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Yellow perch strain evaluation I: Genetic variance of six broodstock populations

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Abstract

As a prelude to strain selection for domestication and future marker assisted selection, genetic variation revealed by microsatellite DNA was evaluated in yellow perch, *Perca flavescens*, from four wild North American populations collected in 2003–2004 (Maine, New York, North Carolina, and Pennsylvania,), and two captive populations (Michigan and Ohio). For the loci examined, levels of heterozygosity ranged from H_e=0.04 to 0.88, genetic differentiation was highly significant among all population pairs, and effective migration ranged from low (N_em=0.3) to high (N_em=4.5). Deviation from Hardy–Weinberg equilibrium was regularly observed indicating significant departures from random mating. Instantaneous measures of inbreeding within these populations ranged from near zero to moderate (median F=0.16) and overall inbreeding levels averaged F_{IS}=0.18. Estimates of genetic diversity, Φ_{ST}, and genetic distance were highest between Michigan and all other broodstock groups and lowest between New York and Ohio. Genetic differentiation among groups did not correlate with geographic distance. Overall, the patterns of variation exhibited by the captive (Michigan and Ohio) populations were similar to patterns exhibited by the other wild populations, indicating that spawning and management practices to date have not significantly reduced levels of genetic variation.
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1. Introduction

The yellow perch, *Perca flavescens*, (Mitchell, 1814) is an ecologically significant component of many North American freshwater food webs including lakes, ponds, creeks, and rivers. Yellow perch have a native distribution throughout the Nearctic ecozone from South Carolina to Nova Scotia, westward throughout the Great Lakes region and the Mississippi Valley, and northward to the Red River Basin (Nelson, 1976). Yellow perch are carnivorous and feed on a wide variety of animals such as zooplankton, insect larvae, crayfish, and small fishes (Hildebrand and Schroeder, 1928). They are common prey to top predators such as the walleye, northern pike, muskellunge, and lake trout and are also consumed by herring gulls and diving ducks (Herman et al., 1964). Dramatic reductions in population sizes have been underway since approximately 1950 in most areas of the continental US (Eshenroder, 1977; Wells and Jorgenson, 1983; McComish, 1986; Marsden and Robillard, 2004) attributed primarily to predation, unusual weather, starvation, competition with other organisms that feed on plankton, novel parasites, and interference from

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exotic organisms, such as zebra mussels in the Great Lakes. Complicating the potential anthropogenic effects on survival, yellow perch populations have been observed in relatively unimpacted, native environments to undergo regular cyclic oscillations that are thought to be precipitated by a combination of demographic factors, intraspecific competition and cannibalism, and predator–prey dynamics (Sanderson et al., 1999). In contrast to reductions observed in native populations, yellow perch have been introduced to a large number of watersheds in the western U.S. and have become established in most areas where they were introduced (Coots, 1956). The most common impact of these introduced populations is competition for food (Coots, 1956) and predation on young native fishes (Echo, 1955).

A number of population genetic studies have been reported for *P. flavescens*. Leclerc et al. (2000) performed a comparison of microsatellite and mtDNA studies of genetic variability. They reported that the genetic variability determined by microsatellite typing was significantly higher than the variability inferred by mtDNA. More recently, Miller (2003) used microsatellites to determine the genetic structure of yellow perch in Lake Michigan and found appreciable polymorphism at the microsatellite loci, whereas prior studies of allozymes and mtDNA had shown little genetic variation.

In addition to its ecological importance, the yellow perch is a popular food item, a common public aquarium fish, a popular recreational angling resource, and it supports commercial fisheries in Lake Michigan, Lake Eerie, and Lake Huron (Malison, 2000). The yellow perch has a mild taste and firm flesh with low fat and phospholipid content, making it appealing to both consumers and restaurant industries and providing for a long-shelf life, resistance to freezer damage, and minimal problems with off-flavor and cooking odors (Malison, 2000). Despite the decline of yellow perch populations in the Great Lakes from 1950 to 1990, market demand for the fish remained high, illustrating strong consumer preferences for this particular seafood product (Malison, 2000; Manci, 2001). Today, yellow perch have a high market value compared to catfish, trout, and other freshwater species that are successfully aquacultured or have significant aquaculture potential (Malison, 2000). The average yellow perch fillet retail value in 2002 was $26/kg in the U.S. as compared to $11/kg retail for catfish (Kentucky State University, 2003) and $8–12/kg for fresh tilapia fillet (Lutz et al., 2003).

