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[Article]

Conservation Genetics of Inland Lake Trout in the Upper Mississippi River Basin: Stocked or Native Ancestry?

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Abstract.—Although stocking for sport fishery enhancement has been practiced by resource managers for decades, the potential genetic effects of these stocking practices have remained largely unknown. We investigated the genetic contributions of stocking lake trout *Salvelinus namaycush* in two inland lakes in Wisconsin (Trout and Black Oak lakes in Vilas County), which represent the only known indigenous lake trout populations in the upper Mississippi River basin. Exogenous sources of lake trout (Lake Michigan and Lake Superior strains) have been stocked into each of these lakes for decades, although the long-term effects of past stocking events on these populations are unknown. We used nine microsatellite loci and polymerase chain reaction–restriction fragment length polymorphism analysis of mitochondrial DNA to determine the distinctiveness and genetic ancestry of lake trout in Trout and Black Oak lakes. Measures of allelic variance indicated that Trout and Black Oak lakes were significantly different ($P < 0.05$) from each other ($F_{ST} = 0.162$) and all other populations evaluated in this study ($F_{ST} = 0.101 - 0.164$). The combined microsatellite and mitochondrial DNA data indicate that upper Mississippi River basin lake trout have been minimally affected by past stocking practices. These populations should be managed as native gene pools, and interlake and interbasin stocking should be avoided.

The conservation and management of exploited or declining fishery stocks requires a fundamental understanding of the genetic population structure of the species involved. For many years, stocking hatchery-reared fish was the most common way to restore declining fishery stocks, with little regard to the ecological or genetic consequences for native stocks (Nielsen 1993). Although a growing number of empirical studies have shown that the introduction of exogenous strains may have negative genetic impacts on native fish stocks through hybridization and introgression (Allendorf 1991; Hindar et al. 1991; Krueger and May 1991; Philipp 1991), other studies have been unsuccessful in documenting considerable stocking effects on native fish populations (Wishard et al. 1984; Vuorinen and Berg 1989; Hansen et al. 2000).

In the last half century, the lake trout *Salvelinus namaycush* has been one of the most heavily cultured and stocked species in North America. Catastrophic declines of lake trout in the Laurentian Great Lakes during the mid-20th century triggered massive stocking and restoration efforts (Selgeby et al. 1995 and references therein). Although most management efforts have focused on rehabilitating Great Lakes populations, introductions and stocking of lake trout have also been widespread in inland lakes (Crossman 1995; Powell and Carl 2003). Only recently has significant attention been

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TABLE 1.—Characteristics of lake trout populations and strains sampled, with additional sampling information.

Population or strain	Abbreviation	*N*	Sampling methodology	Size (cm)	Date of sampling	Basin	Lake size (km^2)
Trout Lake, Wisconsin	TL	48	Fyke netting	51.6–77.5	Fall 2001 (wild)	Mississippi River	15.2
Black Oak Lake, Wisconsin	BO	40	Fyke netting	41.1–85.9	Fall 2001 (wild)	Mississippi River	2.3
Big Green Lake[a], Wisconsin	BG	43	Fyke netting	39.6–58.9	Fall 2001 (wild)	Lake Michigan	29.3
Lewis Lake[b], Wyoming	LL	37	Hatchery	Unknown	Fall 2001 (hatchery)	Lake Michigan	11.4
Gull Island Shoal, Wisconsin	GIS	36	Fyke netting	58.2–91.2	Fall 2002 (wild)	Lake Superior	82,100
Marquette[c], Michigan	MQ	50	Hatchery	Unknown	Fall 2001 (hatchery)	Lake Superior	82,100

[a] Original source in Lake Michigan (Hacker 1957).
[b] Sampled population was derived from F_1 fish from the Saratoga National Fish Hatchery; the original stocking source was Lake Michigan.
[c] Broodstock hatchery source originally derived from Lake Superior.

focused on issues relating to the sustainability of inland lake trout populations in their own right (Evans and Olver 1995; Gunn et al. 2003; Powell and Carl 2003).

Genetic research and monitoring of lake trout has similarly had a strong Great Lakes focus. Genetic studies of lake trout in the Great Lakes over the last quarter century have employed a range of molecular markers, from allozymes and mitochondrial DNA (mtDNA) to nuclear sequences and microsatellite DNA (Dehring et al. 1981; Grewe and Hebert 1988; Krueger et al. 1989; Phillips et al. 1989; Grewe et al. 1993; Kincaid et al. 1993; Guinand et al. 2003; Page et al. 2003). By contrast, comparatively few studies have assessed the genetic structure and variation among inland populations. Although Ihssen et al. (1988) and Wilson and Hebert (1996, 1998) used allozymes and whole-molecule mtDNA, respectively, to identify the genetic structure of inland lake trout populations, these markers do not offer sufficient resolution for investigating fine-scale population genetic differentiation. Hypervariable microsatellite DNA markers, by contrast, have been used extensively in salmonids to examine genetic variation over both temporal and spatial scales (Nielsen et al. 1997; Hansen 2002; Guinand et al. 2003). In particular, microsatellites have proven useful in identifying the structure and admixture of genetic stocks (Olsen et al. 2000; Beacham et al. 2001) and in providing genetic information to monitor unique stocks or strains (Nielsen et al. 1999). Comparatively few studies have used microsatellite markers to study lake trout, although recent work has demonstrated their utility for delineating population structure in this species (Guinand et al. 2003; Page et al. 2003).

