

## Genetic Structure and Diversity among Brook Trout from Isle Royale, Lake Nipigon, and Three Minnesota Tributaries of Lake Superior

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*Abstract.*—Brook trout *Salvelinus fontinalis* from Isle Royale, Michigan, three Minnesota tributaries of Lake Superior, and Lake Nipigon in Ontario were analyzed for genetic variation at 12 microsatellite DNA loci. Analysis of molecular variance, genetic distance measures, and cluster analysis were used to examine the diversity, gene flow, and relatedness among the samples. The diversity estimates for the samples from Isle Royale were similar to those for the samples collected from Minnesota tributaries of Lake Superior, and all estimates were lower than those reported in other studies of brook trout from eastern North America. Genetic differences were detected among the brook trout at Isle Royale, Lake Nipigon, and the Minnesota tributaries of Lake Superior. Further, the population in Tobin Harbor at the eastern end of Isle Royale was distinct from the populations from tributaries at the southwestern end of the island. The Minnesota tributary population formed a group that was genetically distinct from those from Isle Royale and Lake Nipigon. The Isle Royale population should be managed to preserve the genetic and phenotypic variation that distinguishes it from the other brook trout populations analyzed to date.

Brook trout *Salvelinus fontinalis* are native to eastern North America, although their range has been extended by human activities such as stocking (Behnke 1972). Brook trout are found most often in freshwater streams and small lakes, but anadromous populations are found on the east coast of North America (Behnke 1972; Scott and Crossman 1973), and potadromous brook trout are found in large lakes such as Lake Superior and Mistassini Lake, Quebec (Power 1980; Becker 1983).

Protection and restoration of brook trout is a goal of management agencies in the Lake Superior region (Newman et al. 2003; Wisconsin Department of Natural Resources and U.S. Fish and Wildlife Service 2005; Schreiner et al. 2006, 2008; Ridgeway 2008). Habitat loss, overfishing, disruption of spawning runs by construction of dams for power generation or water-

level, control disruption of spawning habitat, and stocking may have reduced the number of brook trout and eroded the genetic integrity of brook trout populations in Lake Superior (Kelso and Demers 1993; Schreiner et al. 2008; Wilson et al. 2008). A strategic plan for brook trout rehabilitation in Lake Superior (Newman et al. 2003) adopted by federal, state, and tribal management agencies described a need for genetic data as well as identification and protection of critical spawning and nursery habitat, stream restoration, population genetics, and hatchery propagation and stocking. Lack of information on life history diversity, habitat use, and genetic population structure has been an impediment to restoration and management actions for brook trout in Lake Superior (Newman et al. 2003; Schreiner et al. 2008).

Brook trout populations are present in numerous locations at Isle Royale National Park, Michigan, in Lake Superior (including Tobin Harbor, Siskiwit Bay, the Big and Little Siskiwit rivers), and in Washington and Grace creeks (J. W. Slade, U.S. Fish and Wildlife

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Service, memorandum to Jack Oelfke, Isle Royale National Park, on the Isle Royale brook trout assessment, 1995; Quinlan 1999; Newman et al. 2003). Brook trout populations along the coast of Isle Royale are believed to be less impacted by disturbances than other populations in Lake Superior (Quinlan 1999). Brook trout gametes from Isle Royale were used to create two new brook trout hatchery strains (Siskiwit Bay and Tobin Harbor). These strains are stocked into American waters of Lake Superior to reintroduce the coaster brook trout (Schreiner et al. 2008). Coaster brook trout are a migratory form of brook trout that spend part of their life in Lake Superior (Becker 1983). Isle Royale supports some of the few remaining populations of coaster brook trout (Newman et al. 2003).

Burnham-Curtis (2001) used mitochondrial DNA (mtDNA) variation to assess the population structure of brook trout from wild collections from Lake Superior and Lake Huron, including Isle Royale. While there was significant variation within Lake Superior collections, the study found little geographic structure among the collections, and the observed patterns of mtDNA diversity appeared to describe patterns of postglacial recolonization by brook trout. Burnham-Curtis (2001) suggested that microsatellite DNA markers be used to further examine the genetic structure of brook trout from Lake Superior, and also suggested that further work should assess the relationship of brook trout from Isle Royale to brook trout from mainland tributaries. Describing and understanding the population structure of brook trout from Isle Royale will be useful to managers developing management and recovery plans (Newman et al. 2003). Park biologists are currently working to develop a fisheries management plan for Isle Royale National Park; any information on stock composition of aquatic species will help identify areas of interest either for protection or research (Lafrancois and Glase 2005). Therefore, we used nuclear DNA markers (microsatellite DNA loci) to evaluate the genetic structure of brook trout from Isle Royale and also analyzed samples from Minnesota tributaries of Lake Superior and Lake Nipigon to place the genetic variation observed at Isle Royale in a broader geographic context.

