larger influence on genetic structure than habitat but (2) over short distances, habitat still explains a modest amount of genetic variance. A more conclusive assessment of how distance, site fidelity, and habitat influence the genetic structure of Smallmouth Bass will require more targeted dispersal and habitat use research.

Distinct river- and lake-spawning ecotypes have been identified previously and may be an important consideration when defining Smallmouth Bass population boundaries (Barthel et al. 2008; Borden 2008). Barthel et al. (2008) found that river and lake ecotypes differed in both age and size at maturation as well as nesting success. Both types also had strong spawning site fidelity to their

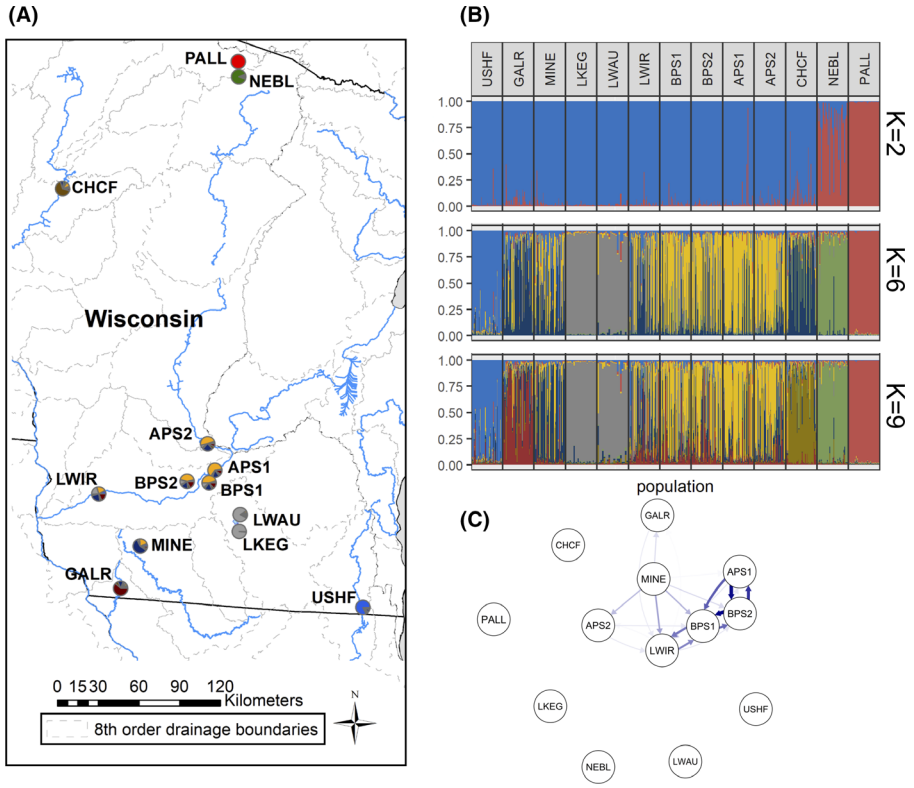


FIGURE 4. Summary of Smallmouth Bass genetic structure identified in the upper Mississippi River drainage, including **(A)** a map of Mississippi River drainage sites (watersheds are delineated by dashed lines; site codes are defined in Table 1); **(B)** bar plots of the most likely number of unique genetic clusters ($K$) identified using Bayesian STRUCTURE analysis; and **(C)** relative migration greater than 0.2, as estimated from Jost's $D$ using methods outlined by Sundqvist et al. (2016). Site locations in panel A are denoted with pie charts of the average cluster membership for fish at each site based on $K = 9$. Each line in the bar plots (panel B) represents the relative admixture from $K$ genetic clusters for a single individual (sample site of origin is indicated at the top of the plot). Relative migration is represented in a network $q$-plot (panel C), with each bubble representing all individuals from a site, and the spatial proximity of each bubble to other bubbles indicates their relative genetic similarity (i.e., not geographic proximity). Migration between sites is shown as arrows connecting the bubbles; the width and shade of arrows indicate the amount of migration (i.e., darker, thicker arrows indicate more migration). [Color figure can be viewed at afsjournals.org.]

respective habitats, which would promote demographic and genetic isolation. Smallmouth Bass tend to have strong spawning site fidelity even within the same habitat. Multi-year tagging studies have indicated that Smallmouth Bass often spawn within 200 m of the prior year's nest (Ridgway et al. 1991) and likely spawn in the same general area where they hatch, given that age-0 fish were observed to stay within 100 m of their hatch site for the first summer (Ridgway et al. 2002). Smallmouth Bass maintain small home ranges outside of the spawning season as well. Populations of Smallmouth Bass in the Beaver Island archipelago, located just north of our study region in Lake Michigan, are believed to have limited exchange with nearby shoreline and river populations less than 30 km away (Kaemingk et al. 2011). Similar small home ranges were identified for Smallmouth Bass and Largemouth Bass in southern Lake Michigan (Savitz and Treat 2007). The apparent life history differences between river and lake Smallmouth Bass, combined with spawning site fidelity and small home ranges, may result in significant genetic differences between individuals that inhabit the same general area but utilize different habitats. This phenomenon has been observed in other lentic–lotic fish populations, such as Threespine Sticklebacks *Gasterosteus aculeatus* (Thompson et al. 1997), Pumpkinseed *Lepomis gibbosus*, and Rock Bass *Ambloplites rupestris* (Brinsmead and Fox 2002).