Wisconsin harbors two native inland lake trout populations, those in Trout and Black Oak lakes in Vilas County. These populations are potentially unique, as they represent the only two extant populations of lake trout indigenous to the upper Mississippi River basin (Greene 1935; Becker 1983; Lyons 1984). Trout Lake is relatively small, with a surface area of 15.2 km^2 (Table 1), and harbors one of the most diverse deepwater assemblages in the Mississippi River basin, which includes cisco *Coregonus artedi*, burbot *Lota lota*, slimy sculpin *Cottus cognatus*, opposum shrimp *Mysis relicta*, and possibly ninespine stickleback *Pungitius pungitius*. Black Oak Lake is much smaller (2.3 km^2) and less speciose than Trout Lake but contains cisco and opposum shrimp in addition to lake trout (Lyons 1984).

Trout and Black Oak lakes were stocked with lake trout from Lake Michigan and Lake Superior sources at various times in an attempt to augment their populations (Table 2) (McKnight 1977; Jahns and Bozek 2000). Trout Lake has been periodically stocked with lake trout since the 1920s, the heaviest period of stocking occurring from 1956 to the present. Stocking records from the Wisconsin Department of Natural Resources (WDNR) indicate that during this period most stocked fish (55.5%) were derived from Lake Superior sources (Marquette and possibly Gull Island Shoal strains), while lake trout derived from Big Green Lake (also known as Green Lake) in central Wisconsin comprised only 2.1% of the total stockings. Although the Big Green Lake lake trout population was originally derived from Lake Michigan populations (Hacker 1957), Lake Superior strains have been stocked in Big Green Lake sporadically from 1951 to the present. Lake Superior strains were last

TABLE 2.—Stocking history for Trout and Black Oak lakes, including the number of lake trout stocked, size of stocked fish, and source population; F refers to fry, f to fingerlings, and y to yearlings.

	Trout Lake			Black Oak Lake		
Year	Number stocked	Size	Source	Number stocked	Size	Source
Pre-1950s	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
1951				31,000	F	Unknown
1959	7,122	f	Trout Lake			
1960	6,448	f	Big Green Lake			
1961	26,250	f	Trout and Big Green lakes			
1962	31,200	f	Big Green Lake			
1963	20,867	y	Lake Superior			
1964	20,000	y	Trout Lake			
1966	7,640	y	Lake Superior			
1967	9,500	y	Lake Superior			
1968	7,936	y	Lake Superior			
1969	25,000	y	Lake Superior			
1970	28,670	y	Trout Lake			
1971	42,650	y	Trout Lake			
1972	6,100	y	Trout Lake	11,120	y	Lake Superior
1973				25,349	Unknown	Lake Superior
1974				12,500	y	Lake Superior
1976	80,000	f	Lake Superior			
1977	95,000	f	Lake Superior	70,000	f	Lake Superior
1978	64,000	f	Lake Superior	80,000	f	Lake Superior
1979	45,000	f	Lake Superior	25,000	f	Lake Superior
1980	40,000	f	Lake Superior	15,000	f	Lake Superior
1983	18,994	y	Trout Lake			
1984	20,800	y	Trout Lake			
1985	15,625	y	Trout Lake			
1986	4,745	y	Trout Lake			
1987	32,000	y	Trout Lake			
1998	88,823	f	Trout Lake			

planted in Trout Lake in 1980, and the Big Green Lake strain was last stocked in 1962. In the 1980s, fisheries managers began supplemental stocking of the Trout Lake strain (42.3% of total stockings). However, the genetic composition or uniqueness of this strain was not investigated prior to culture, rearing, and stocking. Subsequent phylogeographic research on lake trout mtDNA variation based on whole-molecule restriction digests (Wilson and Hebert 1998) revealed that Trout Lake was comprised solely of fish of Mississippian ancestry. By contrast, Great Lakes stocks and hatchery strains showed mixed phylogenetic ancestry, with representation from all three major phylogenetic mtDNA lineages (Grewe and Hebert 1988; Wilson and Hebert 1996, 1998).

Black Oak Lake has also been stocked to a lesser degree with nonnative strains of lake trout from Lake Superior. From 1951 to 1980, approximately 270,000 progeny from were planted in Black Oak Lake from Lake Superior (WDNR, unpublished data). The most intensive period of stocking was from 1972 to 1980, and the last stocking event occurred in 1980 (Table 2). Undocumented stockings may also have occurred in the late-19th and early-20th centuries (Becker 1983). Recent investigations have indicated that although the population is small, natural lake trout reproduction occurs in the lake (Wes Jahns, WDNR, personal communication). Although management programs aimed at restoring inland populations of lake trout in Wisconsin to self-sustaining levels have been implemented, detailed information regarding the genetic ancestry and population structure for upper Mississippi River basin populations is lacking. If Trout and Black Oak lakes represent genetically unique or remnant native populations despite multiple exogenous stocking events, a more aggressive restoration and protection program may be appropriate.

We used complementary genetic marker systems (microsatellite DNA and mtDNA) to determine whether lake trout in Trout and Black Oak lakes were comprised of mixed-ancestry stocks (i.e., admixed indigenous and exogenous gene pools) or were still native, indigenous populations. The study presented several challenges owing to the lakes' complex stocking history and lack of historical (prestocking) tissue samples. Rather than directly comparing current samples with historical

or archival ones to assess the contribution of past stocking events, we had to resolve the genetic ancestry and integrity of the inland populations through indirect evidence. We therefore characterized the genetic variation for the most likely source populations and applied several analytical methods to determine the genetic ancestry of the inland populations. The combined results support the hypothesis of largely intact native gene pools and provide baseline genetic data for the restoration and sustainable management of these potentially unique inland lake trout populations.