### Methods

*Sample collection.*—We analyzed samples of brook trout from Lake Nipigon (1997) and three tributaries (the Onion River, Kadunce Creek, and the Cross River) along the Minnesota shoreline to compare genetic variation of Isle Royale populations with that of populations from nearby Lake Superior tributaries (Figure 1). Most of the samples were collected during

the fall and were not classified as either coaster or resident brook trout. Details of field sampling programs are found in Slade (1995), Tilma et al. (1999), Quinlan (1999), and Burnham-Curtis (2001). Tobin Harbor was sampled in September and October of 1996, 1998, and 2001. The Big Siskiwit River was sampled in October 1994 and 1995. Grace and Washington creeks were sampled in October 1994. Three Minnesota tributaries of Lake Superior were sampled below barriers to Lake Superior during fall surveys; the Onion River was sampled in October 1995 and 1997, Kadunce Creek in October 1997, and Cross River in October 1997. Fin clips from brook trout were stored in a 95% solution of ethanol or Queen's buffer (Seutin et al. 1991) and transported to the Great Lakes Science Center, U.S. Geological Survey, for genetic analysis.

*Laboratory analysis.*—We extracted DNA using the Qiagen protocol and reagents. The extracted DNA was examined for quality and quantified, and microsatellite DNA loci were amplified using the polymerase chain reaction (PCR). Twelve microsatellite DNA loci designed for brook trout were used in the survey: *SfoB52*, *SfoC24*, *SfoC28*, *SfoC38*, *SfoC79*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC115*, *SfoC129* (King et al. 2002), *Sfo12*, and *Sfo18* (Angers et al. 1995). Polymerase chain reaction was carried out in 15- $\mu$ L volumes using 1 U *Taq* DNA polymerase (Promega), the manufacturer's buffer, 0.3 mM each deoxynucleotide triphosphate, 0.4  $\mu$ M of each primer, 1.5 mM  $MgCl_2$ , and 90–120 ng template DNA. The PCR thermal profile was similar for all loci; only the annealing temperature was altered (Table 1). An initial denaturation step of 2 min at 94°C was performed, followed by 35 cycles of 1 min at 94°C, 1 min at the annealing temperature, and a 1-min extension at 72°C.

The PCR products were prepared according to manufacturer's guidelines (Applied Biosystems) for capillary electrophoresis. Each PCR product was diluted with 9  $\mu$ L of water, and then formamide and a 400-base-pair size standard (labeled with ROX) were added to 1  $\mu$ L of the diluted PCR product. Samples were denatured for 4 min and chilled for 3 min before they were loaded on the ABI Prism 3100-AVANT genetic analyzer. Fragment size data were collected and genotyped using the manufacturer's software (Applied Biosystems).

*Data analysis.*—Sample sites and loci were tested for deviation from Hardy-Weinberg equilibrium using the probability test described by Raymond and Rousset (1995a). The significance level for multiple tests was adjusted using the sequential Bonferroni technique (Rice 1989). Heterozygosity, standardized allelic richness per locus (Goudet 1995), and number of alleles per locus were calculated for each locus and

