

Genetic Population Structure of Brook Trout Inhabiting a Large River Watershed

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Abstract.—The genetic population structure of brook trout *Salvelinus fontinalis* inhabiting the Miramichi River, New Brunswick, a large (14,000-km²) river system composed of three main stems, was assessed using six microsatellite DNA loci. Samples from 12 sites incorporating four temporal replicates were analyzed. An individual-based assignment method without a priori knowledge of geographic origin suggested the presence of five candidate source populations within the 12 sites. Drainage structuring based on the 12 sampling sites did not explain the observed patterns of genetic population structure (analysis of molecular variance: 0.74% of variance explained; not significant). Conversely, the five candidate source populations estimated under the assignment approach significantly explained the genetic population structure observed (3.47% of variance explained; $P < 0.001$), the level of population fragmentation within sampling sites increasing significantly with proximity to the mouth of the watershed ($P = 0.011$). These results suggested elevated levels of brook trout dispersal within a large river watershed where geographic distance among sampling sites did not have a significant impact on the genetic population structure. Brook trout populations inhabiting a large river watershed may therefore be more influenced by ecological variables affecting the observed patterns of divergence, such as alternative life history strategies (e.g., anadromy) and habitat selection.

Brook trout *Salvelinus fontinalis* are native salmonids found throughout the coldwater streams, rivers, and lakes of eastern North America (Behnke 1972). The species displays a variety of life history strategies that contribute to an extremely complex ecology (Behnke 1980; Power 1980). Although brook trout are considered primarily a freshwater species (Scott and Crossman 1973), anadromous forms are common to rivers where access to marine environments exists (Power 1980). Recently, the brook trout has become the focus of numerous studies due to its capacity for dispersal and movement in both freshwater (Gowan and Fausch 1996; Curry et al. 2002) and saltwater (Castric and Bernatchez 2003).

Empirical genetic studies concerned with the conservation of fish populations have stressed the importance of understanding genetic diversity and population structure in defining the evolutionary potential of fish populations, particularly when the species is threatened and there is a need to resolve the processes that have produced or influenced its genetic structure for purposes of responsible man-

agement (Schonewald-Cox et al. 1983; Bernatchez et al. 1995; Fraser and Bernatchez 2001; but see Youngson et al. 2003). To this end, neutral genetic markers (e.g., microsatellites) have provided unparalleled perspectives on the patterns and processes promoting and maintaining intraspecific diversity. For example, while genetic analyses based on allozymes and mitochondrial DNA have suggested that the genetic variance observed among brook trout populations is the result of historical structuring among major drainages or association with distinct glacial refugia (Schmidt 1986; Perkins and Kreuger 1993; Danzmann et al. 1998), microsatellite DNA has resolved the contemporary factors (such as drainage partitions) that appear to influence genetic structure on a microgeographic scale (Angers et al. 1995, 1999; Angers and Bernatchez 1998; Castric et al. 2001). However, with the exception of Hébert et al. (2000), these studies have focused primarily on brook trout inhabiting lakes within closed systems. Lacustrine environments represent only a fraction of the habitat range for this species and focusing on them is probably leading researchers to underestimate its capacity to move within alternative home ranges, such as river systems.

In an open-river system, there is a much greater potential for dispersal by brook trout inhabiting

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different drainages. Brook trout are highly mobile and can disperse long distances within freshwater systems (O'Connor and Power 1973). Spatial and temporal studies have revealed that these trout have the capacity to move well over 100 km within a season, an activity that is often thought to be associated with accessing foraging or reproductive habitats (Curry et al. 2002). Brook trout routinely exhibit anadromous migrations that include traversing long distances to reach productive marine environments (Power 1980; Ryther 1997; Curry et al. 2002). Population relationships may also entail anadromous and resident behaviors that occur concomitantly within a system (Curry et al. 2002). These alternative life history tactics probably play a major role in shaping the genetic population structure of a system. For example, Hébert et al. (2000) revealed that the extent of genetic structure both within and among drainages in Kouchibouguac National Park, New Brunswick, (40°N, 60°W; 239 km²) was low, offering some of the first genetic evidence that nongeographic factors such as anadromy may maintain gene flow among trout populations from neighboring rivers and thereby reduce interdrainage divergence. Studies have also shown that resident brook trout may exhibit equally high levels of dispersal that are thought to be associated with habitat selection (see Gowan and Fausch 2002). However, there is little information on how brook trout movements in open-river systems impact genetic population structure, which may have implications for the conservation and management of trout populations that are currently undergoing tremendous exploitation and habitat loss.