Because of the sustained high demand (despite the reduction in domestic supplies of yellow perch) and due to concern over micro-contaminant levels in Great Lakes fishes, there has been a tremendous increase in the interest in yellow perch aquaculture (Malison, 2000). However, despite the recent technical advancements in yellow perch aquaculture methods (Manci, 2001), this species still is considered in most areas as an "alternate aquaculture species." As part of the effort to enhance aquaculture production of yellow perch, Ohio State University has undertaken an O'GIFT (Ohio Genetic Improvement of Farmed-fish Traits) program to improve aquaculture production traits for yellow perch and other species. One component of our broodstock selection efforts entailed examination of genetic diversity in 6 geographically disparate stocks of yellow perch. The objectives of our study were to locate and optimize a large number of polymorphic microsatellites within the yellow perch genome, to utilize these molecular markers to conduct a population genetic analysis among relevant broodstock groups collected from a geographically broad range of native populations, and to utilize estimates of molecular genetic variation to discriminate among stocks.

2. Materials and methods

2.1. Broodstock collection

Similarly aged adult yellow perch were collected live during 2003–2004 from wild populations (Fig. 1) in Maine (ME, Sebasticook River; $n=96$), North Carolina (NC, Perquimans River, $n=62$), New York (NY, Erie Canal; $n=76$), Pennsylvania (PA, Lake Wallenpaupack; $n=97$), and from captive populations held in Michigan (MI, F_1 of an original broodfish collected from Saginaw Bay; $n=88$) and at Ohio State University South Centers (OH, F_x originally from Lake Erie; $n=73$). All broodfish were maintained in the Wet Laboratory, Ohio State University South Centers. Non-lethal biopsy (fin clip) specimens were collected upon arrival at the Center and preserved immediately in 70% ethanol.

2.2. Microsatellite identification and optimization

For each specimen, DNA was extracted from 50 mg of tissue according to the methods outlined by Waters et al. (2000). A microsatellite-enriched library was prepared according to the methods outlined by Li et al. (2007). From a suite of 200 microsatellite-containing sequences, 30 loci produced amplicons displaying at least 4 different alleles. Eleven of these polymorphic loci were selected for broodstock analysis to complement data collected for eight loci previously published by Leclerc et al. (2000), Kapuscinski and Miller (2000), Borer et al. (1999), and Wirth et al. (1999). Ultimately, sixteen loci yielded sufficient data for analysis of the 6 broodstock populations.