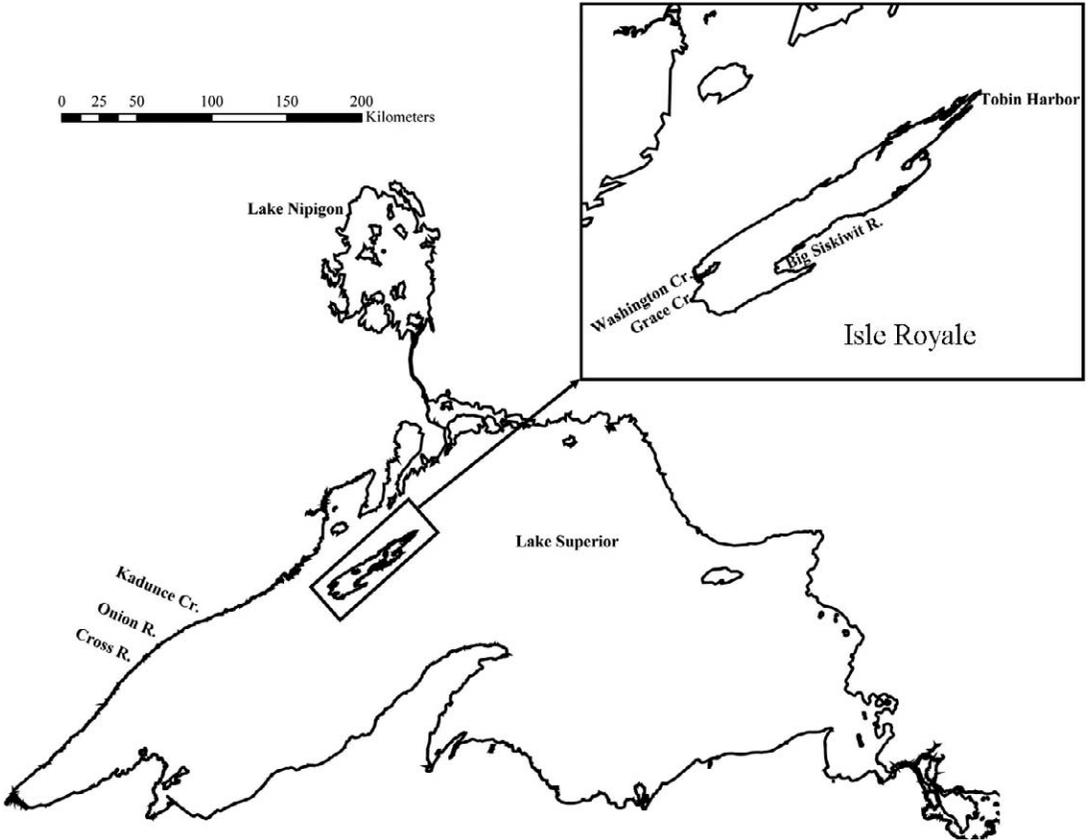


FIGURE 1.—Sampling locations for brook trout collected from Lake Superior and Lake Nipigon for genetic analysis.

collection site. The GENEPOP software package (Raymond and Rousset 1995b) was used to test the homogeneity of allele frequencies between all pairs of populations, and the *P*-values were also adjusted with the sequential Bonferroni technique (Rice 1989).

Genetic distances among the samples were estimated using the Cavalli-Sforza–Edwards chord distance measure (Cavalli-Sforza and Edwards 1967). The

neighbor-joining method was used to construct a dendrogram of the genetic relationships among samples (Saitou and Nei 1987). The software package PHYLIP (Felsenstein 1993) was used to perform the analyses. The data set was resampled using the bootstrapping procedures available in PHYLIP, and 1,000 replicates were used to create a consensus tree. The resulting dendrogram was visualized using Tree-View (Page 1996), showing bootstrap values for branches of 60% or greater support.

We also employed an individual-based approach to explore the relationships among brook trout in our sample set. We used a Bayesian approach implemented in the software package STRUCTURE (Pritchard et al. 2000) to determine how many genetically distinct clusters existed in the data set. A cluster may contain one or more collections of brook trout. The number of clusters (*K*) is inferred from the posterior probability distribution calculated using the posterior probability of the genotypes of each individual. However, Pritchard et al. (2000) warned that the posterior probability distribution is only a guide to the number of clusters

TABLE 1.—Annealing temperatures for the microsatellite loci used in the analysis of Lake Superior brook trout.

Locus	Temperature (°C)	Reference
<i>Sfo12</i>	60	Angers et al. 1995
<i>Sfo18</i>	56	Angers et al. 1995
<i>SfoB52</i>	58	T. King, unpublished data
<i>SfoC113</i>	58	T. King, unpublished data
<i>SfoC115</i>	58	T. King, unpublished data
<i>SfoC129</i>	58	T. King, unpublished data
<i>SfoC24</i>	58	T. King, unpublished data
<i>SfoC28</i>	58	T. King, unpublished data
<i>SfoC38</i>	58	T. King, unpublished data
<i>SfoC79</i>	58	T. King, unpublished data
<i>SfoC86</i>	58	T. King, unpublished data
<i>SfoC88</i>	58	T. King, unpublished data

TABLE 2.—Sample sizes and diversity statistics for brook trout collections analyzed for microsatellite DNA variation. Averages are for the 12 loci used in the analysis. Abbreviations are as follows:  $N$  = sample size,  $H_e$  = expected heterozygosity,  $H_o$  = observed heterozygosity,  $N_a$  = number of alleles,  $R_s$  = allelic richness.

Collection	$N$	$H_e$	$H_o$	$N_a$	$R_s$
Big Siskiwit River	18	0.47	0.42	3.64	3.36
Cross River	24	0.68	0.57	5.18	4.64
Grace Creek	29	0.56	0.55	4.91	4.12
Kadunce Creek	30	0.68	0.57	5.18	4.50
Lake Nipigon	33	0.34	0.29	3.82	2.83
Onion River, 1995	23	0.61	0.55	4.18	3.75
Onion River, 1997	52	0.66	0.57	6.45	4.60
Tobin Harbor, 1996	57	0.56	0.52	4.82	3.90
Tobin Harbor, 1998	30	0.49	0.45	4.09	3.43
Tobin Harbor, 2001	53	0.57	0.52	4.55	3.67
Washington Creek	29	0.59	0.60	5.55	4.45

and users should not choose a value of  $K$  based solely on the value of the posterior probability, but should also look at how much information is gained as  $K$  changes. This consideration is important when the data set does not conform to an isolation by distance model, when there is a hierarchical arrangement among the populations, or when gene flow is uneven (Rosenberg et al. 2002; Garnier et al. 2004; Evanno et al. 2005). For a data set with a complex structure or a large number of clusters,  $K$  will increase quickly to reach a value that accounts for the most divergent groups and then the rate of change will decrease until the most likely value of  $K$  is reached (Pritchard et al. 2000; Rosenberg et al. 2002; Garnier et al. 2004). We expected a hierarchical arrangement among the collections because there are geographical barriers among them. Examples of barriers to brook trout movement include: barriers on rivers, the distance among sites, and restrictions on movement due to temperature, oxygen, or depth preferences (Mucha 2004).

