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[Article]

Admixture Analysis of Florida Largemouth Bass and Northern Largemouth Bass using Microsatellite Loci

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Abstract.—Microsatellite DNA variation was examined at 11 loci in five populations of Florida largemouth bass *Micropterus salmoides floridanus* ($N = 175$) and eight populations of northern largemouth bass *M. s. salmoides* ($N = 249$). Distinct allele frequencies with 33 private alleles between subspecies (threshold frequency, ≥ 0.05) and 19 private alleles among three geographic regions distinguished between Florida largemouth bass and northern largemouth bass as well as between northern largemouth bass from northern and southern latitudes in North America. Variation at microsatellite loci also provided sufficient resolution to discriminate among some populations within regions. Allele frequencies indicated that of 37 trophy bass (defined here as fish weighing 5.90 kg or more) donated to the Texas Parks and Wildlife Department (TPWD) between 2004 and 2005, all had more than 50% Florida largemouth bass influence with ancestry genetically similar to that of populations sampled in western Florida. Some fish ($N = 24$) were direct descendents of Florida largemouth bass (either remnants or direct descendants from introductions), whereas others ($N = 13$) were admixed with northern largemouth bass. Of the 13 admixed fish, 11 had ancestry in lineages of southern-latitude northern largemouth bass. Genetic variation within northern largemouth bass populations was depressed at northern latitudes (mean heterozygosity, 0.37; SD, 0.26; mean number of alleles per locus, 2.91; SD, 1.51) relative to southern latitudes (mean heterozygosity, 0.52; SD, 0.25; mean number of alleles per locus, 4.57; SD, 2.88); Florida largemouth bass exhibited intermediate heterozygosity (mean, 0.41; SD, 0.32) and an allelic richness (mean, 4.51; SD, 4.58) similar to that of southern-latitude northern largemouth bass. Overall, the variation observed at these loci is greater than that at other codominant markers explored in this species, providing additional power to detect admixture in populations and individuals.

The divergence of lineages within largemouth bass *Micropterus salmoides* (Bailey and Hubbs 1949) was largely influenced by Pliocene sea level fluctuations (Near et al. 2003) and Pleistocene glaciation events (Nedbal and Philipp 1994). The native range of the Florida largemouth bass *M. s. floridanus* consisted of peninsular Florida, while the northern largemouth bass *M. s. salmoides* extended throughout northeastern Mexico, southeastern Canada, and the U.S. corridor in between (MacCrimmon and Robbins 1975). Furthermore, genetic distinctions between northern largemouth bass at northern and southern latitudes have been identified (Philipp et al. 1983; Nedbal and Philipp 1994). The genetic distinctions among these groups, however, are continually being diluted by introgression (Philipp et al. 1983). The resulting ambiguity over the genetic composition of populations poses problems for researchers and resource agencies working with largemouth bass, particularly when it is of interest to quantify stocking events, identify gene flow within or from targeted systems, or resolve the identity or origins of individuals.

Morphological characters useful for discriminating between the two subspecies (Bailey and Hubbs 1949) are uninformative for resolving admixture proportions given their predominantly polygenic basis and the influence of environment on their expression (Philipp et al. 1985). Allozyme analysis has been the standard technique used to assess the genetic composition of largemouth bass populations (Philipp et al. 1983; Williamson et al. 1986), but Philipp et al. (1983) caution against the use of these markers with individual fish. Additionally, although DNA analysis techniques including the use of restriction fragment length poly-

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morphisms of mitochondrial DNA (mtDNA; Nedbal and Philipp 1994) and randomly amplified polymorphic DNA (RAPD) (Williams et al. 1998) have been used to evaluate largemouth bass populations, these methods rely on maternally inherited or dominant genetic markers, which in the latter case may not be reproducible.

Microsatellites offer an alternative that may be better suited for estimating the proportions of contributions to the genomes of putative hybrids and the origins of individuals (Primmer et al. 2000; Randi et al. 2001). Microsatellites are repetitive, codominant, neutral sequences that offer a greater number of loci and higher level of polymorphism (Jeffreys et al. 1994). Unlike with allozyme analyses, the resolution of microsatellites requires minimally invasive, nonlethal sampling of small amounts of tissue (e.g., fin clips) from which all potential loci may be resolved. Additionally, collected tissues may be stored under an array of conditions (e.g., from room temperature to liquid nitrogen) making field sampling efficient and long-term storage effective. Microsatellites also can be used to analyze degraded and archived samples, allowing assessment of genetic variation over relatively long time frames (Nielsen et al. 1999).

Although Texas is within the native range of the northern largemouth bass, Florida largemouth bass have been systematically introduced to the state since 1972 as part of an effort to enhance fisheries by introducing lacustrine-adapted lineages to newly created reservoirs (Forshage and Fries 1995). These introductions resulted in numerous reservoir populations with varying levels of Florida largemouth bass influence and fewer isolated populations of northern largemouth bass. Following the introduction of Florida largemouth bass, there were increases in the size of the state record largemouth bass (6.12–8.25 kg), the number of reservoirs yielding trophy fish (defined here as fish weighing 5.90 kg or more), and the number of trophy fish harvested annually by anglers.

Since 1986, the Texas Parks and Wildlife Department (TPWD) has accepted donations of trophy-sized largemouth bass for research and spawning before returning the fish to their original population. To date, all donated fish have been harvested from reservoirs or private water bodies to which Florida largemouth bass were introduced. Thus, each fish may have been a Florida largemouth bass from the initial introduction, a direct descendant of introduced fish, a resident northern largemouth bass, or a hybrid from some generation since the introduction. It is of interest to TPWD to be able to resolve the admixture proportions and ancestry of these trophy individuals while retaining the option of returning a viable fish to the wild.

In this study we quantified allele frequency variation at 11 microsatellite loci in samples from three geographically distinct regions. Samples encompassed both subspecies as well as populations of northern largemouth bass at northern and southern latitudes. Our objectives were as follows: (1) to determine whether microsatellite genetic structure concurs with sampling three distinct geographic regions and the results of previous studies of population structure in the species, allowing the resolution of individual geographic origins; (2) to quantify allele frequency differences between species and among regions; and (3) to resolve the admixture proportions and ancestry of trophy fish donated to TPWD during 2004 and 2005. Based on previous work showing that Florida largemouth bass attain a larger overall size than northern largemouth bass at southern latitudes (Bottroff and Lembeck 1978) and the locations of our samples, we expected that a majority of the donated fish would have substantial Florida influence and that admixed individuals would have ancestry derived from northern largemouth bass at southern latitudes. Additionally, given that hybrid vigor in the F_1 generation is thought to be followed by outbreeding depression (Lynch 1996), we expected to detect F_1 hybrids at a greater rate than later-generation hybrids in the individuals donated to TPWD.