Measuring brook trout movements directly by techniques such as radiotelemetry (e.g., Curry et al. 2002) can be labor intensive, thereby leading to limited field observations and sample size. However, molecular markers can be employed as an indirect method of estimating movements through individual-based, multilocus genotypic assignment tests that assign individuals probabilistically to candidate source populations (Cornuet et al. 1999; Pritchard et al. 2000; Blanchong et al. 2002). These individual-based assignment methods are widely believed to hold the potential to estimate contemporary rates of gene flow and dispersal (Cornuet et al. 1999; Berry et al. 2004) and have proved highly amenable to identifying candidate source populations and estimating movements as well as identifying individuals (Waser and Strobeck 1998; Goudet et al. 2002; Manel et

al. 2002; Berry et al. 2004; Castric and Bernatchez 2004).

Gene flow among trout populations inhabiting an open-river system is inevitably dependent on the interactions among populations in different tributaries, yet the patterns of brook trout genetic structure within river watersheds remain poorly understood. Moreover, genetic population structure for brook trout may not always be dictated by geographic or historical factors that have typically been found to influence patterns of genetic divergence within a system (Angers and Bernatchez 1998; but see Castric et al. 2001). Therefore, our objective was to investigate the genetic population structure of brook trout inhabiting a large, open-river watershed. As no prior information on brook trout population structure existed for our system, we first estimated the most likely number of candidate source populations, for which 12 sites were sampled without any a priori assumptions as to the number of such populations using a Bayesian individual-based assignment approach. We then attempted to discern factors that may have influenced the observed genetic structure for the candidate brook trout source populations inhabiting the system.

Methods

Study Site

Brook trout inhabit virtually every area within the large (14,000-km²) Miramichi River watershed of central New Brunswick (Figure 1). There are no barriers to movement, and the sea is accessible and used by brook trout from all areas of the catchment (P. Cronin, New Brunswick Department of Natural Resources and Energy, unpublished data). Four sites (6MS, 5MS, 4MS, and 3MS) were sampled by electrofishing, netting, and angling in September and early October of 1997 before the spawning period (Table 1). In 1998, we resampled these four sites to assess the temporal stability of the samples, along with eight additional sites to ensure incorporation of samples from all of the major stems encompassing the Miramichi River ($N = 441$ samples). Tissue samples (~50 mg; caudal fin) from 30–51 brook trout were collected and stored in a 95% solution of ethyl alcohol for subsequent laboratory analyses.

Genetic Analyses

We extracted DNA from tissue using a modification of the Bardacki and Skibinski (1994) protocol. Six primer pairs that have been successfully implemented in previous studies were used in our