We used two approaches to determine what the most likely value(s) of  $K$  would be. First, we calculated mean log-likelihood probabilities ( $\log_e[P\{X | K\}]$ ) for the 10 replications at each level of  $K$ . Then we used the approach suggested by Evanno et al. (2005) and calculated the second-order rate of change of  $K$  ( $|L'K| = |L'[K + 1] - L'[K]|$ , where  $L'[K] = L[K] - L[K - 1]$  and  $L[K]$  is the mean likelihood over 10 runs at each value of  $K$ ). We calculated the percent of the total rate of change that was associated with each value of  $K$ . We sought the value of  $K$  that captured the most information or the point at which the change in the posterior probability starts to level off. It is possible that several solutions may exist for  $K$ , representing different levels of substructure. We calculated the variance components for different values of  $K$  using

analysis of molecular variance (AMOVA). For each level of clustering we discovered, the data set was split into the corresponding groups and then analyzed again. We ran the initial simulations (with  $K = 1-12$ ) and did 10 replications for each value of  $K$ . We ran the simulations without using the collection site information and using an admixture model. We ran 100,000 replications before data were collected (burn-in) and ran 100,000 replications after the burn-in period. Finally, we also used AMOVA (Schneider et al. 1997) to measure how diversity was partitioned within and among the groups for each value of  $K$ . A well-supported value of  $K$  will be characterized by a low within-group variance and high between-group variance components.

## Results

All 12 loci were polymorphic within and among brook trout collections from Isle Royale, Minnesota, tributaries of Lake Superior, and Lake Nipigon. The small sample sizes taken in two separate years from the Big Siskiwit River were combined for the analysis because no significant differences in allele frequencies were observed between the two samples (data not shown). A total of 378 brook trout were used in the analysis, and the sample size per collection site ranged from 18 to 57 (Table 2). Genotype data, basic biological data, and details of the laboratory analysis for all the brook trout are stored in the genetics database maintained by the Great Lakes Science Center.

Hardy-Weinberg equilibrium was tested for each locus-collection combination. One hundred thirty-two tests were performed and 16 (12.1%) were significant at the 0.05 level, which is more than what is expected by chance alone. Twelve tests remained significant after Bonferroni correction; four of these were observed at *Sfo18*, three at *Sfo12*, and the rest were single tests at other loci. Three of the four significant tests at *Sfo18*, and all of the tests for *Sfo12* were due to heterozygote deficiencies. The remaining significant tests at the other loci were due to both heterozygote deficiency and excess. None of the collections were characterized by deficiencies across all loci. Heterozygote deficiencies in microsatellite DNA loci can be caused by a number of factors, including the presence of null alleles, nonrandom sampling, sampling more than one population, or scoring errors; however, it is often difficult to determine what the cause is (Castric et al. 2002). Because the deviations were not the result of collection-level effects, we did not adjust the raw data to fit to Hardy-Weinberg equilibrium.

The average observed heterozygosity over all loci was lowest in the samples from Lake Nipigon (0.29;

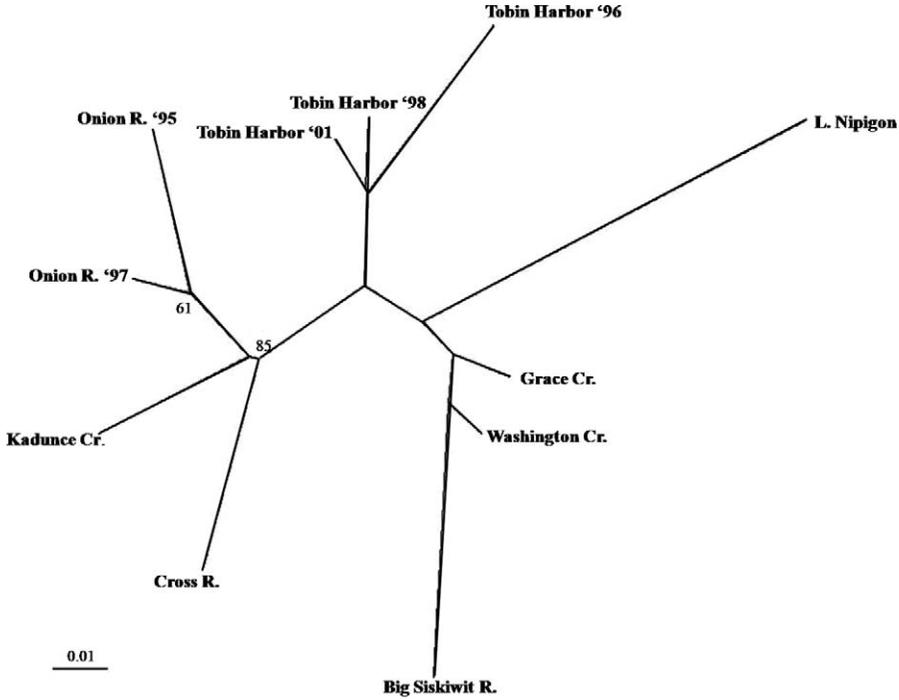


FIGURE 2.—Neighbor-joining diagram of Cavalli-Sforza-Edwards distances among brook trout collections from Lake Superior and Lake Nipigon. The numbers on the branches indicate the degree of bootstrap support. Only branches with more than 60% support are labeled.