Methods

Sample collection and DNA extraction.—Pectoral fin clips of Florida largemouth bass ($N = 175$) and northern largemouth bass ($N = 249$) were obtained from geographic regions known to be divergent at allozyme loci (Figure 1). Samples of Florida largemouth bass were obtained from the Hillsborough River (Hill), Lake Kissimmee (Kiss), East Lake Tohopekaliga (Toho), Medard Reservoir (Meda), and Lake Dora (Dora), Florida (Table 1). All of these populations either exist in peninsular Florida within a range known to contain pure Florida largemouth bass or were previously assessed by means of other genetic techniques to ensure their genetic integrity (Philipp et al. 1983; Nedbal and Philipp 1994; Alvarado-Bremer et al. 1998; Kassler et al. 2002). Samples of northern largemouth bass were obtained from two regions. Populations at southern latitudes included Lake Kickapoo (Kick), Twin Oaks Reservoir (Twin), Lake Fryer (Fryr), and the Devils River (Devr), Texas, and Lake Charlotte (Char), Oklahoma. Populations at northern latitudes included Lake Minnetonka (Minn) and Lake Pepin (Pepn), Minnesota, and Pike Lake (Pike), Wisconsin (Table 1). The sample locales of northern

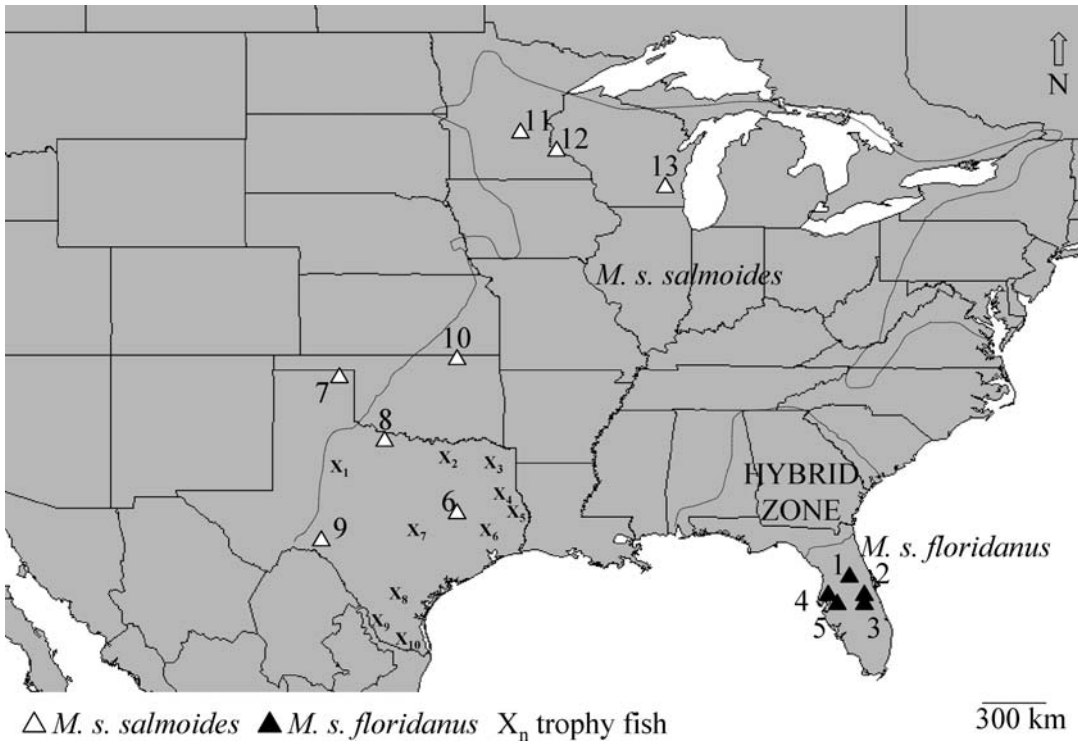

FIGURE 1.—Sampling locations for populations of Florida largemouth bass *M. s. floridanus* and northern largemouth bass *M. s. salmoides* as well as the origins of trophy fish. Native ranges (thin lines) are from MacCrimmon and Robbins (1975). The numbers correspond to the populations listed in Table 1.

largemouth bass were chosen based on stocking records indicating that no divergent lineages were introduced to these populations. Additionally, 37 trophy bass (XXX-352 to XXX-388, where the notation XXX indicates a lineage of uncertain origin) from 10 introgressed populations were donated to TPWD by anglers during 2004 and 2005 (Figure 1).

Fin clips obtained from each sample were preserved in 70% solutions of ethanol and stored at room temperature. Total DNA was extracted from a portion

TABLE 1.—Subspecies designations and sample sizes of the five Florida largemouth bass and eight northern largemouth bass populations sampled for this study.

	Population				
Subspecies	Number	Name	Abbreviation	Location	Sample size
Florida largemouth bass	1	Lake Dora	Dora	Lake County, Florida	27
	2	East Lake Tohopekaliga	Toho	Osceola County, Florida	51
	3	Lake Kissimmee	Kiss	Osceola County, Florida	28
	4	Hillsborough River	Hill	Hillsborough County, Florida	35
	5	Medard Reservoir	Meda	Hillsborough County, Florida	34
Northern largemouth bass	6	Twin Oaks Reservoir	Twin	Robertson County, Texas	31
	7	Lake Fryer	Fryr	Ochlitree County, Texas	30
	8	Lake Kickapoo	Kick	Archer County, Texas	27
	9	Devils River	Devr	Val Varde County, Texas	37
	10	Lake Charlotte	Char	Osage County, Oklahoma	50
	11	Lake Minnetonka	Minn	Hennepin County, Minnesota	27
	12	Pepin Lake	Pepn	Le Sueur County, Minnesota	23
	13	Pike Lake	Pike	Washington County, Wisconsin	24
Unknown[a]	X_1–X_{10}		XXX	Texas	37

[a] Thirty-seven trophy fish (>5.90 kg) obtained from 10 introgressed populations.

of each fin clip following a modified version of the Puregene protocol for extraction from fish tissue (Gentra Systems, Inc., Minneapolis, Minnesota). Briefly, 3–5 mm^3 of fin tissue was placed in 300 μL of cell lysis solution (10 mM tris–HCl, 10 mM EDTA [pH 8.0], and 2% sodium dodecyl sulfate) with 3 μL of proteinase K (20 mg/mL) and incubated at 55°C for 1.5–2.0 h. After incubation, 120 μL of ammonium acetate (7.5 M) was added and mixed. The solution was then incubated at 0°C for 10–15 min followed by centrifugation at 13,000 × gravity (*g*) for 5 min. The supernatant was added to 1,000 μL of 100% ethanol, stored at −80°C for 10 min, and recentrifuged at 13,000 × *g* for 10 min. The supernatant was then decanted and the pellet washed with 600 μL of 70% ethanol and centrifuged at 13,000 × *g* for 5 min, decanted, and dried at room temperature for 15 min. The pellet was resuspended in 200 μL of deionized H_2O and left to rehydrate at room temperature for 24 h. Quantification of DNA concentration was performed by comparison to standards on 0.8% agarose gels.

Microsatellite optimization and genotyping.—Polymerase chain reaction (PCR) optimization and amplification was attempted for 45 microsatellite loci originally isolated in other centrarchids (Colbourne et al. 1996; Neff et al. 1999; DeWoody et al. 2000; Malloy et al. 2000). Reactions were 10 μL, consisting of 1× buffer containing 0.75–1.75 mM $MgCl_2$, 0.04 μM of an M13 (−29) 5'-adapted forward primer, 0.20 μM of a reverse primer, 0.20 μM of 25% labeled M13 (−29) infrared dye 700/800 sequence (LI-COR, Inc., Lincoln, Nebraska), 0.20 mM of each deoxynucleotide triphosphate, 10–20 ng of genomic DNA, and 0.25 U *Taq* DNA polymerase (Promega, Madison, Wisconsin). Standard PCRs of 94°C for 60 s, the annealing temperature for 60 s, and 72°C for 180 s were performed on an MJ Research PTC-200 thermocycler (MJ Research, Waltham, Massachusetts) for 30 cycles. Annealing temperatures were ± 8°C of the primer's calculated melting temperatures. The PCR products were denatured by heat and formamide and analyzed on an NEN 4200 Global IR^2 DNA Sequencer (LI-COR) using a size standard of 50–350 base pairs. Allele sizes were estimated using GeneImagIR (Scanalytics, Billerica, Massachusetts).