Methods

Genetic data collection.—Lake trout were captured in Trout Lake (TL) and Black Oak Lake (BO), nonlethally sampled (adipose or caudal fin clip), and released. In an effort to determine the strain composition of the inland lakes, tissue samples were also collected from all potential source populations, including the Gull Island Shoal (GIS) and Marquette (MQ) strains from the Lake Superior basin and from the Big Green Lake (BG; Green County, Wisconsin) and Lewis Lake (LL; Saratoga National Fish Hatchery, Wyoming) strains, which were originally derived from Lake Michigan stocks. All populations of wild lake trout were collected with fyke nets during fall 2001 sampling; total length ranged from approximately 40 to 91 cm. The Lewis Lake samples in this study were taken from first generation (F_1) hatchery fish that were created by crossing 68 wild females with 85 males collected from Lewis Lake in Yellowstone National Park in 2001. The Marquette strain sample was obtained from Michigan state broodstocks. The stocking history of other strains of lake trout used in this study has been discussed elsewhere in greater detail (Krueger et al. 1983; Krueger and Ihssen 1995; Page et al. 2003). All tissue samples were preserved and stored in 95% solutions of ethanol until analysis.

Genomic DNA was extracted from fin clips using the DNeasy tissue extraction kit (Qiagen). Two mtDNA segments, cytochrome *b* (cyt *b)* and subunits 3 and 4 of the NADH dehydrogenase gene (ND 3/4), were amplified for discrimination of phylogeographic ancestry. All mtDNA and microsatellite loci were amplified on an M-J Research PTC-100 Thermal Cycler. A 505-base-pair (bp) segment of the cyt *b* gene was amplified with a conserved teleost primer (5′-GTGACTTGAAAAACCACCG TTG-3′) from Song et al. (1998) and an unpublished internal degenerate primer (5′-GARAABCCNCC YCARATTCATTG-3′). Unpublished ND 3/4 primers provided by R. Phillips (Washington State University) provided a second informative 724-bp amplicon. Both mtDNA segments were amplified via the polymerase chain reaction (PCR) in 10-μL reaction volumes containing 8 ng of template DNA, 1 μL of 10× PCR buffer, 2 mM of $MgCl_2$, 0.6–1.5 pmol of primers (cyt *b* and ND3/4, respectively), 1 mM of deoxynucleotide triphosphates (dNTPs), and 1 U of *Taq* polymerase. The PCR profiles for both amplicons involved a single denaturation step of 94°C for 2 min, followed by 30 cycles at 92°C for 45 s, 50°C for 1 min, and 72°C for 1 min, followed by a single extension cycle of 72°C for 6 min. The PCR amplicons for each gene were digested with 1 U *Bam* HI restriction enzyme for 1 h at 37°C in separate reactions. The digested fragments were photographed and sized in comparison with a known size standard. The fragment patterns generated by the restriction digests were assigned letter codes following the designations in Wilson and Hebert (1998).

Nine microsatellite loci were resolved for lake trout using primers originally developed for use in other salmonid species. These loci were *Sfo8, Sfo12, Sfo18, Sfo23* (brook trout *S. fontinalis*; Angers et al. 1995), *SfoC24, SfoC28, SfoD75* (brook trout; T. King and M. Burnham-Curtis, U.S. Geological Survey, unpublished data), *Sco19* (bull trout *S. confluentus*; Taylor et al. 2001), and *Ots-1* (Chinook salmon *Oncorhynchus tshawytscha*; Banks et al. 1999). Microsatellite loci were coamplified using fluorescently labeled primers in three 25-μL multiplex reactions (the particular dye labels are given in parentheses): (1) *Sfo23* (NED), *SfoD75* (6-FAM), and *SfoC24* (6-FAM); (2) *Sfo8* (NED), *Sfo12* (HEX), and *SfoC28* (6-FAM); and (3) *Ots-1* (6-FAM), *Sfo18* (6-FAM), and *Sco19* (HEX). Each 25-μL multiplex reaction contained approximately 8 ng of template DNA, 1.5 mM of $MgCl_2$, 200 μM dNTPs, 0.2–0.4 pmol of each primer pair, and 0.4 U of *Taq.* The PCR profiles for all multiplexes were identical and consisted of an initial denaturation step of 95°C for 11 min, followed by 27 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min, followed by a single extension step of 60°C for 45 min. The resultant amplicons were electrophoresed and visualized by means of an ABI 377 (Applied Biosystems) or MegaBace 1000 (Amersham-Bioscience) DNA sequencer. To test for ambiguities between sequencers, a subset of the amplicons were run on both sequencers and compared visually and with MicroChecker (van Oosterhout et al. 2004). Gels were scored using Genotyper (Applied Biosys-

tems) or MegaBace Genetic Profiler (Amersham-Bioscience) software but were verified manually for accuracy.

Genetic data analyses.—The frequencies of the mtDNA polymerase chain reaction–restriction fragment length polymorphism digest haplotypes were summarized for each population and compared with published data. Mitochondrial divergence among populations was estimated with FSTAT (Goudet 2001) using the author's recommendations for F_{ST} estimation with haploid data.

Statistical analysis of population parameters, including allele frequencies, observed and expected heterozygosities, and the number of alleles per locus, was performed for each population and microsatellite locus with version 3.3 of GenePop (Raymond and Rousset 1995). Comparative allelic richness within populations was calculated with FSTAT (Goudet 2001) to correct for unequal sample sizes. The genotypes at each locus for each population were tested for conformance to Hardy–Weinberg equilibrium (HWE) using the heterozygote deficiency module in GenePop. Significance levels for the HWE tests and all other multiple comparison tests were adjusted using sequential Bonferroni methods (Rice 1989) with an initial α value of $0.05/k$, k being the number of tests.