Table 2) and highest in Washington Creek (0.60) samples. Estimates of observed heterozygosity over all collections from Isle Royale (0.51) were lower than the estimates for collections from the three Minnesota tributaries (0.55; Cross Creek, Kadunce Creek, Onion River). We used estimates of allelic richness to account for variation in sample size (effective sample size = 11) and found that the results were similar to those observed using the heterozygosity estimates (i.e., the allelic richness was highest in the Cross River [4.64; Table 2] samples and lowest in samples from Lake Nipigon [2.83, Table 2]). The mean value for all the Isle Royale collections (3.82) was a little lower than the value for the collections from Minnesota (Cross Creek, Kadunce Creek, and Onion River; 4.37). The average number of alleles per locus was lowest in the Big Siskiwit River samples and highest in the Onion River (1997) samples.

Genetic relationships among the sampled brook trout populations were summarized in a neighbor-joining diagram (Saitou and Nei 1987) based on Cavalli-Sforza-Edwards chord distances (Cavalli-Sforza and Edwards 1967). Comparisons were made among 1,000 bootstrap replicates of the original frequency distributions to estimate a consensus tree (Figure 2). Two of

the branch nodes received greater than 60% support on the consensus tree. The samples from the southwestern end of Isle Royale (Big Siskiwit River, Washington Creek, and Grace Creek) grouped together. There was separation between the Big Siskiwit River and other sites at the southwestern end of Isle Royale, and among collections from the southwestern end and Tobin Harbor at the northeastern end of Isle Royale. The collections from Minnesota (Cross River, Kadunce Creek, and Onion River) grouped together on the consensus tree. The samples from Lake Nipigon were grouped with the Isle Royale samples on a distinct branch.

We calculated the posterior probability distribution for different values of *K* in STRUCTURE and found that the posterior probability distribution reached a maximum value at *K* = 7, declined and then reached another maximum at *K* = 10 (Table 3). These values of *K* correspond roughly to the number of sites sampled (*N* = 8) and the total number of collections taken over the entire study (*N* = 11), indicating that there is some differentiation among all the collections analyzed in this study. When we examined how *K* changed over the course of the simulation, we were able to identify four clusters of populations; *K* = 4 is the lowest value of *K*

TABLE 3.—Results of Bayesian clustering analysis to infer the number of clusters among brook trout samples collected from Lake Superior. Averages are over 10 replicates of the simulation at each value of  $K$ ;  $L'(K)$  is the first-order rate of change and  $L''(K)$  is the second-order rate of change. The results also include AMOVA results for each value of  $K$ . Abbreviations are as follows: ISRO = collections from Isle Royale, WISRO = Big Siskiwit + Grace + Washington, MN = Cross + Kadunce + Onion, On = Onion River, and TH = Tobin Harbor (see Figure 1); the two-digit numbers denote years. All the variance components were significant at the 0.05 level.

$K$	Average of $\log_e[P(\bar{X} K)]$	$L'(K)$	$L''(K)$	Percent of change in $L'(K)$	$F_{SC}$	$F_{CT}$	Inferred clusters
1	-10,475.7						
2	-9,952.45	523.26	120.73	39.1	0.097	0.066	(ISRO), (Nipigon, MN)
3	-9,549.92	402.53	142.74	30.0	0.067	0.090	(WISRO, Nipigon), (TH), (MN)
4	-9,290.13	259.79	151.59	19.4	0.039	0.118	(WISRO), (Nipigon), (TH), (MN)
5	-9,181.93	108.20	30.52	8.1	0.038	0.112	(WISRO), (Nipigon), (TH), (Kadunce + Cross), (Onion)
6	-9,104.25	77.68	69.24	5.8	0.043	0.102	(WISRO), (Nipigon), (TH96 + TH01), (TH98), (Kadunce + Cross), (Onion)
7	-9,095.81	8.44	173.76	0.6	0.040	0.103	(BSiskiwit), (Grace + Washington), (Nipigon), (TH96 + TH01), (TH98), (Kadunce + Cross), (Onion)
8	-9,261.13	-165.32	168.71	-12.3	0.042	0.096	(BSiskiwit), (Grace + Washington), (Nipigon), (TH96), (TH01), (TH98), (Kadunce + Cross), (Onion)
9	-9,257.74	3.39	146.39	0.3	0.037	0.099	(BSiskiwit), (Grace + Washington), (Nipigon), (TH96), (TH01), (TH98), (Kadunce + Cross), (On95), (On97)
10	-9,107.96	149.78	175.18	11.2	0.026	0.109	(BSiskiwit), (Grace + Washington), (Nipigon), (TH96), (TH01), (TH98), (Kadunce), (Cross), (On95), (On97)
11	-9,133.36	-25.40	22.71	-1.9			
12	-9,136.05	-2.69	2.69	-0.2			