Analyses of genetic variation.—Deviations from Hardy–Weinberg equilibrium (HWE; (Guo and Thompson 1992) and linkage equilibrium (LE; Slatkin and Excoffier 1996) were tested for all locus–population and locus–locus combinations using ARLEQUIN version 2.0 (Schneider et al. 2000). Significance levels were adjusted for multiple comparisons using the sequential Bonferroni method (Rice 1989). Genetic variation was measured as observed heterozygosity and the number of alleles per locus within populations, regions, and species. Private alleles among groups were calculated using GenAlEx 6 (Peakall and Smouse 2005). The absence or presence and frequency of null alleles at each locus were resolved using the methods of Brookfield (1996) as applied in MICRO-CHECKER version 2.2.3 (Oosterhout et al. 2004).

Genetic structure and admixture.—Genetic structure among populations was evaluated in terms of the Cavalli-Sforza and Edwards chord distance (1967). A consensus neighbor-joining tree (1,000 bootstraps) was constructed based on chord distance matrices using the programs NEIGHBOR and CONSENSE in PHYLIP version 3.6 (Felsenstein 1989). Genetic structure among individuals was evaluated using the model-based Bayesian clustering method applied in STRUCTURE (Pritchard et al. 2000). The Bayesian model used multilocus genotypes to infer population structure when there was an undefined number of genetic clusters (*K*) characterized by allele frequencies at multiple loci, constrained by HWE and LE. Additionally, for each *K* the model was used to probabilistically assign admixed individuals to an inferred group or groups. These membership coefficients were used to approximate admixture proportions. Thus, tautological influence was eliminated from the characterization of structure and assignment of individuals to clusters in the data set. The posterior probability of each number of genetic clusters was estimated for values of $K = \text{I}$ to XIV using all genotypes ($N = 424$; 20,000 burn-in steps; 200,000 Markov chain Monte Carlo steps). The most likely structure of the data set was identified as the maximum mean likelihood value for *K* (10 iterations). Conformity of the Bayesian and distance analyses was evaluated as the order of formation and composition of clusters inferred by each technique.

After choosing $K = \text{VII}$, which showed the highest mean likelihood score, we evaluated the mean membership coefficients of all known (sampled) populations to the seven inferred clusters. Additionally, the membership coefficients (*q*) of all known populations were assessed at $K = \text{II}$ to VI, populations being assigned to the cluster with the greatest mean membership coefficient. The proportion of each genotype that was inferred to originate from each cluster for clustering solutions from $K = \text{II}$ to VII was also assessed. Individuals with membership coefficients of 0.95 or more were assigned to a single cluster; otherwise they were assigned to more than one cluster (admixed). Individuals with membership coefficients less than 0.95 at $K = \text{II}$ and III were removed from subsequent admixture analyses of unknowns. The admixture proportions and recent ancestry of trophy fish were inferred with respect to genetic structure implied in the

TABLE 2.—Microsatellite primer sequences, annealing temperatures (T_{ann}), and mean levels of genetic variation within populations, represented as (1) observed heterozygosity (H_O) and (2) the number of alleles per locus (N_a) with allele sizes (base pairs) in parentheses. The sequence for the primer M13 (−29) is also shown.

Locus	Primer sequences (5′–3′)[a]	Source	T_{ann} (°C)	H_o	N_a[b]
Lma10	GTCTGTAAGTGTGTTTGCTG GAAACCCGAAACTTGTCTAG	Colbourne et al. 1996	57.7	0.46	3.6 (139–151)
Lma12	CTGCTCAGCATGGAGGCAG TTCTTCCACAATATTCTCGCC	Colbourne et al. 1996	45.6	0.31	3.1 (124–154)
Lma21	CAGCTCAATAGTTCTGTCAGG ACTACTGCTGAAGATATTGTAG	Colbourne et al. 1996	47.5	0.81	11.5 (146–244)
Lma120	TGTCCACCCAAACTTAAGCC TAAGCCCATTCCCAATTCTCC	Neff et al. 1999	59.6	0.45	3.3 (204–224)
Lar7	GTGCTAATAAAGGCTACTGTC TGTTCCCTTAATTGTTTTGA	DeWoody et al. 2000	47	0.49	5.9 (121–193)
Mdo3	AGGTGCTTTGCGCTACAAGT CTGCATGGCTGTTATGTTGG	Malloy et al. 2000	46.2	0.44	3.3 (119–143)
Mdo4	TCTGAACAACTGCATTTAGACTG CTAATCCCAGGGCAAGACTG	Malloy et al. 2000	48.6	0.31	2.2 (151–155)
Mdo6	TGAAATGTACGCCAGAGCAG TGTGTGGGTGTTTATGTGGG	Malloy et al. 2000	55	0.17	1.8 (160–176)
Mdo7	TCAAACGCACCTTCACTGAC GTCACTCCCATCATGCTCCT	Malloy et al. 2000	53	0.43	3.7 (179–205)
Msal21	CACTGTAAATGGCACCTGTGG GTTGTCAAGTCGTAGTCCGC	DeWoody et al. 2000	58	0.28	1.8 (214–224)
Msal25	CAATATTGCCAAAGCATC CATTTGATACTGAATTTATTG	DeWoody et al. 2000	47.5	0.41	5.3 (198–230)
M13 (−29)[c]	CACGACGTTGTAAAACGAC				

[a] The first line for each locus shows the forward primer sequence, the second line the reverse primer sequence.
[b] Alleles per locus are reported as within-population averages. Allele size ranges are for the entire data set.
[c] Primer sequences are reported prior to 5′ adaptation with the M13 (−29) sequence on the forward sequence.

data set at $K = \text{III}$ (our sampling design) and VII (the most likely clustering solution). In each case reference clusters were identified a priori by using the prior population information option to classify all known samples. Unknowns identified as admixed ($q < 0.95$) under this model were tentatively allocated to the genetic cluster of most likely origin, again using the prior population information option. Each of these individuals was further evaluated to determine whether it was most likely a product of the cluster to which it was tentatively allocated, was a first-generation immigrant, or had recent ancestry (parent or grandparent) in an alternative cluster. If it was still found that an admixed individual was more likely to be from an allocated cluster than to have ancestry in any alternative cluster, admixture was estimated to have occurred more than two generations earlier.

Results

Genetic Variation

Eleven of 45 primer pairs were optimized for amplification of microsatellite loci in Florida and northern largemouth bass (Table 2), no multilocus duplicate genotypes being resolved in 424 fish. Although tests of HWE indicated heterozygote deficits across allelic classes within some Florida largemouth bass populations at the locus *Msal25* owing to the presence of null alleles (Table 3) as well as at *Lma10* in Kiss, these were not significant after Bonferroni correction. With the exception of comparisons with alleles at *Msal25*, pairwise combinations of alleles between loci were not significantly different within populations. Subsequent analyses were performed with and without *Msal25* to resolve spurious results.