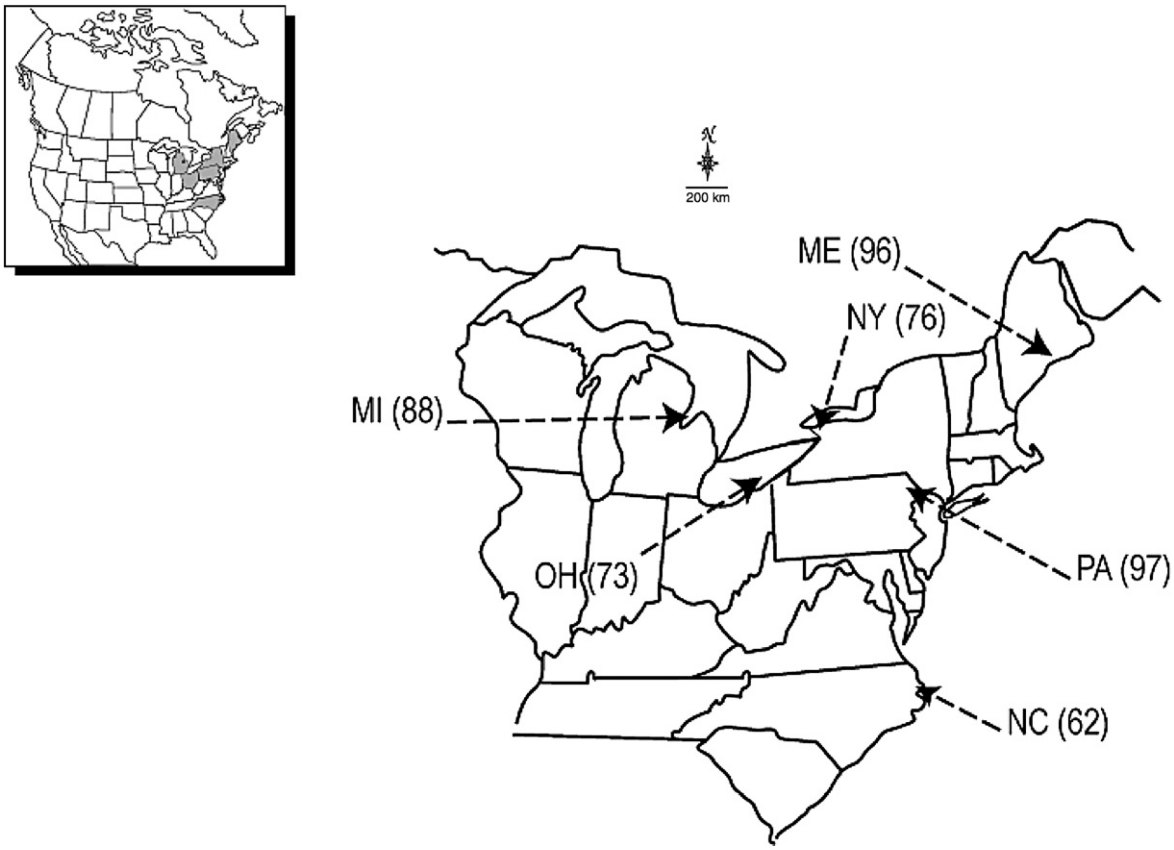

Fig. 1. Sites of capture for yellow perch *Perca flavescens* broodstock groups.

2.3. Broodstock genotyping

All primer sets were modified as described by Boutin-Ganache et al. (2001) with the addition of a unique sequence to the 5′ end of one of each pair (referred to hereafter as modified primer). PCR was performed using MJ Research PTC100 thermal cyclers as described by Li et al. (2007). The 5′-modified primers allowed use of the third fluorescently labeled primer in PCR, which facilitated pooling of PCR reactions and automated detection and genotyping using a BaseStation 51™ DNA fragment analyzer (MJ Research). Each lane of each ultra thin gel contained a 70–400 base pair ROX-labeled molecular marker (BioVentures). Genotypes were automatically scored using Cartographer® and individual genotypes were checked for accuracy and consistency.

2.4. Statistical tests

To calculate allele frequencies and genotypic proportions, GENEPOP Version 3.4 (Raymond and Rousset, 1995) was used. Linkage disequilibrium was tested with the probability test using a Markov chain method (Guo and Thompson, 1992) and global tests were performed across all populations with Fisher's method. Pairwise genetic differentiation among populations was calculated using exact tests for each locus. The significance of deviation from Hardy–Weinberg expectations was examined with exact *P*-values that were estimated using a Markov chain method, and where significant deviations occurred, tests for heterozygote excess and heterozygote deficiency for each locus were conducted. All Markov chain runs consisted of 1000 dememorization steps, 100 batches, and 1000 iterations. In each instance where multiple independent tests were performed, significance levels (α) were revised by Bonferroni correction (Rice, 1989). Multilocus inbreeding estimates, originally described by Ayres and Balding (1998) and subsequently illustrated by Dyer (2005) to be useful in consideration of inbreeding in wild populations, were examined in each of the 6 yellow perch samples. The distribution of inbreeding coefficients, *F*, was plotted to compare estimated levels of inbreeding.

Table 1
Microsatellite [1] genetic variation in *Perca flavescens* from six North American populations categorized as the number of alleles observed (*A*), heterozygosity observed (H_o) and expected (H_e), and the *P*-values for exact tests of fit to Hardy–Weinberg equilibrium (HWE)