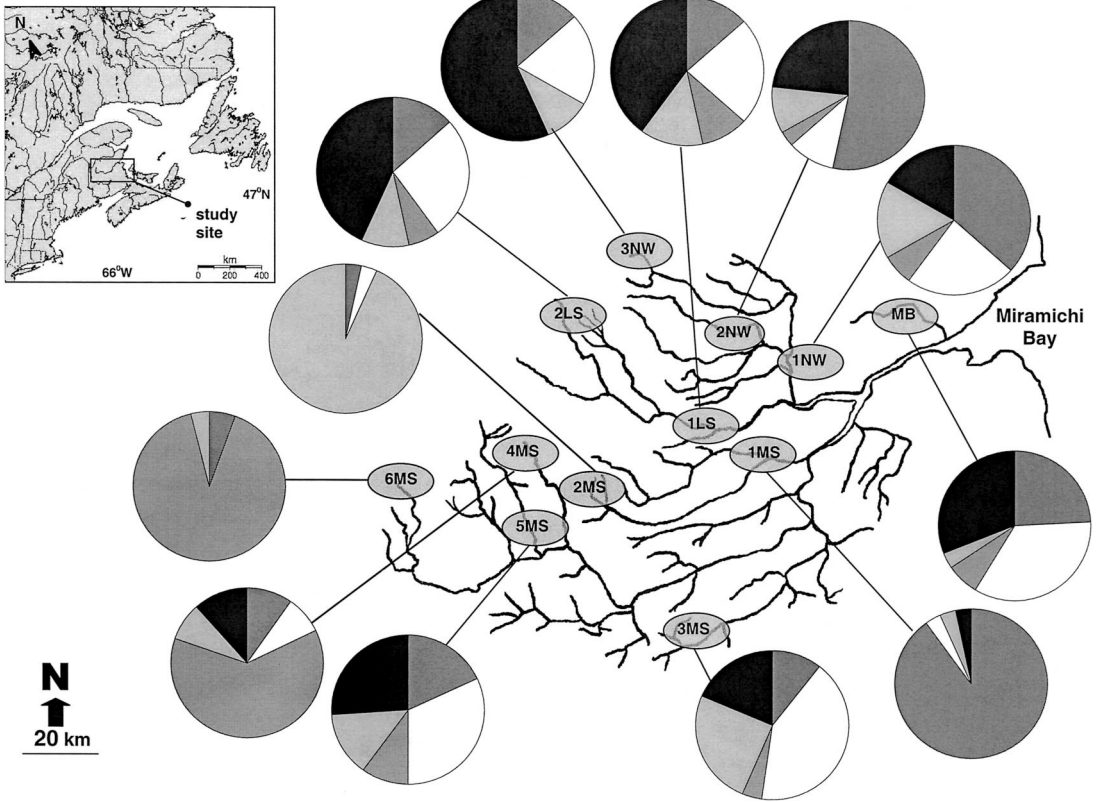


FIGURE 1.—Percentages of brook trout at sampling sites in the Miramichi River (see Table 1 for site codes) that were assigned to the five candidate source populations identified by means of individual-based assignment. Populations are denoted by shading as follows: white, Cains; light gray, Southwest; medium gray, Renous; dark gray, Dungarvon; and black, Northwest (see Table 4 for details).

TABLE 1.—Summary of the 12 sites in the Miramichi River at which brook trout were sampled.

Site code	Main stem	Drainage	Tributary	Location
MB	Miramichi Bay	Bartibog River	Green Brook	47°12'N, 65°34'W
1NW	Northwest Miramichi River	Northwest Miramichi River	Trout Brook	47°10'N, 65°50'W
2NW	Northwest Miramichi River	Big Sevogle River	Big Sevogle River	47°20'N, 66°15'W
3NW	Northwest Miramichi River	Northwest Miramichi River	Gill Brook	47°24'N, 66°13'W
1LS	Little Southwest Miramichi River	Little Southwest Miramichi River	Otter Brook	46°53'N, 66°02'W
2LS	Little Southwest Miramichi River	Little Southwest Miramichi River	North Pole Stream	47°08'N, 66°40'W
1MS	Main Southwest Miramichi River	Renous River	McGraw Brook	46°49'N, 66°07'W
2MS	Main Southwest Miramichi River	Dungarvon River	Dungarvon River	46°52'N, 66°41'W
3MS	Main Southwest Miramichi River	Cains River	Cains River	46°32'N, 66°29'W
4MS	Main Southwest Miramichi River	Main Southwest Miramichi River	Clearwater Brook	46°67'N, 66°79'W
5MS	Main Southwest Miramichi River	Main Southwest Miramichi River	Burnthill Brook	46°67'N, 66°90'W
6MS	Main Southwest Miramichi River	Main Southwest Miramichi River	Beadle Brook	46°63'N, 67°18'W

analyses, namely, those for *Sfo-8*, *Sfo-12*, *Sfo-18*, and *Sfo-23* (developed specifically for brook trout; Angers et al. 1995), *MST-85* (developed for brown trout *Salmo trutta*; Presa and Guyomard 1996), and *Ssa-197* (developed for Atlantic salmon *Salmo salar*; O'Reilly et al. 1996). Amplification of microsatellite fragments was completed using a Perkin-Elmer 2400 thermocycler with a protocol initially developed by Angers et al. (1995). Electrophoresis was completed using an ABI 310 sequencer with the ROX 500 size standard (Applied Biosystems, Inc.). Analyses were completed with the Genescan analysis software (Applied Biosystems).

Genetic diversity at each of the 12 sampling sites was quantified using the software GENEPOP (Raymond and Rousset 1995) by calculating the number of alleles per locus, observed heterozygosity, and expected heterozygosity. Hardy-Weinberg equilibrium (HWE) was tested using the Markov chain method implemented in GENEPOP to estimate the probability of wrongly rejecting the null hypothesis of heterozygote deficiency for each locus in each sample (Guo and Thompson 1992). Values of F_{IS} , a correlation measure of the heterozygote deficiency, were used to perform a Kendall coefficient of concordance rank test in the presence of Hardy-Weinberg (HW) disequilibrium. This test compares the relatedness among all loci to determine whether any observed heterozygote deficiencies are locus specific (David et al. 1997). Tests of linkage disequilibrium for all possible pairs of loci were analyzed in GENEPOP as well. Sequential Bonferroni adjustments were used to maintain the probability of a type I error at 0.05 (Rice 1989).