Within sampled populations, 85% (122/143) of all locus–population combinations were polymorphic, expressing 1–22 alleles per locus within populations (mean, 4.10; SD, 2.70), with levels of heterozygosity ranging from 0 to 0.96 (mean, 0.41; SD, 0.16). The mean heterozygosity within Florida largemouth bass populations (0.37; SD, 0.32) was similar to that in northern largemouth bass populations at northern latitudes (0.37; SD, 0.26) and lower than that in northern largemouth bass populations at southern latitudes (0.52; SD, 0.25). When *Msal25* was removed from the analysis, however, the levels of heterozygosity in Florida largemouth bass populations were intermediate to those of northern largemouth bass populations at southern and northern latitudes (mean, 0.41; SD, 0.32). The number of alleles per locus was similar in Florida largemouth bass populations (mean, 4.51; SD, 4.58) and southern-latitude northern largemouth bass populations (mean, 4.57; SD, 2.88) but lower in populations of northern-latitude northern largemouth bass (mean, 2.91; SD, 1.51). Overall, comparisons between subspecies yielded 33 private alleles with

TABLE 3.—Observed heterozygosity (H_O), with adherence to Hardy–Weinberg expectations (P_{HWE}) in parentheses, and the number of alleles per locus (N_a) for five Florida largemouth bass and eight northern largemouth bass populations. Abbreviations and sample sizes given in Table 1. Asterisks indicate $P < 0.05$; no result was significantly different from Hardy–Weinberg expectations after Bonferroni correction.

Locus and statistic	Dora	Toho	Hill	Kiss	Meda	Twin	Kick	Fryr	Devr	Char	Pepn	Pike	Minn
Lma10													
H_O	0.333 (0.013)*	0.074 (0.176)	0.229 (0.234)	0.143 (0.001)*	0.118 (0.045)*	0.839 (0.645)	0.704 (0.798)	0.800 (0.699)	0.757 (0.585)	0.360 (0.695)	0.370 (0.199)	0.625 (1.000)	0.609 (0.032)*
N_a	2	2	2	2	2	5	6	5	5	5	4	4	3
Lma12													
H_O	0.444 (0.123)	0.549 (0.437)	0.229 (0.181)	0.556 (0.156)	0.471 (0.310)	0.226 (1.000)	0.111 (1.000)		0.243 (1.000)	0.080 (1.000)	0.556 (0.209)	0.458 (0.232)	0.130 (1.000)
N_a	5	4	3	5	4	4	2	1	2	2	3	3	2
Lma21													
H_O	0.926 (0.676)	0.922 (0.468)	0.800 (0.135)	0.963 (0.988)	0.912 (0.059)	0.903 (0.826)	0.926 (0.597)	0.800 (0.324)	0.892 (0.819)	0.880 (0.416)	0.444 (0.248)	0.542 (0.561)	0.652 (1.000)
N_a	17	22	12	13	16	9	13	10	11	9	5	4	8
Lma120													
H_O	0.259 (1.000)		0.343 (0.557)	0.296 (1.000)	0.206 (1.000)	0.355 (1.000)	0.667 (0.156)	0.567 (0.160)	0.622 (0.654)	0.660 (0.486)	0.519 (0.667)	0.750 (0.379)	0.609 (0.294)
N_a	4	1	2	3	3	2	5	4	3	5	6	3	2
Lar7													
H_O	0.808 (0.681)	0.765 (0.365)	0.543 (0.061)	0.963 (0.853)	0.765 (0.365)	0.581 (0.514)	0.407 (0.730)	0.633 (0.680)	0.730 (0.100)	0.120 (1.000)			
N_a	12	9	9	14	9	6	4	5	4	2	1	1	1
Mdo3													
H_O	0.630 (0.733)	0.667 (0.823)	0.657 (0.790)	0.556 (0.798)	0.471 (0.191)	0.548 (0.536)	0.630 (0.585)	0.733 (0.887)		0.180 (0.465)	0.074 (1.000)	0.167 (0.078)	0.435 (0.809)
N_a	3	3	4	3	3	4	5	4	1	3	5	3	2
Mdo4													
H_O	0.148 (1.000)	0.137 (1.000)				0.065 (1.000)	0.519 (0.887)	0.500 (0.603)	0.459 (0.692)	0.620 (0.671)	0.593 (0.457)	0.417 (0.622)	0.565 (1.000)
N_a	2	2	1	1	1	2	3	3	2	3	3	3	2
Mdo6													
H_O		0.059 (0.098)	0.029 (1.000)	0.111 (1.000)	0.059 (1.000)	0.419 (0.646)	0.630 (0.234)	0.533 (0.693)	0.081 (1.000)	0.300 (0.253)			
N_a	1	2	2	3	2	2	2	2	2	2	1	1	1
Mdo7													
H_O	0.111 (1.000)		0.143 (1.000)	0.185 (0.358)	0.324 (0.563)	0.935 (0.708)	0.778 (0.530)	0.700 (0.704)	0.351 (1.000)	0.680 (0.503)	0.259 (0.135)	0.500 (1.000)	0.565 (0.431)
N_a	2	1	2	2	2	8	9	8	3	4	2	3	2
Msal21													
H_O	0.667 (0.312)	0.778 (0.183)	0.571 (0.311)	0.778 (0.183)	0.588 (0.746)			0.233 (0.501)					
N_a	4	2	3	2	3	1	1	2	1	1	1	1	1
Msal25													
H_O	0.000 (0.011)*	0.000 (0.028)*		0.000 (0.203)	0.000 (0.021)*	0.742 (0.234)	0.630 (0.403)	0.700 (0.588)	0.730 (0.409)	0.780 (0.296)	0.778 (0.549)	0.417 (0.743)	0.609 (0.154)
N_a	3	4	1	4	3	11	6	9	4	9	5	5	5

frequencies ranging from 0.05 to 0.98 (mean, 0.31; SD, 0.28), and pooled frequencies for private alleles at the loci *Mdo6* and *Msal21* were 1.00 in both species. Private alleles summed to 0.99 at *Mdo7* and 0.88 at *Lma12* in northern largemouth bass and to 0.84 and 0.97 at *Lma10* in Florida and northern largemouth bass, respectively. There were 19 private alleles partitioned among clusters at $K = \text{III}$ (threshold, ≥ 0.05; maximum frequency, 0.964; mean, 0.27; SD, 0.28).

Genetic Structure and Admixture Analyses

Clustering populations based on the Cavalli-Sforza and Edwards (1967) chord distance indicated three major groups: Florida largemouth bass and northern largemouth bass at southern and northern latitudes. There was also strong bootstrap support for subsequent delineation of specific populations and groups (Figure 2). To cluster individuals, we started with the assumption that each clustering solution was equally likely and estimated the posterior probability that each