In this study, null (nonamplifying) homozygotes were detected for one locus (*SfoC28*) and all populations exhibited some null homozygotes and fewer heterozygotes than expected at this locus. The occurrence of null alleles can confound the analysis of microsatellite data (Brookfield 1996), and several methodologies have been proposed to adjust allele frequencies in the presence of null alleles (Chakraborty et al. 1992; Brookfield 1996). Null allele frequencies for *SfoC28* were estimated using equation (4) of Brookfield (1996), which treats null homozygotes as observations rather than as missing data. The adjusted allele frequencies were re-input into GenePop and the population genetic statistics were regenerated for each locus and population. Except for individual assignment tests (see below), all subsequent analyses included adjusted values for the *SfoC28* locus. To test for differentiation among lake trout populations, pairwise F_{ST} values based on adjusted allele frequencies were obtained with FSTAT (Goudet 2001).

Individual assignment tests.—Individual assignment tests of the microsatellite data were conducted to estimate the probable origins of the lake trout in Trout and Black Oak lakes. Source populations for each fish from Trout and Black Oak lakes were inferred from similarities in multilocus genotypes using version 1.0.02 of GeneClass (Cornuet et al. 1999). The *SfoC28* locus was not included in the assignment tests owing to the existence of null alleles. We employed a Bayesian approach (Rannala and Mountain 1997) with the leave-one-out procedure to assign individuals. We incorporated the simulation procedure in independent assignments to provide a measure of assignment confidence (10,000 simulations with a rejection level of $P < 0.01$). Assignment methods incorporating distance or frequency measures result in the classification of unknowns to a single probable source population, whereas assignment methods that use a Bayesian approach allow for one of several assignment possibilities (Cornuet et al. 1999). The simulation analysis allowed for several possible outcomes, including (1) the assignment of unknowns to a single population of origin, (2) the assignment of unknowns to an unresolved Great Lakes group (LL, BG, GIS, and/or MQ), and (3) the assignment of unknowns to a mixed-ancestry group ([LL, BG, GIS, and/or MQ] and [TL or BO]).

Results

Three mitochondrial lineages observed among populations of lake trout corresponded to the major lineages identified by Wilson and Hebert (1996, 1998). Digestion of the cyt *b* and ND 3/4 amplicons with *Bam* HI identified lake trout as having Mississippian (haplotype A), Atlantic (haplotype B), or Mississippian–Missourian–Beringian ancestry (haplotype C) (Figure 1). All specimens from Trout Lake possessed mtDNA characteristic of lake trout of Mississippian lineage. With the exception of one specimen that possessed haplotype C, all individuals in Black Oak Lake were characterized by the Mississippian (A) mtDNA. By contrast, samples from the potential source populations contained all three mitochondrial lineages with frequencies similar to those observed by Grewe and Hebert (1988).

Nine microsatellite loci were scored for at least 37 individual lake trout per population (Table 3; Appendix 1). The results indicated that there were no differences in allele size calls between DNA sequencers. All microsatellite loci were polymorphic; the total number of alleles per locus (excluding null alleles) ranged from 4 (*Sfo12*) to 34 (*Sfo23*) across all populations, and the average number of alleles per locus was 15.2. The two Great Lakes populations (Gull Island Shoal and Marquette) showed the greatest allelic diversity at

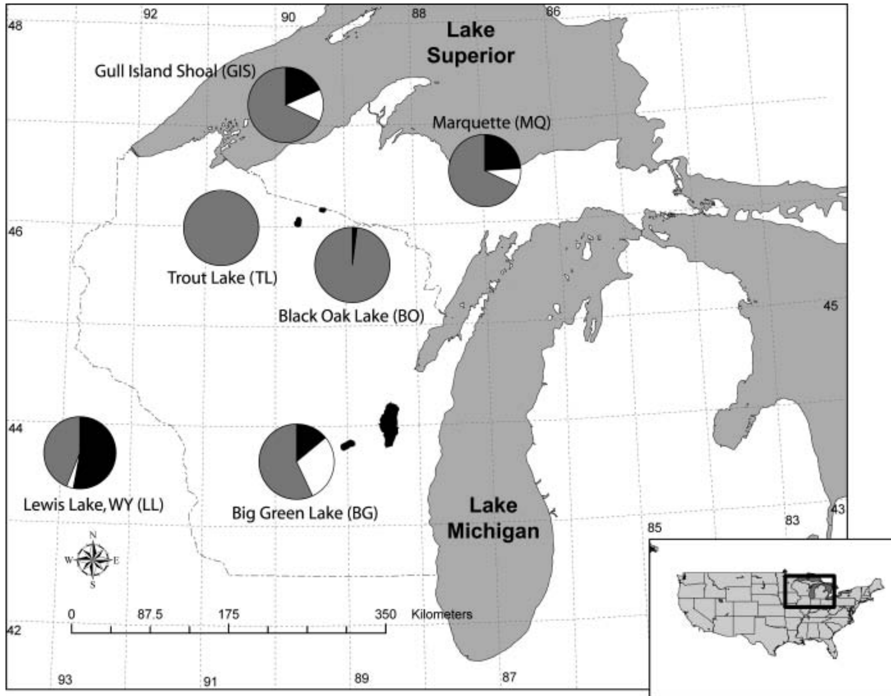

Figure 1.—Distribution of lake trout mtDNA haplotypes for six lake trout populations; sample sizes are given in Table 1. Within the pie charts, gray shading refers to the Mississippian, white to the Atlantic, and black to the Mississippian–Missourian–Beringian phylogeographic lineages (Grewe and Hebert 1988; Wilson and Hebert 1996, 1998).

five of the microsatellite loci, despite differences in sample size. Although allelic richness was lowest for Trout and Black Oak lakes independent of sample size, the differences in allelic richness among populations were not significant (P = 0.072).