that maximized the second-order rate of change (Table 3). Again, there is more than one solution, but that is to be expected when the number of collections is similar to or greater than the number of loci analyzed or when there is a hierarchical structure. The value of  $K = 4$  is the highest value with a significant increase in the gain in percent change in  $L'(K)$ . Changes in the posterior probability distribution are high from  $K = 2$  to  $K = 3$ , and again from  $K = 3$  to  $K = 4$ ; but then the change decreases sharply (Table 3). The AMOVA analysis supported solutions at  $K = 4$  and  $K = 5$  (Table 3) because the values of the among-group ( $F_{CT}$ ) and within-group ( $F_{SC}$ ) variance were maximized or minimized at these values. At  $K = 4$ , the clusters of individual brook trout corresponded to brook trout from Lake Nipigon, the Minnesota samples (Cross, Kadunce, and Onion), samples from the southwestern end of Isle Royale, and Tobin Harbor. The membership coefficients of brook trout in each of the four clusters were high (mean coefficient = 0.860; see Figure 3a).

During the simulation analysis, Tobin Harbor samples formed their own cluster even before the samples from Lake Nipigon at  $K = 3$  (data not shown), which was surprising. We suspected that the relatively large collection from Tobin Harbor might have caused this result. Therefore, we ran the simulation three more times; each time we used only one of the Tobin Harbor collections (i.e., only samples collected in 1996, only those collected in 1998, etc.). In these simulations, the Lake Nipigon samples formed their own cluster before the Tobin Harbor samples (data not shown). However,

the Tobin Harbor samples still formed a cluster that was distinct from the rest of the Isle Royale collections, Lake Nipigon, and the Minnesota collections.

The analysis was repeated using subsets of data to identify genetic clusters that might be missed in the larger data set because large genetic differences associated with major barriers to gene flow may obscure differences that exist on a finer scale. Based on the results from the initial analysis, we conducted separate simulations for the collections from Minnesota and Isle Royale. We included the Tobin Harbor samples in the Isle Royale simulations to see if they remained distinct from the western end of the island when a smaller geographic scale was considered. We did not further analyze the samples from Lake Nipigon. The posterior probability distribution among the four sites sampled at Isle Royale was maximized at  $K = 5$ , and the second-order rate of change was highest at  $K = 2$ . As with the initial analysis, this corresponded to a cluster of samples from Tobin Harbor and another cluster that consisted of samples from the western end of the island (Figure 3b). The average membership coefficient of the samples from the cluster that corresponded to Tobin Harbor was 0.844, and the cluster of the other sites had an average membership coefficient of 0.948. The membership coefficients could be used to distinguish between the two clusters. When we used a value of  $Q \geq 0.80$  as a cutoff value, all of the fish from the western end of Isle Royale were assigned to the same cluster; none were assigned to the cluster associated with Tobin Harbor. Eight of the 140

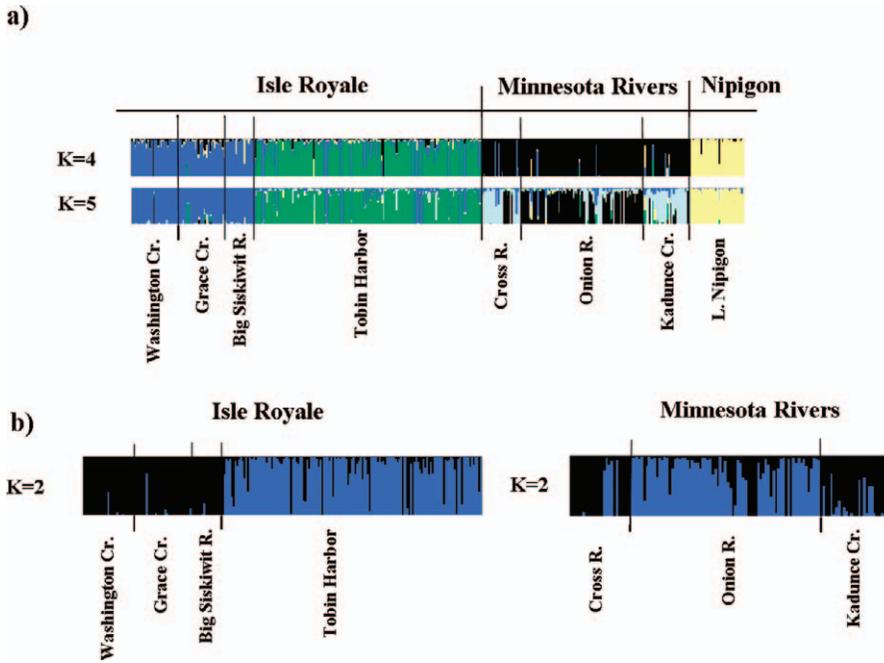


FIGURE 3.—Clustering results for (a) all brook trout collections (for  $K=4$  and 5) and (b) the Isle Royale ( $K=2$ ) and Minnesota Rivers clusters ( $K=2$ ). The vertical lines represent individual brook trout. Each colored segment of each line represents the estimated membership coefficient for each brook trout.