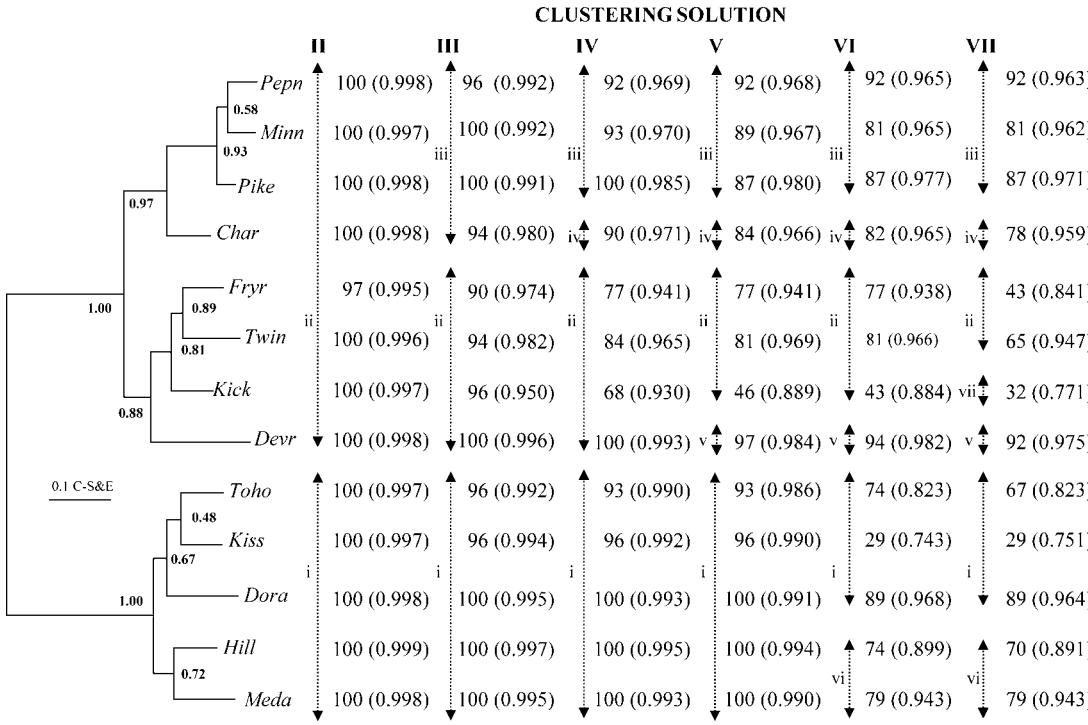

FIGURE 2.—Inferred genetic structure of sampled Florida and northern largemouth bass populations based on Cavalli-Sforza and Edwards (C-S&E 1967) chord distance (1,000 bootstaps; proportion support reported at each node) and Bayesian clustering (Pritchard et al. 2000). Abbreviations are given in Table 1. Vertical arrows identify the assembled populations under the clustering solutions obtained by assuming that values for the number of genetic clusters (K) range from II to VII. The percentage of individuals from each population assigned to each cluster (i to vii) with a membership coefficient of 0.95 or more is followed in parentheses by the mean membership coefficient of all individuals from each population in the cluster.

solution would account for the genetic variation in the data set with respect to HWE and LE. Bayesian clustering based on genotypes at 10 and 11 loci indicated that the sample ($N = 424$) included at least seven differentiable genetic clusters using no a priori population information (Tables 4, 5). The likelihood values of the data ranged from $-14{,}553.4 \pm 2.1$ ($K = \text{I}$) to $-8{,}164.8 \pm 80.6$ ($K = \text{VII}$) (Table 4; Figure 2). The assignment of populations to clusters was identical to the composition of the clades in the NJ tree, and the order of nodes was similar to the order of formation of clusters at $K = \text{II}$ to VII (Figure 2).

Individuals were assigned to a single cluster ($q \geq 0.95$) or jointly to two or more clusters if admixed ($q < 0.95$) (Figure 2). The assignment of individuals at $K =$ II produced clusters of Florida largemouth bass (cluster_i: frequency [$f_{0.95}$], 100%) with no admixed individuals (mean q value [q_mean], 0.998) and northern largemouth bass (cluster_ii: $f_{0.95}$, 99%; q_mean, 0.997) with a single admixed individual from Fryr (q, 0.893). This individual was removed from subsequent analyses using reference populations to resolve the admixture proportions in trophy fish. Three clusters ($K =$ III) partitioned individuals into groups with Florida largemouth bass (cluster_i: $f_{0.95}$, 100%; q_mean, 0.995) and northern largemouth bass at northern ($\text{cluster}_\text{iii}$: $f_{0.95}$, 99%; q_mean, 0.989) and southern latitudes (cluster_ii: $f_{0.95}$, 95%; q_mean, 0.976), except that 94% of the individuals sampled from Char were assigned to $\text{cluster}_\text{iii}$ (q_mean, 0.980) (Table 5; Figure 2).

Clustering at $K =$ VII (the most likely clustering solution) partitioned Florida largemouth bass into two clusters (cluster_i and cluster_vi) that discriminated between samples collected from the mideastern and western portions of peninsular Florida. Samples obtained from western Florida populations (Meda and Hill) were predominantly assigned to cluster_vi ($f_{0.95}$, 75%; q_mean, 0.917), the remaining individuals being identified as admixed with cluster_i ($f_{0.95}$, 0%; q_mean, 0.074). Samples obtained from the middle to eastern portion of the peninsula were predominantly assigned to cluster_i ($f_{0.95}$, 62%; q_mean, 0.846), the remaining individuals being identified as admixed with cluster_vi ($f_{0.95}$, 3%; q_mean, 0.145). Under the same clustering

TABLE 4.—Likelihood of each clustering solution (K) for all sampled genotypes in a Bayesian clustering analysis of Florida largemouth bass and northern largemouth bass samples (424 individuals; 11 loci) using STRUCTURE (Pritchard et al. 2000). The most likely solution (K = VII) is indicated by bold italics.

K	Likelihood[a]
I	−14,553.4 ± 2.1
II	−10,069.1 ± 2.2
III	−8,960.6 ± 1.7
IV	−8,660.1 ± 36.2
V	−8,473.8 ± 108.3
VI	−8,249.2 ± 78.7
VII	***−8,164.8 ± 80.6***
VIII	−8,214.1 ± 54.9
IX	−8,257.7 ± 83.2
X	−8,299.7 ± 118.3
XI	−8,360.8 ± 119.9
XII	−8,435.7 ± 76.6
XIII	−8,526.6 ± 31.2
XIV	−8,564.6 ± 29.4

[a] Likelihood is defined as $\log_e P(x|K)$, where P is the probability of the data (x) under each clustering solution (K).

solution, 87% of the fish sampled from northern latitudes were assigned to $\text{cluster}_{\text{iii}}$ (q_{mean}, 0.965), admixed individuals being associated with $\text{cluster}_{\text{iv}}$ (Char). Samples from southern-latitude northern largemouth bass populations were partitioned into three clusters that included Fryr and Twin as a single group ($\text{cluster}_{\text{ii}}$: $f_{0.95}$, 54%; q_{mean}, 0.894) and Kick ($\text{cluster}_{\text{vii}}$: $f_{0.95}$, 32%; q_{mean}, 0.771) and Devr ($\text{cluster}_{\text{v}}$: $f_{0.95}$, 92%; q_{mean}, 0.975) as isolated groups. The membership coefficients among individuals in these clusters, when admixed, were partitioned within the southern-latitude northern largemouth bass cluster defined at K = III ($\text{cluster}_{\text{ii}}$; Table 5; Figure 2).