Tests of heterozygote deficiency showed significant deviations from Hardy–Weinberg equilibrium in 6 of 54 tests ($P < 0.05$; $k = 54$) after adjustment for null alleles. Despite this correction, most deficits (5/6) were at the *SfoC28* locus. Null alleles (i.e., null–null homozygotes) were detected in all populations at the *SfoC28* locus and were confirmed by repeated amplification efforts alongside positive controls. The proportion of null allele homozygotes ranged from 0.075 (Black Oak Lake)

Table 3.—Observed genetic diversity at nine microsatellite loci for six populations of lake trout, including the total number of alleles, mean number of alleles per population (N_A), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), and allele size range.

Locus and mean	Total number of alleles	N_A	H_E	H_O	Allele size range
Ots1	25	14.33	0.834	0.884	219–263
Sfo18	7	4.17	0.546	0.545	172–188
SfoC24	10	4.33	0.566	0.548	81–114
SfoD75	19	10.67	0.841	0.741	272–350
Sfo23	34	19.00	0.912	0.899	169–247
Sco19	11	6.33	0.609	0.589	158–178
SfoC28[a]	11	4.83	0.578	0.193	251–293
Sfo8	16	8.83	0.828	0.897	263–297
Sfo12	4	2.83	0.218	0.210	253–261
Mean	15.22	8.40	0.659	0.612	

[a] Unadjusted for the presence of null alleles.

TABLE 4.—Pairwise F_{ST} comparisons among six lake trout populations in the upper Mississippi River and Great Lakes basins for polymerase chain reaction–restriction fragment length polymorphism mtDNA (above diagonal) and microsatellite DNA analyses (below diagonal; mean values across 9 loci). Asterisks indicate estimates that were significantly different from zero ($P < 0.05$) after sequential Bonferroni correction for multiple comparisons. See Table 1 for abbreviations.

Population	TL	BO	BG	LL	GIS	MQ
TL		−0.0004	0.3476*	0.4702*	0.2223*	0.2428*
BO	0.162*		0.3098*	0.4374*	0.1837*	0.2119*
BG	0.164*	0.101*		0.1129*	0.0130*	0.0459
LL	0.138*	0.149*	0.085*		0.1043	0.0435
GIS	0.117*	0.127*	0.051*	0.023		−0.0037
MQ	0.111*	0.109*	0.067*	0.060*	0.025	

to 0.729 (Trout Lake) (mean = 0.381); the adjusted r values based on the Brookfield (1996) null allele adjustment method ranged from 0.382 (Black Oak Lake) to 0.807 (Trout Lake).

Population Differentiation

Estimates of population divergence based on mtDNA haplotype frequencies and microsatellite allelic variances (F_{ST}) showed sharply contrasting patterns for pairwise differentiation among all pairwise comparisons of the lake trout populations (Table 4). Trout Lake and Black Oak Lake were very similar based on mtDNA, but both differed significantly from all other populations, with F_{ST} estimates ranging from 0.18 to 0.47. In comparison, the Big Green Lake and Lewis Lake populations were significantly different from each other, but neither differed significantly from those of Great Lakes sources (Table 4).

By contrast, F_{ST} estimates based on microsatellite data showed significant differentiation among all population pairs (Table 4). Pairwise F_{ST} values ranged from 0.023 (LL and GIS) to 0.164 (BG and TL). The Trout Lake and Black Oak Lake populations were significantly distinct from each other ($F_{ST} = 0.162$; $P < 0.05$) and other populations ($F_{ST} = 0.101 - 0.164$; $P < 0.05$), whereas Great Lakes (or Great Lakes–derived) populations showed much lower levels of genetic differentiation ($F_{ST} = 0.023 - 0.085$; $P < 0.05$).

Individual Assignment

Individual assignments using multilocus genotypes showed that Trout and Black Oak lakes were comprised of relatively pure populations. The greatest assignment success in simulations was found for Trout Lake, 90% (43/48 fish) being assigned back to Trout Lake as the most likely source (log-likelihood ratios ≥ 2, equivalent to $P \leq 0.01$). Lake trout sampled from Black Oak Lake were similarly recognizable as a distinct population (85% of fish with log-likelihood ratios ≥ 2 compared with the next most probable source). Gull Island Shoal had the lowest proportion of self-assigned individuals (21%), perhaps reflecting the high genetic diversity within this population and its relatedness to the Marquette strain and introduced populations (Table 5). A single individual in Trout Lake was assigned to Gull Island Shoal (a population previously stocked in Trout Lake), and two had mixed ancestry. One fish in Black Oak Lake had mixed ancestry, and four were misassigned to Trout Lake. By contrast, lake trout

TABLE 5.—Proportional assignment of individual lake trout from each population based on individual multilocus genotypes and population allelic frequencies at nine microsatellite loci. Correct assignments are shown in bold italics. Assignment probabilities are based on Bayesian assignments using 10,000 simulations per population and a rejection probability of 0.01. The abbreviation UGL (unresolved Great Lakes) refers to Great Lakes populations or populations originally derived from Great Lakes sources that could not be identified further; the term ''mixed'' refers to individual genotypes that could be assigned with equal probability to more than one source. See Table 1 for other abbreviations.

Population	N	TL	BO	BG	LL	GIS	MQ	UGL	Mixed
TL	48	***0.90***				0.02			0.08
BO	40	0.10	***0.85***						0.05
BG	43			***0.42***				0.53	0.05
LL	37			0.03	***0.35***	0.05		0.49	0.08
GIS	39					***0.21***	0.03	0.73	0.03
MQ	50						***0.58***	0.42	

sampled from putative source populations were never solely misassigned to inland populations.