Genetic substructure within the Mississippi River drainage was largely partitioned by watersheds (Seaber et al. 1987). Smallmouth Bass in the Mississippi River drainage were collected from seven different watersheds, and each watershed approximately corresponded with a cluster identified via STRUCTURE at a $K$-value of 9 (Figure 4). The only exceptions were PALL and NEBL, which are located in the same watershed but formed two distinct clusters. These sites are both small, isolated lakes with little opportunities for gene flow, which may increase the likelihood of experiencing genetic drift and subsequent genetic

differentiation with neighboring populations (Gillespie 2010). Although these factors do likely influence the genetic structure observed between PALL and NEBL, the magnitude of the genetic distance is more likely an artifact of the low heterozygosity observed in PALL Smallmouth Bass. The low genetic diversity in PALL, which could be the result of a population bottleneck or a founder effect, appears to have caused increased estimates of genetic distance between PALL and all other sites. In the Wisconsin River, where we had paired sample sites, there appears to be a high degree of migration among sites, even between river and impoundment sites (Ruzich et al. 2019). This supports previous studies indicating that Smallmouth Bass in rivers migrate farther than those in lakes (Langhurst and Schoenike 1990; Kaemingk et al. 2011). Our sampling design did not include any other lake–river comparisons in the same watershed, but we did find that lake sites tended to have lower genetic diversity and appeared to be more similar to other nearby lakes than to river sites. This may indicate that there is a similar genetic difference between river and lake Smallmouth Bass in both the Lake Michigan and upper Mississippi River drainages.

Black bass are usually managed at a regional scale within a single small lake or river (Long et al. 2015). As such, most research comes from populations living entirely in lentic or lotic systems and from systems that are much smaller than the Laurentian Great Lakes or the Mississippi River (Gerber and Haynes 1988; Savitz et al. 1993). Our results and those of others indicate that Smallmouth Bass use a wide range of habitats, and in systems like the Great Lakes, the populations likely mix between both lentic and lotic environments. Therefore, consideration of habitat heterogeneity may be important when developing black bass management plans at a larger scale, which may encompass multiple bass subpopulations. Smallmouth Bass in our study showed substantial genetic variation over a small scale. Therefore, reducing artificial gene flow and limiting the movement of individuals among distinct Smallmouth Bass populations by stocking and angling may help to retain fine-scale genetic structure.

During tournaments in large lakes such as Lake Michigan, fish may be released hundreds of kilometers from where they were captured (Maynard et al. 2013; Slagle et al. 2020). However, some species of black bass do not always migrate back to their original location after they are released, instead remaining within just a few kilometers of their release location (Wilde 2003; Maynard et al. 2017). We found significant genetic differentiation at small spatial scales of tens of kilometers and unique genetic populations in each surveyed watershed. Therefore, moving Smallmouth Bass from their capture location, even within the same lake or river, could result in subtle degradation of genetic structure and the loss of local adaptation in the subpopulation if even a subset of these individuals fails to return to their home population (Vrijenhoek 1998). Additionally, moving fish among habitats has the potential to further degrade local adaptation. Unlike other species of bass, which return to fluvial habitats after translocation (e.g., Shoal Bass; Taylor and Peterson 2015), Smallmouth Bass are often considered habitat generalists and naturally inhabit both lotic and lentic environments. However, this does not mean that populations are not adapted to the local habitat, and there is some evidence that fluvial populations of Smallmouth Bass can suffer after the creation of impoundments (Brewer and Long 2015). Although Smallmouth Bass have demonstrated an ability to maintain genetic structure on a small scale, the most conservative management approach would be to limit translocations of fish, at least until the mechanisms maintaining local structure are better understood.

Smallmouth Bass populations regularly contain strong fine-scale genetic structure and an apparent relationship between habitat and genetic substructure at neutral loci (Rogers et al. 2006; Philipp et al. 2008; Taylor et al. 2018a). Additionally, some black bass have been successful invaders of freshwater systems worldwide and have shown a penchant for adapting and thriving in novel environments (Takamura 2007; Bangs et al. 2018). Given that the genetic structure of black bass at neutral loci has been well described, we suggest that the inclusion of nonneutral loci in future studies could help to determine whether differences between adjacent populations are facilitated through natural selection or are simply a product of low dispersal and migration (Luikart et al. 2003; Guinand et al. 2004). The scope of taxonomic and genetic diversity in black bass has only recently been recognized (Taylor et al. 2019), and the advent of modern genomic techniques offers new opportunities to investigate speciation and the evolution of these species in novel habitats. Genomic techniques have already been used to successfully identify genes associated with Largemouth Bass growth in a captive population (Li et al. 2017), and similar techniques could be used to uncover signals of natural selection and identify adaptive loci in wild populations (Nadeau and Jiggins 2010). Information about natural selection can be important for guiding management decisions and for understanding threats to sustainable fisheries (Waples et al. 2008). By advancing past descriptions of how populations are structured, as we do here, and focusing on how Smallmouth Bass maintain genetic differentiation without substantial barriers to gene flow, new research will facilitate conservation of black bass diversity and an improved understanding of fish speciation and evolution.

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## SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.