brook trout from Tobin Harbor were assigned to the cluster associated with fish from the southwestern end of Isle Royale. A simulation using just the collections from the western end of Isle Royale (Big Siskiwit River, Washington Creek, and Grace Creek) did not reveal any clustering (i.e., the posterior probability was maximized at  $K=1$ ). The posterior probability distribution among the three sites from Minnesota was maximized at  $K=4$ , and the second-order rate of change was highest at  $K=2$ . In this simulation, the clusters were not as well defined by geography as the clusters in the simulation using all the Isle Royale collections, but corresponded to a cluster consisting of the Cross River and Kadunce Creek samples and a second cluster of the Onion River samples. The average membership coefficient of the samples from the cluster that corresponded to Onion River and for the Cross–Kadunce cluster was 0.761 and 0.759, respectively. These samples could not be assigned to the clusters with the same level of accuracy observed for the Isle Royale samples. Again, using 0.80 as the threshold, we found that 5 of the 54 samples from Cross River or Kadunce Creek were assigned to the cluster associated with Onion River, and 8 of the 75 Onion River samples were assigned to the Cross–Kadunce cluster.

## Discussion

We used microsatellite DNA loci to evaluate the genetic structure of brook trout from Isle Royale, Lake Nipigon, and Minnesota tributaries of Lake Superior and found evidence of genetic population structure at broad and fine geographic scales. Hierarchical population structure was observed in a relatively small number of collections. Southwestern Isle Royale, Tobin Harbor, Kadunce Creek–Cross River, Onion River, and Lake Nipigon were identified as major clusters separated by a significant disruption to gene flow. Differences among samples from Isle Royale, Lake Nipigon, and Minnesota tributaries were supported by both the population- and individual-based analysis methods. Lake Nipigon was distinct from the Lake Superior collections in the individual-based approach, but was grouped with the Isle Royale samples in the population analysis; however, the support for the grouping was weak (Figure 2). Previous analyses of life history data also indicated that brook trout from Isle Royale represent discrete stocks when compared with brook trout from Lake Nipigon (Quinlan 1999).

When the Isle Royale collections were considered separately, both analytical methods indicated that collections from the southwestern end of the island

grouped together and were distinct from Tobin Harbor. Gorman et al. (2008) found that brook trout in Siskiwit Bay and Washington Harbor had habitat associations that were distinct from those for brook trout in Tobin Harbor. Although all the Isle Royale collections had different genotypic frequencies, the individual-based analysis indicated that there may be some gene flow among Grace Creek, Washington Creek, and the Big Siskiwit River. Geographic barriers may not inhibit brook trout movement along Lake Superior shorelines (Mucha 2004), where suitable spawning and rearing habitats may be widely spaced. However, the physical characteristics of the southwestern shoreline of Isle Royale may make gene flow between Tobin Harbor and other sites on the island unlikely (Quinlan 1999). A telemetry study of brook trout from Isle Royale found that fish tagged in Tobin Harbor moved very small distances as compared with other salmonids (Newman 2000). Tobin Harbor fish were also less mobile than those tracked in Nipigon Bay (Mucha and Mackereth 2008). This may be due, in part, to the long narrow shape of Tobin Harbor and the deep, open lake outside the harbor. In another study of brook trout population genetics and movement, D'Amelio et al. (2008) examined three rivers in Nipigon Bay, Ontario (Cypress, Jackpine, and Little Cypress rivers), and found that they had high levels of gene flow among them even though they were not geographically proximate. Coaster brook trout in Lake Superior may link rivers into a network of populations (D'Amelio and Wilson 2008).

The three populations from Minnesota formed one and possibly a second genetic group; the samples from Kadunce Creek and Cross Creek were in a separate cluster from the Onion River samples (Figures 2, 3b). This is interesting because the Onion River is located between the other two sites. Disruptions in genetic integrity of wild populations of salmonids is a possible consequence of stocking (Krueger and Menzel 1979) and may have had an effect on Lake Superior brook trout populations (Newman and Dubois 1997; Waters 1977). Many of the Minnesota tributaries of Lake Superior have been stocked extensively for over 150 years. Early records are scarce, but in the last 30 years several different strains have been stocked, including strains originating from the east coast of North America (Waters 1977; Goodier 1982; GLFC 2008). Although extensive stocking of brook trout populations has occurred in eastern North America, including Lake Superior (Krueger and Menzel 1979; Hayes et al. 1996; Jones et al. 1996; Newman and Dubois 1997; Kerr 2000; Schreiner et al. 2008), genetic studies indicate that a substantial amount of natural genetic variation remains (Danzmann and Ihssen 1995; Danzmann et al.

1998; Burnham-Curtis 2001; Hall et al. 2002; Rogers and Curry 2004; D'Amelio and Wilson 2008; Wilson et al. 2008). Preliminary work using mtDNA has indicated that little introgression has resulted from past stocking events (Burnham-Curtis 2001). More samples from other collections along the north shore of Lake Superior would provide a more complete picture of the structure of brook trout populations in Lake Superior and of the impacts of stocking.