Discussion

This study focused on determining the genetic ancestry of lake trout in Trout and Black Oak lakes, two systems that have been influenced by stocking from exogenous sources. Data from both mitochondrial and microsatellite DNA markers suggest that stocking has had relatively little influence on the genetic composition of these inland lakes. Despite the absence of historical (prestocking) samples to compare with the extant populations, all lines of evidence suggest that introgression from stocking has had no impact on the genetic characteristics of the Trout and Black Oak Lake populations of lake trout.

Mitochondrial DNA Diversity

The data from this study indicate that Great Lakes populations or those originally derived from Great Lakes sources (i.e., BG and LL) are mitochondrially more diverse than inland populations (Figure 1). Mitochondrially, three distinct groups occur among all the populations examined in this study and correspond to those identified in previous studies (Wilson and Hebert 1996, 1998). Possession of multiple haplotypic lineages within a single lake trout strain implies either secondary contact among historic lineages during postglacial re-colonization (Wilson and Hebert 1996) or human-mediated contact via stocking. Although Grewe and Hebert (1988) detected only lineage A lake trout from Big Green Lake in their study (n = 15 fish from the Jordan National Fish Hatchery), all three lineages were detected in the larger sample (n = 50) taken for this study (Figure 1).

Trout Lake was comprised solely of the Mississippian mtDNA lineage, a result that corroborated observations by Wilson and Hebert (1996). This sample lineage was similarly predominant in Black Oak Lake, which had not been previously studied. The single lake trout from Black Oak Lake that exhibited the lineage C haplotype could also indicate that Black Oak Lake has a pure native ancestry based on mtDNA, as Wilson and Hebert (1998) reported that some lake trout that exhibited the mitochondrial C haplotype dispersed from a southern (Mississippian or Missourian) refuge. Conversely, this fish could represent some genetic contribution of past stocking to Black Oak Lake, as the Lake Superior sources used to stock the lake contain moderate percentages of lake trout with haplotype C (Wilson and Hebert 1998). Even if this individual represents a mitochondrial contribution from a stocking source, introgression from exogenous sources appears to have been minimal.

By contrast, the mitochondrial data from the introduced populations (Big Green Lake and Lewis Lake) indicated their origin from Great Lakes sources. All three major mtDNA phylogeographic lineages observed by Wilson and Hebert (1996, 1998) were present in both introduced populations, which is concordant with data from Great Lakes populations and hatchery stocks (Grewe and Hebert 1988; Wilson and Hebert 1998). Although the different frequencies of the mtDNA lineages within Big Green Lake and Lewis Lake may reflect some genetic drift since these populations were established, there has been insufficient time and large enough population sizes to obscure their stocked origins (Table 4).

Microsatellite DNA Differentiation

Microsatellite data revealed strong levels of genetic differentiation between upper Mississippi River basin populations and Great Lakes (or Great Lakes–derived) sources. Although inland populations of lake trout (Trout and Black Oak lakes) contained the lowest levels of allelic variation, they were the most differentiated populations examined in this study. Their constrained population sizes and long isolation (10,000–12,000 years; Lyons 1984) have probably enhanced their differentiation through genetic drift and local adaptation. The Great Lakes populations were somewhat differentiated from each other based on microsatellite-based F_{ST} estimates, but they were more similar to each other than to the inland lakes because of their shared ancestry, geographic proximity (GIS and MQ), and recent historical connections through stocking (BG and LL).

The apparently contradictory results for the two genetic marker systems reinforce the probable native status of the lake trout populations in Trout and Black Oak lakes. Both populations were fixed or nearly so for the Mississippian mtDNA lineage and have retained their phylogeographic (Mississippian) ancestry (Wilson and Hebert 1998). By contrast, the diverse mtDNA composition of the introduced inland populations (Big Green Lake and Lewis Lake) reflects their founding from diverse ancestral (Great Lakes) sources (Grewe and Hebert 1988). Similarly, the substantial nuclear DNA differentiation between Trout Lake and Black Oak Lake (F_{ST} = 0.162) reflects their historical isolation and subsequent divergence,

whereas the lower F_{ST} values among the other populations reflect substantial gene flow, either through natural or anthropogenic means. Interestingly, both sets of genetic data reflect the close affinities between the introduced inland populations (Big Green Lake and Lewis Lake) and the Great Lakes hatchery strains, indicating their recent establishment and comparative lack of divergence.

Other aspects of the genetic data similarly support the hypothesis of native gene pools and argue against introgression from stocked fish. Both Trout Lake and Black Oak Lake populations had substantially lower allelic richness than Great Lakes strains or the introduced inland populations with respect to both mtDNA haplotypes and microsatellite loci, which is consistent with genetic drift or coalescence within limited populations over long periods of time. The comparable levels of heterozygosity across populations (Appendix 1) indicate that this is not an artifact of sampling effort or inbreeding but reflects the genetic diversity present within the studied populations.

The ability to identify distinct strains is contingent on the degree of genetic differentiation among populations (Cornuet et al. 1999). In this study, the inland populations were highly differentiated from stocking source populations and each other based on the combined mitochondrial and microsatellite DNA data. As a result, the individual assignment tests based on the microsatellite loci resulted in high assignment success for highly differentiated populations, including Trout Lake and Black Oak Lake. The largest proportion of misclassifications was attributed to the GIS strain, which was poorly differentiated from the other populations. Guinand et al. (2003) provided evidence that contemporary Lake Superior populations, including GIS and Isle Royale, had closer genetic affinities with contemporary hatchery-reared fish than with historic (prestocked) wild fish, indicating that these populations may have introgressed with native stocks. Conversely, successful large-scale facilitated gene flow through the introduction or introgression of genes from donor (''source'') populations would also reduce assignment power. Situations such as these can confound assignment tests through the homogenization of formerly distinctive strains, resulting in poor assignment success for individual populations.