Locus	Population						Mean
*Pfla*L2	ME	MI	NY	NC	OH	PA	
A	10	7	12	16	14	2	11
H_o	0.58	0.75	0.86	0.94	0.75	0.91	0.80
H_e	0.70	0.71	0.88	0.83	0.84	0.87	0.80
HWE	0.0136	0.5417	0.7702	0.0568	0.1067	0.9836	
*Pfla*L4							
A	29	25	18	23	16	21	22
H_o	0.58	0.57	0.64	0.88	0.90	0.63	0.70
H_e	0.87	0.91	0.91	0.94	0.92	0.88	0.91
HWE	0.0000	0.0000	0.0000	0.0000	0.0219	0.0000	
*Pfla*L5							
A	9	8	6	8	2	6	7
H_o	0.47	0.47	0.62	0.63	0.50	0.23	0.49
H_e	0.57	0.45	0.70	0.80	0.40	0.34	0.54
HWE	0.0000	0.0146	0.2125	0.0895	1.0000	0.0000	
*Pfla*L6							
A	5	10	13	14	13	15	12
H_o	0.27	0.68	0.73	0.51	0.83	0.56	0.60
H_e	0.25	0.59	0.81	0.68	0.87	0.75	0.66
HWE	0.8457	0.0459	0.0000	0.0286	0.0004	0.0000	
*Pfla*L9							
A	4	9	12	10	11	18	11
H_o	0.24	0.73	0.29	0.55	0.86	0.64	0.55
H_e	0.59	0.76	0.81	0.88	0.86	0.85	0.79
HWE	0.0000	0.0165	0.0000	0.0043	0.6077	0.0000	
YP1							
A	7	3	6	8	4	9	6
H_o	0.40	0.04	0.31	0.07	0.18	0.72	0.29
H_e	0.67	0.04	0.70	0.41	0.46	0.80	0.52
HWE	0.0000	1.0000	0.0000	0.0000	0.0014	0.0009	
YP6							
A	9	2	2	3	2	5	4
H_o	0.45	0.06	0.55	0.38	0.24	0.42	0.35
H_e	0.53	0.06	0.43	0.45	0.21	0.51	0.37
HWE	0.0000	1.0000	0.0293	0.3243	1.0000	0.0000	
YP7							
A	4	4	4	5	10	7	6
H_o	0.68	0.64	0.30	0.27	0.34	0.67	0.49
H_e	0.49	0.49	0.59	0.50	0.57	0.74	0.56
HWE	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	
YP9-1							
A	5	6	5	7	4	5	5
H_o	0.28	0.91	0.20	0.31	0.78	0.54	0.50
H_e	0.24	0.63	0.26	0.32	0.71	0.56	0.45
HWE	0.5666	0.0000	0.0013	0.0982	0.0000	0.0000	

(continued on next page)

Table 1 (*continued*)

Locus	Population						Mean
YP13	ME	MI	NY	NC	OH	PA	
A	12	6	11	12	7	14	10
H_o	0.43	0.45	0.43	0.44	0.36	0.70	0.47
YP13							
H_e	0.54	0.42	0.83	0.78	0.73	0.88	0.70
HWE	0.0015	0.9383	0.0000	0.0000	0.0000	0.0000	
YP16							
A	4	3	2	3	3	5	3
H_o	0.42	0.20	0.51	0.54	0.39	0.51	0.43
H_e	0.56	0.26	0.50	0.58	0.44	0.47	0.47
HWE	0.0000	0.0671	1.0000	0.3978	0.3004	0.6979	
YP17							
A	5	5	5	4	4	5	5
H_o	0.48	0.65	0.39	0.57	0.70	0.48	0.55
H_e	0.49	0.57	0.70	0.59	0.63	0.61	0.60
HWE	0.0000	0.0279	0.0000	0.7337	0.1012	0.0005	
YP30							
A	4	5	4	3	3	5	4
H_o	0.68	0.82	0.79	0.76	0.87	0.97	0.81
H_e	0.66	0.53	0.57	0.59	0.65	0.66	0.61
HWE	0.0621	0.0000	0.0000	0.0131	0.0003	0.0000	
YP66							
A	5	3	5	6	5	5	5
H_o	0.21	0.13	0.33	0.56	0.29	0.47	0.33
H_e	0.44	0.15	0.79	0.80	0.73	0.67	0.60
HWE	0.0000	0.0266	0.0000	0.007	0.0000	0.0000	
YP73							
A	6	4	6	4	4	5	5
H_o	0.08	0.09	0.07	0.22	0.27	0.04	0.13
H_e	0.70	0.32	0.58	0.74	0.56	0.35	0.54
HWE	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
YP79							
A	6	3	7	3	3	5	5
H_o	0.36	0.03	0.58	0.76	0.54	0.51	0.46
H_e	0.50	0.25	0.64	0.56	0.65	0.47	0.51
HWE	0.0085	0.0000	0.0005	0.0000	0.0000	0.0243	

Population abbreviations are ME: Maine, MI: Michigan, NY: New York, NC: North Carolina, OH: Ohio, and PA: Pennsylvania as described under "Sample collection".