Significant microsatellite DNA diversity remains among and within brook trout populations at all the locations we examined. The levels of heterozygosity observed in this study are within the range reported in a previous study of brook trout from Nipigon Bay, Lake Superior (D'Amelio and Wilson 2008). However, they are somewhat lower than those reported in studies of brook trout from eastern North America (Hébert et al. 2000; Boula et al. 2002), but this may reflect differences in the structure of the respective river systems sampled (Hébert et al. 2000). The stream systems studied in eastern North America tend to be more complex with many tributaries. Associations among several small populations may result in a higher level of genetic variation than in a single collection (Kimura and Crow 1963; Hébert et al. 2000). In contrast to the mtDNA data for these same samples (Burnham-Curtis 2001), the Grace Creek and Washington Creek populations had the highest values of observed heterozygosity and allelic richness among the Isle Royale collections. Burnham-Curtis (2001) found mtDNA diversity to be lower in the Isle Royale samples than in samples from other Lake Superior tributaries; mtDNA diversity among the samples from Tobin Harbor was among the lowest recorded in a study of 33 collections from Lake Superior (Burnham-Curtis 2001). A lack of agreement between the two genomes has been noted in another study of brook trout (Ferguson et al. 1991). The mtDNA genome is inherited maternally and is sensitive to the effective size of a population, population bottlenecks, and founding population size (Moritz et al. 1987). Scarce spawning habitat may reduce the number of females spawning successfully and may have an impact on diversity that is more pronounced in the mitochondrial than in the nuclear genome. For example, only one redd has been located to date in Tobin Harbor (Quinlan 1999). While we know that each of these populations persists in low numbers, it is not clear which, if any, of the factors noted above has influenced these populations.

Migratory behavior is an example of how salmonids exhibit differential use of habitat through variation in migratory behavior or morphological variation associated with different ecological niches (Balon 1980;

Castonguay et al. 1982; Bourke et al. 1997). The work by D'Amelio and Wilson (2008) suggested that some rivers produce more coasters possibly as a consequence of habitat supply or population dynamics. In this study, no data were collected that allowed us to distinguish between river resident and lake run fish; therefore, we were unable to directly address this issue. However, we have no reason to suspect that any coaster-specific genotypes were found among the samples analyzed. Neutral markers like microsatellites would have deviations from Hardy–Weinberg equilibrium in a mixed population of genetically different coasters and resident fish. Deviations from Hardy–Weinberg equilibrium were not specific to any locus or any collection, and the brook trout clustered along clear geographic lines in both the individual and population-based analyses. Burnham-Curtis (2001) also found no evidence of a distinct lineage associated with the coaster brook trout using mtDNA, and many of the brook trout used in that study were analyzed in this study. It should be noted that the genetic studies cited above used neutral markers. It is possible that life history or morphological variants for traits that have a genetic basis may not seem genetically different if the markers used are not linked to the functional genes or quantitative genetic variation and differentiation is possible that is not observed in neutral marker systems (Thrower et al. 2004; Leinonen et al. 2008; Nichols et al. 2008). It is possible that there is a genetic basis for the behavior of coaster brook trout that is mediated by environment or by population dynamics. Examples of functional genes affecting behavior have been documented in insects (Sokolowski et al. 1997; Ben-Shahar et al. 2003), and a genetic basis for anadromy has been demonstrated in brook trout (Boula et al. 2002). Alternatively, in a geologically young system such as the Great Lakes region, insufficient time has elapsed for genetic differences to develop (Bailey and Smith 1981).

Completed and ongoing studies of brook trout populations in Lake Superior have suggested that regional management strategies that account for the hierarchical structure of brook trout populations should be employed (D'Amelio et al. 2008; Wilson et al. 2008). The genetic population structure of Isle Royale brook trout populations we observed also supports a regional approach to management. Differences between the northeastern and southwestern ends of Isle Royale have been captured in the recently developed hatchery strains. Ongoing work to assess the success of the Tobin Harbor and Siskiwit Bay hatchery strains (Sloss et al. 2008; Stott and Quinlan 2008) will provide useful information to managers who employ stocking as part of a rehabilitation program and will provide

additional insights in the development of the coaster phenotype.

This study was designed to survey and compare genetic variation among brook trout from Isle Royale, Lake Nipigon, and three Minnesota tributaries to Lake Superior. The analysis of the genetic data indicates that significant genetic variation remains in Lake Superior and at Isle Royale. Isle Royale brook trout are genetically and geographically distinct from the other sites we surveyed. No evidence of a coaster genotype or mitochondrial DNA lineage was observed among the brook trout sampled to date using microsatellite markers (D'Amelio et al. 2008; Scribner et al. 2008; Wilson et al. 2008) or mitochondrial DNA (Burnham-Curtis 2001); however, this does not rule out the possibility that there is a genetic basis for the behavior. Future research needs to combine studies of functional markers, neutral markers, and ecology to produce better understanding of the genetic–environment interactions that produce life history variants such as the coaster brook trout.

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