The admixture proportions and recent ancestry of unknown fish were estimated by incorporating prior population information into each model. Under the first model, assuming K = III, the results showed that of the 37 trophy fish from admixed populations, 24 were assigned to the Florida largemouth bass cluster ($q \geq 0.95$), 13 were admixed, and none were assigned to northern- or southern-latitude northern largemouth bass clusters (Table 6). Additionally, of the 13 admixed fish, all individuals had greater membership coefficients in the Florida largemouth bass cluster than in either cluster of northern largemouth bass, and 11 of 13 had greater membership coefficients in $\text{cluster}_{\text{ii}}$ (southern-latitude northern largemouth bass populations) than $\text{cluster}_{\text{iii}}$ (northern-latitude northern largemouth bass populations, including Char). When assessed under the assumption of K = VII, the results showed that of the 24 fish resolved as monophyletic in the Florida largemouth bass lineage (at K = III), all were more closely associated with $\text{cluster}_{\text{vi}}$ (western Florida; $f_{0.95}$, 58%; q_{mean}, 0.942) than $\text{cluster}_{\text{i}}$ (mideastern Florida; $f_{0.95}$, 0%; q_{mean}, 0.029) and only 3 of these individuals produced membership coefficients to $\text{cluster}_{\text{i}}$ exceeding 0.05 (Table 7). Of two individuals previously resolved as admixed with greater influence from northern- than southern-latitude northern largemouth bass ($\text{cluster}_{\text{iii}}$ at K = III), one (XXX-376) had a greater membership coefficient in $\text{cluster}_{\text{iv}}$ (Char) and the other (XXX-365) in $\text{cluster}_{\text{iii}}$ (Pepn, Pike, Minn). A third individual (XXX-357) had substantial genetic contributions from the northern-latitude cluster at K = III and from the Kick cluster at K = VII, and under both models it had some ancestry in southern- and northern-latitude clusters.

Ancestry analyses of the 13 admixed individuals assuming K = III showed that 6 fish were more likely to be members of the Florida largemouth bass cluster to which they were allocated than to be first-generation

TABLE 5.—Mean membership coefficients for individuals in each population to each cluster assuming K = III and K = VII. Population abbreviations are given in Table 1. See text and Table 4 for additional details. The greatest coefficients for each population are indicated by bold italics.

	Inferred clusters at K = III			Inferred clusters at K = VII						
Population	i	ii	iii	i	ii	iii	iv	v	vi	vii
Dora	***0.994***	0.003	0.003	***0.964***	0.002	0.001	0.002	0.002	0.027	0.002
Toho	***0.992***	0.004	0.004	***0.823***	0.002	0.002	0.002	0.003	0.165	0.003
Kiss	***0.994***	0.003	0.003	***0.751***	0.002	0.002	0.004	0.002	0.237	0.002
Hill	***0.997***	0.002	0.001	0.101	0.002	0.001	0.001	0.002	***0.891***	0.002
Meda	***0.995***	0.003	0.002	0.047	0.002	0.002	0.001	0.002	***0.943***	0.003
Twin	0.002	***0.982***	0.016	0.002	***0.947***	0.008	0.01	0.013	0.002	0.018
Fryr	0.002	***0.974***	0.024	0.004	***0.841***	0.008	0.029	0.03	0.003	0.085
Kick	0.006	***0.950***	0.044	0.002	0.140	0.021	0.017	0.047	0.002	***0.771***
Pepn	0.002	0.006	***0.992***	0.002	0.006	***0.963***	0.020	0.003	0.002	0.004
Minn	0.003	0.005	***0.992***	0.002	0.005	***0.962***	0.023	0.003	0.002	0.003
Pike	0.002	0.007	***0.991***	0.001	0.008	***0.971***	0.007	0.004	0.002	0.007
Devr	0.002	***0.996***	0.002	0.001	0.008	0.003	0.003	***0.975***	0.001	0.009
Char	0.002	0.018	***0.980***	0.001	0.007	0.018	***0.959***	0.005	0.002	0.008

TABLE 6.—Admixture proportions and inferred ancestry of trophy fish donated to the Texas Parks and Wildlife Department as estimated using STRUCTURE assuming $K = III$. Admixture proportions are for each individual. Individuals with membership coefficients of 0.95 or more were assigned to a cluster; individuals with membership coefficients less than 0.95 were identified as admixed and tentatively allocated to the cluster of most likely origin for subsequent analysis. Inferred ancestry is the probability that each admixed individual belongs in the allocated cluster (i) versus the probability that it is a first-generation immigrant or has a parent or grandparent in an alternative cluster (first, second, and third values in the columns for clusters ii and iii, respectively).

	Cluster		
Sample	i[a]	ii[b]	iii[c]
	Admixture proportions		
XXX-352	0.798	0.159	0.043
XXX-353	0.711	0.286	0.003
XXX-354	0.993	0.003	0.004
XXX-355	0.986	0.011	0.003
XXX-356	0.955	0.017	0.028
XXX-357	0.598	0.283	0.119
XXX-358	0.695	0.268	0.037
XXX-359	0.993	0.003	0.004
XXX-360	0.537	0.458	0.005
XXX-361	0.955	0.027	0.018
XXX-362	0.572	0.380	0.048
XXX-363	0.992	0.005	0.003
XXX-364	0.975	0.019	0.006
XXX-365	0.768	0.011	0.221
XXX-366	0.994	0.003	0.003
XXX-367	0.995	0.003	0.002
XXX-368	0.668	0.304	0.028
XXX-369	0.606	0.377	0.017
XXX-370	0.982	0.016	0.002
XXX-371	0.995	0.003	0.002
XXX-372	0.954	0.026	0.020
XXX-373	0.993	0.003	0.004
XXX-374	0.990	0.005	0.005
XXX-375	0.994	0.003	0.003
XXX-376	0.740	0.044	0.216
XXX-377	0.707	0.282	0.011
XXX-378	0.993	0.004	0.003
XXX-379	0.802	0.171	0.027
XXX-380	0.990	0.005	0.005
XXX-381	0.989	0.004	0.007
XXX-382	0.994	0.003	0.003
XXX-383	0.996	0.002	0.002
XXX-384	0.993	0.004	0.003
XXX-385	0.992	0.004	0.004
XXX-386	0.993	0.003	0.004
XXX-387	0.989	0.008	0.003
XXX-388	0.700	0.292	0.008
	Inferred ancestry		
XXX-352	0.923	0.000, 0.000, 0.076	0.000, 0.000, 0.001
XXX-353	0.618	0.000, 0.000, 0.380	0.000, 0.000, 0.002
XXX-357	0.003	0.000, 0.001, 0.257	0.000, 0.000, 0.739
XXX-358	0.284	0.000, 0.000, 0.588	0.000, 0.000, 0.128
XXX-360	0.005	0.001, 0.012, 0.973	0.000, 0.000, 0.009
XXX-362	0.035	0.000, 0.122, 0.822	0.000, 0.000, 0.020
XXX-365	0.895	0.000, 0.000, 0.012	0.000, 0.000, 0.094
XXX-368	0.423	0.000, 0.000, 0.536	0.000, 0.000, 0.041
XXX-369	0.224	0.000, 0.000, 0.737	0.000, 0.000, 0.039
XXX-376	0.909	0.000, 0.000, 0.034	0.000, 0.000, 0.057
XXX-377	0.613	0.000, 0.000, 0.378	0.000, 0.000, 0.008
XXX-379	0.985	0.000, 0.000, 0.012	0.000, 0.000, 0.004
XXX-388	0.033	0.000, 0.000, 0.948	0.000, 0.000, 0.020

[a] Populations Kiss, Dora, Toho, Meda, and Hill (see Table 1).
[b] Populations Kick, Fryr, Twin, and Devr.
[c] Populations Pepn, Pike, Minn, and Char.

immigrants or to have a parent or grandparent in an alternative cluster, suggesting that in these individuals the initial hybridization event occurred several generations in the past (Table 6). Six of the 7 remaining fish were most likely to have grandparents or more distant ancestors from $cluster_{ii}$, and 1 fish (XXX-357) was more likely to have a grandparent (probability [p], 0.739) or earlier ancestor in $cluster_{iii}$. Analyses of these individuals assuming $K = VII$ showed that 10 were more likely to be members of the Florida largemouth bass cluster to which they were allocated than recent immigrants, 2 (XXX-360 and XXX-362) were more likely to have grandparents or later in $cluster_{ii}$, and 1 (XXX-357) had similar probabilities of having a grandparent or later in $cluster_{iii}$ or $cluster_{vii}$ (Table 7).