[1] GenBank accession: P*fla*L2 (AF211827), P*fla*L4 (AF211829), P*fla*L5 (AF211830), P*fla*L6 (AF211831), P*fla*L9 (AF211834), YP1 (DQ826677), YP6 (DQ826678), YP7 (DQ826679), YP9-1 (DQ826680), YP13 (DQ826683), YP16 (DQ826685), YP17 (DQ826686), YP30 (DQ826689), YP66 (DQ826700), YP73 (DQ826704), YP79 (DQ826706).

Genetic structure was examined using Φ_{ST} calculated by AMOVA (Excoffier et al., 1992) as implemented by the population genetic software GeneticStudio (Dyer, 2005) and by estimating *F*-statistics with GENEPOP (Weir and Cockerham, 1984; Wright, 1946). In a manner similar to that proposed by Weitzman (1993), the relative contribution of each "strain" to the total genetic variance of the broodstock collection was evaluated by eliminating each from the AMOVA analysis. Molecular variance estimates were then utilized to determine the potential proportion of genetic variation that would be lost by disincorporating each group from the broodstock collection. F_{IS} was calculated across and within each stock. F_{ST} was calculated across all populations and for each population pair (Cockerham, 1973; Weir and Cockerham, 1984). As a further indication of population structure, inbreeding

coefficients were determined using GeneticStudio. Finally, Nei's standard genetic distance (D_S; Nei, 1987) was calculated for each population pair using MICROSAT Version 1.5d (Minch, 1997) and PHYLIP phylogenetic software (Felsenstein, 1993) was used to obtain a neighbor-joining tree (Saitou and Nei, 1987) based on D_S-values.

Isolation by distance was estimated using map distances (km) between each pair of populations and the relationship between genetic distance (D_S) and geographic distance was tested across all stocks with Mantel's (1967) general regression test (Z) as implemented by the population genetic software GeneticStudio. To further evaluate historical gene flow, effective migration rate (N_em) was computed with GENEPOP using private allele frequencies (Barton and Slatkin, 1986; Slatkin, 1985). This method relied on the expectation that private alleles reach high frequencies in populations when the migration rate is low enough to prevent homogenizing effects of out breeding.

3. Results

3.1. Genetic variation within broodstock groups

Across all stocks, a total of 223 alleles were detected at the 16 loci analyzed, 37% of which occurred at a frequency of 5% or lower. The average total number of alleles per locus ranged from a low of 3 for locus P2 to a high of 22 alleles for P4, with an overall mean of 7 alleles per locus. The frequency of private alleles was relatively low and averaged 5% across all broodstock samples tested. Only the Michigan broodstock exhibited population genetic results expected of a healthy wild population (conformation to HWE and both F and F_{IS} near zero). The remaining populations showed significant deviations from the Hardy–Weinberg equilibrium (Table 1). In all cases except MI, the broodstock populations were characterized by significant heterozygote deficits ($P<0.0001$) indicating departures from random mating. Bimodal distribution of allele frequencies for loci *Pfla*L2, *Pfla*L4, *Pfla*L5, YP1, YP9-1, YP13, YP17, YP30 and YP32 (not all populations for each locus) indicated the possibility of null alleles, subpopulation structure, or recent supplemental stocking in the ancestral populations. Although linkage disequilibrium over all loci was not prevalent, there were incidences of significant linkage disequilibrium in five of the 6 groups. Four instances of linkage were observed in PA, three in OH, two each in MI and NY, and one in NC, strongly indicating the possibility of null alleles at *Pfla*L6 and YP7. Single-locus inbreeding estimates, F_{IS}, ranged from a low of −0.55 (MI: YP30) to a high of 0.90 (MI: YP79) and averaged approximately 0.20 for all populations except MI (where average F_{IS} was 0.01). No evidence for inbreeding was detected for the Michigan

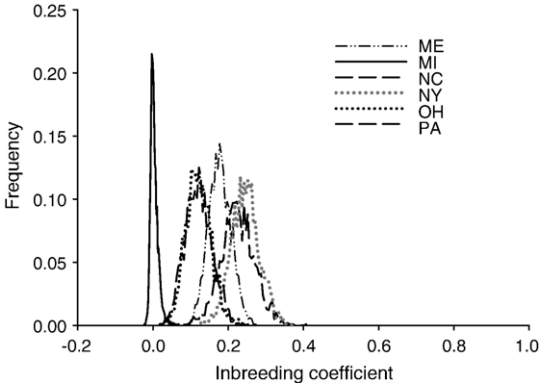

Fig. 2. Distribution of inbreeding coefficients within six *Perca flavescens* broodstock groups collected in North America (abbreviated as described in Table 1). Frequencies appear on the vertical axis and inbreeding coefficient values, F, along the horizontal axis.