Conservation and Management Implications

Based on both direct and indirect estimates of gene flow, our results show that there was minimal genetic contribution of exogenous lake trout in the Trout and Black Oak Lake populations. This contrasts with the results from numerous studies that have documented introgression and negative impacts of cultured salmonids on congeneric or conspecific populations through hybridization, introgression, and competition (Allendorf and Leary 1988; Krueger and May 1991). Although it is not known why so little introgression occurred in these populations, Evans and Olver (1995) and Powell and Carl (2003) discuss several ecological hypotheses that could merit investigation with neutral genetic markers.

The results from this study have several conservation and management implications. The populations of lake trout in Trout and Black Oak lakes are small and isolated and may constitute the most representative (unmixed) remnants of the Pleistocene Mississippian refugial race of lake trout. As such, these populations may represent unique biodiversity elements for the species and should be given a high level of protection. A concerted effort should be put made to shield Trout and Black Oak lakes from additional human-mediated impacts, including overfishing, habitat loss, and genetic degradation.

The current management plan for Trout Lake includes angling harvest regulations that protect 95% of the population and closed fishing seasons (Jahns and Bozek 2000). A similar plan does not currently exist for Black Oak Lake, but one is being developed. Additional research should focus on the life history and population structure of lake trout within these lakes. Collecting data for existing life history and exploitation models for lake trout, as in Shuter et al. (1998), could provide valuable and cost-effective management information.

Future management plans should include safeguarding the genetic integrity of these populations and exclude exogenous stocking or transfers. Presently, Black Oak Lake has a self-sustaining population at the average lake trout density for a lake of its size, whereas Trout Lake is below average in density and fish numbers are sustained by supplemental (same-source) stocking. Unfortunately, supplemental stocking can have long-term negative genetic impacts through decreases in effective population size (Krueger and May 1991; Ryman et al. 1995; Laikre and Ryman 1996; but see Duchesne and Bernatchez 2002). However, continued genetic monitoring can help to detect these effects. Alternatively, an annual monitoring program addressing the differential contribution of hatchery and wild fish to future generations, through either

the incorporation of additional genetic studies or continued usage of traditional mark–recapture methodologies, should be implemented.

As lake trout populations face increasingly diverse pressures across the species' range, resolving the ancestry and status of individual-lake populations should help fisheries professionals and management agencies assess options for regional, lake-specific management strategies. Regardless of stocking or exploitation histories, management agencies should make a concerted effort to identify native populations in order to protect their long-term viability and safeguard these evolutionary and adaptive resources for the species as a whole.

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Appendix: Genetic Variability of Lake Trout

TABLE A.1.—Estimates of allele frequencies and measures of genetic variability for lake trout populations by locality. Population abbreviations are as follows: TL, Trout Lake; BO, Black Oak Lake; BG, Big Green Lake; LL, Lewis Lake; GIS, Gull Island Shoal; and MQ, Marquette. See Table 1 in the text for more information on these populations.

		Population					
Locus and statistic	Allele	TL	BO	BG	LL	GIS	MQ
Ots1	215	0	0	0	0.014	0	0
	217	0	0	0	0	0	0.070
	219	0	0	0.198	0.108	0.385	0.420
	221	0.010	0.013	0.279	0.014	0.026	0.100
	223	0	0.013	0.035	0	0.038	0.040
	225	0.010	0	0.186	0.230	0.115	0.040
	227	0	0	0.023	0.068	0.026	0.010
	229	0.010	0	0.012	0.027	0.064	0
	231	0.073	0.013	0.047	0.149	0.103	0.020
	233	0	0.038	0.012	0.095	0	0.040
	235	0.021	0.050	0	0.014	0.038	0
	237	0.031	0	0	0	0	0
	239	0.010	0	0.023	0.014	0.013	0.030
	241	0	0	0	0.014	0	0
	243	0.010	0	0	0	0	0.060
	245	0	0	0.047	0.054	0.051	0.050
	247	0	0	0.023	0.122	0.013	0
	249	0.010	0.075	0	0	0.026	0
	251	0.063	0.325	0	0	0.013	0.010
	253	0.302	0.088	0	0	0	0.060
	255	0.219	0.088	0.070	0.027	0.051	0.030
	257	0.125	0.238	0.035	0.054	0	0
	259	0.104	0.050	0.012	0	0.026	0.020
	261	0	0	0	0	0.013	0
	263	0	0.013	0	0	0	0
Sfo18	172	0.177	0.213	0.593	0.649	0.577	0.540
	174	0.188	0.063	0	0	0	0
	176	0	0	0	0	0.013	0.040
	182	0.635	0.700	0.314	0.216	0.282	0.300
	184	0	0.025	0.093	0.108	0.026	0
	186	0	0	0	0.027	0.013	0.010
	188	0	0	0	0	0.090	0.110
SfoC24	81	0	0	0	0	0.013	0
	84	0	0	0	0	0.013	0
	90	0	0	0	0	0	0.020
	93	0	0	0	0.027	0	0
	96	0	0	0.023	0	0	0.010
	102	0.635	0.463	0.233	0.243	0.218	0.380
	105	0.365	0.238	0.605	0.622	0.667	0.440

TABLE A.1.—Continued.