Discussion

The analysis of admixted proportions in trophy fish donated to TPWD showed that a majority of the genetic contribution was from Florida largemouth bass, particularly lineages similar to those obtained from western Florida (Meda and Hill). Furthermore, a majority of the trophy fish showed no significant influence from northern largemouth bass. Given the time it takes to attain trophy size (Crawford et al. 2002), these fish are original remnants of, or direct descendents from, Florida largemouth bass stocking events at least a decade in the past. Post hoc analysis of the sparse broodfish acquisition records over the past two decades indicated that Florida largemouth bass were collected from several sources. These include populations from western Florida's Homosassa River (L. T. Fries, personal observation) and Lake Hollingsworth and mideastern Florida's Lake Kissimmee and West Lake Tohopekaliga, populations introduced into California whose origin is unknown (D. Campbell, TPWD, personal observation), and populations from Cuba. Without more extensive sampling within Florida and more complete records of these acquisitions, however, we cannot confirm that the ancestors of these trophy fish derive from a geographic location in western Florida. All we can say is that Florida largemouth bass similar to those we collected in western Florida were used as broodfish.

Admixed trophy fish were largely influenced by southern lineages of northern largemouth bass, a majority probably having a grandparent or earlier relative in the southern-latitude northern largemouth bass cluster. Genetic contributions from southern-latitude northern largemouth bass were expected based on the location (Texas) yielding these individuals, but two fish (XXX-365 and XXX-376) were unexpectedly resolved as having greater genetic contributions from northern-latitude clusters and one fish (XXX-357) as

TABLE 7.—Admixture proportions and inferred ancestry of trophy fish donated to the Texas Parks and Wildlife Department as estimated using STRUCTURE assuming $K = \text{VII}$. See Table 6 for more details. Note that there is only one set of inferred ancestry values for cluster vi, versus three sets for the other clusters.

	Cluster						
Sample	i[a]	ii[b]	iii[c]	iv[d]	v[e]	vi[f]	vii[g]
				Admixture proportions			
XXX-352	0.013	0.088	0.015	0.028	0.014	0.812	0.030
XXX-353	0.005	0.060	0.003	0.009	0.132	0.741	0.050
XXX-354	0.014	0.004	0.004	0.004	0.004	0.966	0.004
XXX-355	0.012	0.004	0.003	0.003	0.017	0.956	0.005
XXX-356	0.017	0.014	0.018	0.017	0.005	0.908	0.021
XXX-357	0.012	0.014	0.021	0.007	0.009	0.492	0.445
XXX-358	0.008	0.075	0.048	0.011	0.026	0.673	0.159
XXX-359	0.013	0.004	0.004	0.004	0.003	0.967	0.005
XXX-360	0.009	0.417	0.004	0.008	0.005	0.538	0.019
XXX-361	0.013	0.014	0.004	0.018	0.004	0.922	0.025
XXX-362	0.018	0.185	0.016	0.057	0.010	0.558	0.156
XXX-363	0.037	0.004	0.003	0.003	0.005	0.943	0.005
XXX-364	0.016	0.007	0.008	0.007	0.026	0.926	0.010
XXX-365	0.009	0.010	0.127	0.058	0.004	0.767	0.025
XXX-366	0.011	0.003	0.003	0.003	0.003	0.974	0.003
XXX-367	0.017	0.003	0.003	0.003	0.003	0.968	0.003
XXX-368	0.116	0.074	0.009	0.013	0.006	0.485	0.297
XXX-369	0.005	0.121	0.007	0.042	0.073	0.634	0.118
XXX-370	0.006	0.003	0.002	0.002	0.031	0.952	0.004
XXX-371	0.008	0.003	0.002	0.002	0.003	0.979	0.003
XXX-372	0.010	0.011	0.009	0.011	0.013	0.935	0.011
XXX-373	0.011	0.004	0.004	0.004	0.004	0.968	0.005
XXX-374	0.011	0.005	0.007	0.006	0.005	0.959	0.007
XXX-375	0.011	0.003	0.003	0.003	0.003	0.974	0.003
XXX-376	0.011	0.058	0.048	0.132	0.011	0.668	0.072
XXX-377	0.012	0.036	0.007	0.007	0.008	0.569	0.361
XXX-378	0.009	0.004	0.003	0.003	0.004	0.973	0.004
XXX-379	0.008	0.027	0.010	0.014	0.043	0.802	0.096
XXX-380	0.016	0.005	0.006	0.006	0.005	0.956	0.006
XXX-381	0.122	0.004	0.003	0.023	0.004	0.839	0.005
XXX-382	0.017	0.003	0.003	0.003	0.003	0.968	0.003
XXX-383	0.010	0.002	0.002	0.002	0.003	0.978	0.003
XXX-384	0.014	0.004	0.003	0.003	0.002	0.970	0.004
XXX-385	0.203	0.004	0.005	0.005	0.004	0.774	0.005
XXX-386	0.075	0.003	0.004	0.004	0.003	0.907	0.004
XXX-387	0.018	0.004	0.004	0.004	0.012	0.953	0.005
XXX-388	0.011	0.259	0.005	0.012	0.008	0.690	0.015
				Inferred ancestry			
XXX-352	0.000, 0.000, 0.000	0.000, 0.000, 0.006	0.000, 0.000, 0.000	0.000, 0.000, 0.000	0.000, 0.000, 0.001	0.993	0.000, 0.000, 0.001
XXX-353	0.000, 0.000, 0.000	0.000, 0.000, 0.014	0.000, 0.000, 0.000	0.000, 0.000, 0.001	0.000, 0.000, 0.052	0.924	0.000, 0.000, 0.010
XXX-357	0.000, 0.000, 0.000	0.000, 0.000, 0.038	0.000, 0.000, 0.483	0.000, 0.000, 0.012	0.000, 0.000, 0.002	0.051	0.000, 0.005, 0.408
XXX-358	0.000, 0.000, 0.000	0.000, 0.000, 0.058	0.000, 0.000, 0.020	0.000, 0.000, 0.005	0.000, 0.000, 0.013	0.828	0.000, 0.000, 0.076
XXX-360	0.000, 0.000, 0.000	0.000, 0.009, 0.866	0.000, 0.000, 0.000	0.000, 0.000, 0.009	0.000, 0.000, 0.000	0.109	0.000, 0.000, 0.006
XXX-362	0.000, 0.000, 0.000	0.000, 0.006, 0.504	0.000, 0.000, 0.000	0.000, 0.000, 0.027	0.000, 0.000, 0.006	0.285	0.000, 0.066, 0.106
XXX-365	0.000, 0.000, 0.000	0.000, 0.000, 0.001	0.000, 0.000, 0.005	0.000, 0.000, 0.003	0.000, 0.000, 0.000	0.989	0.000, 0.000, 0.002
XXX-368	0.000, 0.000, 0.002	0.000, 0.000, 0.123	0.000, 0.000, 0.003	0.000, 0.000, 0.004	0.000, 0.000, 0.003	0.677	0.000, 0.001, 0.187
XXX-369	0.000, 0.000, 0.000	0.000, 0.000, 0.056	0.000, 0.000, 0.001	0.000, 0.000, 0.016	0.000, 0.000, 0.022	0.855	0.000, 0.000, 0.049
XXX-376	0.000, 0.000, 0.000	0.000, 0.000, 0.007	0.000, 0.000, 0.006	0.000, 0.000, 0.005	0.000, 0.000, 0.001	0.976	0.000, 0.000, 0.004
XXX-377	0.000, 0.000, 0.000	0.000, 0.000, 0.029	0.000, 0.000, 0.001	0.000, 0.000, 0.001	0.000, 0.000, 0.002	0.849	0.000, 0.002, 0.116
XXX-379	0.000, 0.000, 0.000	0.000, 0.000, 0.000	0.000, 0.000, 0.000	0.000, 0.000, 0.000	0.000, 0.000, 0.000	0.998	0.000, 0.000, 0.001
XXX-388	0.000, 0.000, 0.000	0.000, 0.000, 0.412	0.000, 0.000, 0.001	0.000, 0.000, 0.026	0.000, 0.000, 0.010	0.527	0.000, 0.000, 0.024