Table 2
Microsatellite genetic variation in six *Perca flavescens* broodstock populations collected in North America categorized as the F_{ST} value (above the diagonal) and estimates of divergence (Φ_{ST} values) are below the diagonal (P-values shown in parenthesis)

Population	ME	MI	NC	NY	OH	PA
ME		0.445	0.185	0.179	0.189	0.207
MI	0.490 (0.000)		0.345	0.220	0.164	0.338
NC	0.251 (0.000)	0.455 (0.000)		0.106	0.122	0.091
NY	0.159 (0.000)	0.315 (0.000)	0.125 (0.000)		0.052	0.091
OH	0.146 (0.000)	0.191 (0.000)	0.064 (0.000)	−0.051 (0.999)		0.154
PA	0.208 (0.000)	0.453 (0.000)	0.198 (0.000)	0.115 (0.000)	−0.006 (0.952)	

Table 3
Microsatellite genetic variation in six *P. flavescens* broodstock populations collected in North America categorized using effective migration rate and genetic distances

Population	ME	MI	NC	NY	OH	PA
ME		1.15	0.36	0.37	0.40	0.45
MI	0.31		0.77	0.35	0.20	0.82
NC	1.06	0.36		0.28	0.31	0.41
NY	0.95	0.65	2.86		0.13	0.20
OH	1.04	0.88	0.68	1.60		0.40
PA	0.61	0.46	1.14	0.96	1.03	

Pairwise estimates of genetic distance (D_S) among populations are above the diagonal and estimates of effective migration rate (N_em) based on private alleles.

population using the multilocus method. Pennsylvania and Ohio demonstrated low estimates of inbreeding ($F<0.2$), the Maine group exhibited moderate inbreeding (median $F=0.2$), and inbreeding levels exhibited by the New York and North Carolina broodstock groups were high (median $F=0.3$). The distribution of these multi-locus inbreeding coefficients, F (Fig. 2), differed significantly between MI and the remaining five populations ($P<0.0001$).

3.2. Genetic variation among broodstock groups

Allele frequencies were not homogeneous among broodstocks for the loci tested, indicating significant genetic differentiation among all broodstock groups (each comparison $\chi^2=\infty$, $P=0.0000$). The overall Φ_{ST} value for the North American *P. flavescens* populations sampled in this study (0.242, $P<0.0001$) indicated that a large proportion of detectable genetic variation was found among the populations rather than within them; approximately 24.2% of the genetic diversity. Indeed, the highest observed value was between Maine and Michigan ($\Phi_{ST}=0.490$; $P<0.0001$). Conversely, a number of broodstock group pairs exhibited low divergence (Table 2), the lowest observed was between Ohio and New York ($\Phi_{ST}=-0.052$; $P=0.999$). By reassessing multilocus variance components, the potential effect on genetic diversity of the program as a result of eliminating any of the broodstock groups was −7% (OH), −14% (both NC and NY), −19% (ME), −22% (MI) and −28% (PA). Genetic subdivision, estimated by F_{ST}, yielded similar results, ranging between a low of 0.05 between Ohio and New York and a high of 0.44 between Maine and Michigan (Table 2). Pairwise estimates of genetic distance (D_S) among populations of *P. flavescens* ranged from 0.13 between New York and Ohio to 1.15 between Michigan and Maine (Table 3 and Fig. 3). Pairwise estimates of effective migration rate (N_em) among populations (Table 3) ranged from a low of 0.3 migrants/generation detected between the Maine and Michigan populations to high gene flow between Ohio and New York ($N_em=1.6$). Across the range of populations sampled for inclusion as broodstock, there was no significant relationship detected between genetic distance (D_S) and geographic distance ($Z=1917$, $P=0.639$).