Locus and statistic	Allele	Population					
		TL	BO	BG	LL	GIS	MQ
	108	0	0	0	0	0	0.010
	111	0	0.300	0.140	0.095	0.090	0.130
	114	0	0	0	0.014	0	0.010
SfoD75	272	0	0	0	0	0	0.020
	274	0	0	0	0	0	0.040
	282	0	0	0	0	0	0.020
	286	0.031	0.038	0	0.041	0.038	0.020
	290	0.010	0.250	0.093	0	0.154	0.100
	294	0.094	0.125	0.128	0.027	0.051	0.130
	298	0	0.125	0.023	0.122	0.103	0.150
	302	0.146	0.038	0.105	0.351	0.167	0.050
	306	0.240	0.225	0.419	0.243	0.179	0.030
	308	0	0	0	0	0	0.020
	310	0.260	0.163	0.093	0.054	0.115	0.110
	312	0	0	0	0	0.026	0
	314	0.083	0.025	0.105	0.081	0.064	0.170
	318	0.104	0.013	0.035	0.068	0	0.060
	322	0.021	0	0	0.014	0.038	0
	326	0	0	0	0	0.026	0.060
	330	0	0	0	0	0.026	0.020
	334	0.010	0	0	0	0	0
	350	0	0	0	0	0.013	0
Sfo23	169	0	0	0	0	0.026	0
	175	0.010	0	0	0	0	0
	179	0	0	0	0.041	0	0
	181	0.052	0.013	0.012	0.027	0.038	0.010
	183	0.010	0	0	0	0.038	0
	187	0.125	0.213	0.151	0.189	0.090	0.010
	189	0	0	0	0.014	0.026	0.050
	191	0.219	0.025	0.023	0.041	0.051	0.020
	193	0	0	0.105	0	0.013	0
	195	0.021	0	0.023	0	0.013	0
	199	0	0	0	0.027	0.038	0
	201	0.010	0	0	0.014	0.051	0
	203	0	0	0	0.014	0.038	0
	205	0	0	0	0.041	0.026	0.030
	207	0.042	0	0.116	0.122	0.026	0.020
	209	0.146	0.038	0.070	0.068	0.051	0.050
	211	0.063	0.075	0	0.041	0.038	0.090
	213	0.063	0.038	0.023	0.027	0.038	0.120
	215	0.052	0	0.105	0.095	0.141	0.130
	217	0.031	0.025	0.081	0.068	0.103	0.030
	219	0.042	0	0.058	0.041	0.051	0.280
	221	0.031	0	0.105	0	0.051	0.070
	223	0.063	0.113	0.058	0.054	0.013	0.020
	225	0.010	0	0.035	0.054	0	0.030
	227	0	0.138	0	0.014	0	0.010
	229	0	0.025	0	0	0.013	0.010
	233	0.010	0.063	0.023	0.014	0.013	0
	235	0	0.025	0	0	0	0
	237	0	0.125	0.012	0	0.013	0
	239	0	0.038	0	0	0	0
	241	0	0.025	0	0	0	0
	243	0	0.013	0	0	0	0
	245	0	0.013	0	0	0	0.010
	247	0	0	0	0	0	0.010
Sco19	158	0	0	0	0	0	0.010
	160	0	0	0.174	0.095	0.179	0.050
	162	0	0.013	0	0	0	0
	164	0	0	0.047	0	0.013	0.070
	166	0.010	0	0.012	0.027	0	0

TABLE A.1.—Continued.

Locus and statistic	Allele	Population					
		TL	BO	BG	LL	GIS	MQ
	168	0	0	0	0	0.026	0
	170	0.865	0.300	0.349	0.230	0.308	0.390
	172	0.094	0.125	0.012	0.027	0.026	0.020
	174	0.031	0.500	0.326	0.595	0.372	0.400
	176	0	0.063	0	0.027	0.051	0.030
	178	0	0	0.081	0	0.026	0.030
SfoC28	251	0.846	0	0.100	0.750	0.603	0.387
	263	0.077	0.027	0.175	0	0.103	0.242
	266	0	0	0	0	0.034	0
	275	0	0.095	0	0	0	0
	278	0	0	0	0.067	0	0
	281	0	0.419	0.550	0	0.103	0
	284	0	0.392	0.025	0.050	0.069	0.145
	287	0	0.068	0	0.133	0.086	0.129
	290	0.077	0	0	0	0	0.097
	293	0	0	0.150	0	0	0
Sfo8	263	0	0	0.012	0	0	0
	265	0	0	0.058	0.014	0	0
	269	0	0	0	0	0	0.020
	271	0	0	0	0	0.013	0
	273	0	0.038	0.081	0	0.026	0
	275	0	0.338	0.047	0.135	0.013	0.050
	277	0.042	0	0.128	0.068	0.026	0.020
	279	0.104	0	0.058	0.176	0.077	0.070
	281	0.083	0.100	0.023	0.108	0.077	0.030
	283	0.188	0.075	0.105	0.081	0.090	0.320
	285	0.385	0.150	0.267	0.257	0.269	0.230
	287	0.073	0.113	0.151	0.095	0.295	0.100
	289	0.125	0.188	0	0.041	0.077	0.070
	291	0	0	0.012	0.014	0.013	0.060
	293	0	0	0	0.014	0.026	0.030
	297	0	0	0.058	0	0	0
Sfo12	253	0.135	0.075	0	0.108	0.077	0.100
	255	0	0.013	0.035	0.081	0.051	0.040
	257	0.865	0.913	0.965	0.797	0.872	0.860
	261	0	0	0	0.014	0	0
N		47	40	43	37	39	50
Observed heterozygosity		0.560	0.640	0.587	0.601	0.648	0.631
Mean number of alleles		7.0	7.2	7.9	8.7	10.3	10.0