[a] Populations Kiss, Dora, and Toho.
[b] Populations Twin and Fryr.
[c] Populations Pepn, Pike, and Minn.
[d] Population Char.
[e] Population Devr.
[f] Populations Meda and Hill.
[g] Population Kick.

having substantial genetic contributions from all three lineages. Two of the three were harvested from private impoundments and may plausibly have had a recent ancestor from a northern-latitude hatchery. When assessed under the seven-cluster model, however, the influence from the northern cluster (K = III) was distributed into the southernmost cluster resolved from what was the northern-latitude cluster and the most proximate cluster resolved from what was the southern-latitude cluster.

We found no evidence of a heterotic effect (in terms of size) resulting from first-generation crosses between Florida largemouth bass and northern largemouth bass. The majority of trophy-sized fish with an admixed genome were later-generation hybrids with a larger percentage of Florida largemouth bass alleles. There was also no observable negative impact on size from the admixed genetic background in these fish, most likely because of the modified environment to which they were introduced and the nonadaptive radiation of micropterids (Near et al. 2003). Although the Florida largemouth bass has poor fitness at northern latitudes outside of its native range (Philipp and Whitt 1991), the fish examined here were relatively elderly, large individuals with high levels of Florida influence. Size has been shown to be positively correlated with fitness by increasing survival and fecundity in fish (Huang and Gall 1990; Roff 1992). If the quality of gametes and resultant zygotes is comparable to that of pure species, the fitness of these fish could be similar, if not superior, to that of the northern largemouth bass in some Texas reservoirs. Our results also support previous work showing that Florida largemouth bass attain a larger overall size than northern largemouth bass and may do so outside of their native range when introduced to relatively warm reservoir environments (Bottroff and Lembeck 1978; Forshage and Fries 1995). It is, however, apparently a unique combination of climatic and anthropogenic influences that have provided Florida largemouth bass with favorable environments in Texas. In fact, each trophy fish examined was obtained from an artificial lentic system, and it is not likely that these same results would be replicated in many environments (Philipp and Whitt 1991).

The variation at microsatellite loci in this species was less than that seen in most other freshwater fishes (DeWoody and Avise 2000) when expressed as the number of alleles per locus (Florida largemouth bass: mean, 7.45; SD, 7.26; northern largemouth bass: mean, 8.55; SD, 7.26) or observed heterozygosity (Florida largemouth bass: mean, 0.35; SD, 0.32; northern largemouth bass: mean, 0.41; SD, 0.23). The values of both of these measures of genetic variation, however, are greater than those reported at other codominant loci for the species (Philipp et al. 1983).

Among northern largemouth bass populations, there was lower heterozygosity and a lower number of alleles per locus within populations at northern latitudes. This pattern of genetic variation is similar to the results of Philipp et al. (1983), whose work, based on the variation at multiple allozymes, showed that among nonintrogressed northern largemouth bass populations the number of alleles per locus, percent polymorphic loci, and observed heterozygosity were dramatically lower in Minnesota and Wisconsin populations than in Texas and Oklahoma populations. Reductions in genetic variation at northerly latitudes have also been reported in other species (Bernatchez and Dodson 1991; Bernatchez and Wilson 1998), which conforms to the predicted genetic consequences of recolonization following Pleistocene glaciation (Hewitt 1996).

The genetic structure among populations supported our sampling design of three geographic regions with the exceptions that Char was placed with the northern-latitude lineage of northern largemouth bass assuming three genetic clusters and seven inferred clusters were determined to be more likely than three. Although the placement of Char was unexpected given its proximity to the sampled southern-latitude northern largemouth bass, the reasons for its placement cannot be resolved without more representative sampling of midwestern locales. While the genetic structure reported herein supports previous analyses (Philipp et al. 1983; Nedbal and Philipp 1994) in the discrimination of species and recognition of genetic differentiation with latitude in northern largemouth bass, we also show that microsatellites may be used to resolve the origins of individuals to some populations with high accuracy. For example, 97% of the fish collected from Devr were assigned to the Devr cluster at $K = \mathrm{V}$ using a membership threshold of 0.95. The ability to distinguish Devr individuals from other samples reflects the relative uniqueness of this population (Figure 2). This is not totally unexpected given that the sample was collected from a drainage basin with a prevalent endemic fish fauna and Edwards (1980) has previously shown that many specimens of largemouth bass in this drainage display glossohyal teeth. This unique morphological characteristic, combined with the genetic differentiation of this population from other populations in the region, suggests that Devr may be a divergent and isolated lineage.

Allozymes, mtDNA haplotypes, and RAPD have been used to distinguish between Florida and northern largemouth bass (Philipp et al. 1983; Nedbal and Philipp 1994; Williams et al. 1998). Our results suggest that microsatellite DNA can provide additional resolu-

tion, whether used alone or with the aforementioned techniques. Philipp et al. (1983) cautioned against the use of allozymes in estimating the genetic composition of individuals given the relatively high probability of type II errors when using one or two diagnostic markers. The loci used here, however, provide a large number of private alleles, yielding greater power to detect introgression within individuals and allowing for more accurate estimates of admixture proportions. The minimal invasiveness of this technique was also particularly useful, allowing the resolution of all 11 markers from a single fin clip without having to sacrifice the donor fish.

The North American populations sampled for this study were from geographically distinct parts of each subspecies' native range, only 1 of 424 individuals showing admixed ancestry. These findings suggest that despite the systematic introduction of Florida largemouth bass into Texas, a number of populations have maintained their genetic integrity or been only minimally affected. The existence of distinct groups based on these markers, however, does not necessarily mean that we sampled "pure" populations with no introgression—rather that we sampled populations that show little evidence of recent ancestry from the other sampled regions. The fact that minimally to unimpacted systems remain, together with the fact that there are a number of markers available to detect introgression between subspecies and among regions, suggests that a full assessment of the variation throughout the range of each subspecies should be performed to identify minimally impacted environments and quantify the levels of gene flow from impacted to nonimpacted water bodies. No such work has been performed for over 20 years (Philipp et al. 1983), and during this time the translocation of stocks has continued.

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