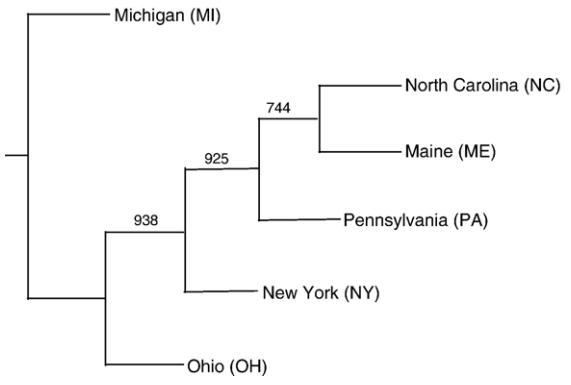

Fig. 3. Extended majority rule consensus neighbor-joining tree constructed from Nei's unbiased distance (D_S) values among six broodstock populations of *Perca flavescens* collected in North America. Bootstrap values at the nodes indicate the number of unambiguous branches at that point out of 1000 resampling events.

4. Discussion

4.1. Yellow perch exhibit relatively low levels of microsatellite polymorphism

The relatedness and genetic distance patterns observed in this study are consistent with previous reports. The average total number of alleles per locus observed was similar to prior published results for *P. flavescens*; Miller (2003) observed similar numbers of alleles (mean 8.7, range 3.2–19.1) and Leclerc et al. (2000 recorded 7.5 alleles per locus (range 2–18). The range of heterozygosity observed in this study ($H_o=0.04$ to 0.88) was broader than the 0.21 to 0.86 range observed by Miller (2003) and the 0.25 to 0.82 range observed by Leclerc et al. (2000) likely due to the larger sample sizes, larger number of populations surveyed, and the increased geographic range covered in the current study. Virtually the same level of population differentiation was recorded by Miller (2003) for eighty samples collected from two native spawning yellow perch populations of Lake Michigan. Miller (2003) also found similarly close genetic relationships among *P. flavescens* populations located in large lakes and river systems of the Lake Michigan and Green Bay regions. These observations imply that the levels of variation detected are likely an accurate reflection of the range of genetic variation in *P. flavescens*.

Given the levels of genetic variation detected within and among the founding populations of the yellow perch broodstock, it is essential to assess whether the base population for selective breeding is sufficiently genetically diverse to achieve the desired selection gain while simultaneously avoiding unintended inbreeding. For comparison, the levels of observed microsatellite podivergencelymorphism in these broodstock populations are somewhat greater than polymorphism observed for several other commercially produced aquaculture fishes. For example, wild populations of trout (*Salmo trutta*) typically exhibit four to 19 alleles per locus with an

average of nine (Was and Wenne, 2003), striped bass, *Morone saxatilis*, average four alleles per locus (Ross et al., 2004), catfish, *Ictalurus punctatus*, average four alleles per locus (Tan et al., 1999), and sunfish, *Lepomis* spp., average five alleles per locus (Neff et al., 1999). Conversely, these yellow perch broodstock groups exhibit lower levels of polymorphism than observed for rainbow trout, *Oncorhynchus mykiss*, where alleles per locus are as high as 17 (Nielsen et al., 1999) and tilapia, *Oreochromis niloticus*, populations, where it is common to detect an average of up to 20 alleles per locus (Fuerst et al., 2000; Romana-Eguia et al., 2004; Hassanien and Gilbey, 2005). Based on multi-locus genetic variance, the Weitzman analysis results suggested that all 6 strains, even the OH, had genetic value to the program. Thus, the current data indicate that for yellow perch, this prerequisite of a genetically diverse base population is met to the extent possible.

4.2. Departures from random mating

The current data indicate that non-random mating is common in extant yellow perch populations. Although Miller (2003) observed few deviations from the Hardy–Weinberg equilibrium in the samples taken from Lake Michigan, Leclerc et al. (2000) observed nine of ten microsatellites described to exhibit heterozygote deficits. Our results for the MI group were consistent with Miller's (2003) observations in that we found this sample to largely conform to Hardy–Weinberg expectations. It is notable that this group originated from a relatively large population, is in the first generation of captivity, and showed very little evidence of inbreeding (Fig. 2). Conversely, the data for other groups we examined conformed to Leclerc's observations in showing significant deviations from Hardy–Weinberg expectations at a majority of the loci tested (Table 1) accompanied by higher estimates of inbreeding *F*. The observed deviations from Hardy–Weinberg equilibrium are not likely a result of sampling bias as similar sampling strategies and sample sizes were employed across these studies. A number of demographic factors, however, are likely contributors to non-random mating in yellow perch. These include high variation in effective population size and unequal numbers of sexes (Shroyer and McComish, 2000), the contemporary dramatic decline in spawning populations (McComish, 1986; Marsden and Robillard, 2004) that could equate to a genetic bottleneck, and regular oscillations in yellow perch population sizes (Sanderson et al., 1999). Subpopulation structure (Wahlund, 1928) is another possible source of the observed reduction in heterozygosity in our samples from these populations. Reproductive isolation within the larger populations of yellow perch could be naturally occurring by means of assortative mating and/or competition. Kin cohesiveness, a type of positive assortative mating previously observed in African cichlids (Thünken and Bakker, 2007) and tilapia in particular (Pouyaud et al., 1999), could occur in yellow perch if related individuals aggregate. Competition during mating, as has been observed for tilapia (Fessehaye et al., 2006), differential timing of reproduction, or other mechanisms yet to be observed for partitioning of reproductive effort in yellow perch are other potential contributors to subpopulation structure. Alternatively, apparent subpopulation structure could be instead a manifestation of admixture due to recent supplemental stocking. Although we found no records of recent secondary or tertiary stocking at the capture sites, such activities are common and could contribute to the observed deviations from random mating. Lastly, harvesting, particularly of the larger females, is expected to result in high levels of inbreeding. In summary, there are a number of possible interrelated explanations that can account for the observed heterozygote deficits, none of which can be definitively confirmed or discounted from the present data. Nevertheless, based on the current analysis, inbreeding arises as an issue of relevance not only in the case of captive yellow perch, but also in the dwindling native populations.

4.3. Ramifications of inbreeding for yellow perch broodstock management and selective breeding

The NY origin of the captive OH population was corroborated by the population parameters as well as the taxonomic analysis. This pair exhibited the lowest genetic distance ($D_S = 0.13$), the highest effective migration rate ($N_e m = 1.6$), and the smallest Φ_{ST} (-0.05) and F_{ST} (0.05) values. Although the observed number of alleles was slightly lower for OH than for its founder NY, there was not a profound Founder Effect. Indeed, the wild populations from which these two groups were derived might both have originated from Lake Erie via the Erie Canal. Thus, an obvious concern in this selective breeding program is the potential for excessive levels of inbreeding in crosses between these two groups. Indeed, one of the most significant problems in aquaculture and fisheries is the decline in productivity of broodstocks due to unintentional inbreeding. It has been suggested that breeding programs of most fish farmers produce inbreeding rates of 3–5% per generation (Tave, 1999). Because yellow perch have been propagated in tanks and ponds at various locations with little or no genetic control since 1970's (Malison, 2000; Manci,

2001), one might suspect this problem to be imminent in commercial-scale production and selective breeding programs for yellow perch. In addition, these elevated levels of inbreeding in the newly captive broodstock populations are of concern because they exceed the conventional cutoff of 0.125, indicative of first cousin mating (Becker, 1992; Tave, 1993; Clark, 1998). Knowledge of inbreeding is extremely valuable to the current O'GIFT program because these estimates highlight the potential for unintentional inbreeding when crossing within and among broodstock populations. The molecular markers identified in this study are therefore being used in combination with standard pedigrees to facilitate use of the simple marker-assisted breeding scheme known as walk back selection (Doyle and Herbinger, 1994) to ensure that unintentional inbreeding is controlled. This strategy allows selection (Sonesson, 2007) for growth, feed conversion efficiency, size at maturity, etc., without negatively affecting the genetic background of the broodstock.

4.4. Utility of microsatellite markers for selective breeding in yellow perch

Prior to this effort, no analysis had been made of the effects of yellow perch broodstock management, particularly inbreeding, during initial stages of yellow perch domestication. The current analysis of microsatellite variation illustrates that the captive Ohio broodstock group (a group likely to show unintentional inbreeding as a result of artificial selection) has not been significantly impacted and shows levels of genetic variation similar to, and in some cases greater than, the wild groups surveyed. Levels of variation revealed by the current set of microsatellites are adequate for constructing molecular pedigrees and for estimating genetic relatedness among potential spawning pairs thereby facilitating efforts to avoid unintentional inbreeding. Combining data for superior phenotypes with data for relatedness will be an effective foundation on which to base efforts designed to increase the economic value of aquacultured yellow perch. However, because of the relatively low numbers of alleles exhibited for these yellow perch microsatellite loci, even for dinucleotides, we now know that to create a reasonably high density genome map, additional higher polymorphism markers (e.g., SNP, AFLP) will likely be necessary.

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