Genetic Structure

Individual-based assignment method.—Microsatellite data for all 12 sampling sites were analyzed using the program STRUCTURE (Pritchard et al. 2000), which employs a Bayesian model-based clustering method for inferring population structure from genotypic data. It makes use of Markov chain Monte Carlo methods to establish the allelic frequencies per locus for a set of K (which may be unknown) candidate source populations and subsequently estimates the probabilities that a given individual will be assigned to each of the K populations. We first tested the probability of 1–12 candidate source populations (12 was chosen as the upper limit because it represented the number of sampling sites, including temporal replicates) using genotypic data for the six microsatellite loci in 441 individuals. Three runs were car-

ried out for each value of K tested to ensure convergence of the Markov chain. By including the four temporal samples (6MS, 5MS, 4MS, and 3MS) in the analysis, it was possible to assess whether STRUCTURE would assign these individuals to the same populations as with samples collected the year before and to compare these results with θ_{ST} temporal estimates (Weir and Cockerham 1984) using ARLEQUIN (Schneider et al. 2000). The analyses were implemented under an admixture and correlated allele frequency model to allow for the possibility that some individuals had a mixed "population" origin. Not only is this model recommended as a starting point when the number of candidate source populations is uncertain (Pritchard et al. 2000), it also provided a reasonably flexible tool for dealing with the possibility that dispersal has had a significant impact on brook trout structure in the Miramichi River.

Genetic differentiation.—After identifying the most likely candidate source populations, we estimated the level of genetic differentiation (F_{ST}) between them using Weir and Cockerham's (1984) θ value calculated from ARLEQUIN (Schneider et al. 2000). The corresponding genetic relationships among candidate source populations were further explored through multidimensional scaling (MDS) employing the matrix of pairwise θ values. A two-dimensional MDS plot of source populations was realized to determine whether there were any meaningful underlying dimensions that might explain the genetic differentiation observed in the pairwise θ matrix.

The hypothesis that genetic structure was influenced by geographic factors (i.e., drainage patterns) was tested via an analysis of molecular variance (AMOVA) in ARLEQUIN via the option of defining the hierarchical grouping of sampling sites by drainage. The most likely estimate of the number of candidate source populations obtained by the assignment method was then applied, whereby individuals within the 12 sampling sites were grouped according to their probabilities of belonging to each of the source populations. This grouping allowed us to compare the percentage of variance explained by genetic differences among strictly geographical tributary groupings with that of the assignment-based candidate source population estimates.

The possibility that the observed genetic population structure can be explained by the principle of isolation by distance (IBD) was assessed following the model of Slatkin (1993), which relates gene flow (defined as the absolute number of mi-

grants between samples to waterway distance. Waterway distance was calculated as the number of kilometers between the source populations' tributaries as measured along the river. Based on IBD assumptions, the prediction that greater distance between two locations would translate into greater genetic divergence was tested (Wright 1943; Castaic et al. 2003). Spearman rank correlation analyses implemented in STATISTICA were used to test the significance of the observed relationship.

Results

Genetic Diversity

The degree of polymorphism at the six microsatellite loci was variable, *Sfo-8* having the most alleles (48) and the tetranucleotide *Ssa-197* having the fewest (8; Table 2). A summary of locus pair tests for gametic disequilibrium was insignificant, suggesting the physical independence of the loci. Genetic diversity among loci (H_E) ranged from 65% (*Sfo-18* and *Ssa-197*) to 91% (*Sfo-23*) over the sampling sites. Three of six loci (*Sfo-23*, *Sfo-8*, and *MST-85*) revealed significant heterozygote deficiencies ($P < 0.0001$; adjusted according to Rice 1989), resulting in HW disequilibrium. A rank test determined the exact influence that the loci had on the HW disequilibrium. Kendall's coefficient of concordance rank test provided a simultaneous test for the relationships between the inbreeding coefficients (F_{IS}) and expressed relatedness between the K correlated samples. The test confirmed that the heterozygote deficiencies were specific to the three loci that deviated from HWE (Kendall's coefficient of concordance = 0.44; average rank $r = 0.378$; $P < 0.001$). The presence of null alleles, nonrandom sampling, or scoring errors might explain the deficiencies observed (Spruell et al. 1999; Pascual et al. 2001), yet the proximate causes of heterozygote deficiencies have been difficult to elucidate in brook trout (Castaic et al. 2002). As the deviations from HWE appeared to be locus specific and not sample-site specific, we did not skew the raw data so that they would conform to HWE, as has been done in previous studies (David et al. 1997; Spruell et al. 1999).

Temporal Stability

We first tested temporal stability among samples by comparing θ_{ST} values between years. The comparison of θ_{ST} between temporal replicates suggested a significant ($P < 0.01$) fluctuation of allelic frequencies at three out of four sampling sites (overall θ_{ST} range = 0.009–0.068) over 1997 and 1998 (Table 3). We subsequently tested the prob-

ability of assignment for these tributaries (see below) and compared the variance in assignment probabilities to each of the five populations for individuals in successive years. In this analysis, a multivariate analysis of variance was concordant ($P < 0.001$) that 4MS samples exhibited significant variation in allelic frequencies among years, suggesting possible temporal fluctuation in genetic population structure at this site. Conversely, the same analysis suggested that the variation in allelic frequencies in the 6MS, 5MS, and 3MS samples was temporally stable, as individuals from these tributaries were assigned to the same respective source populations in different years ($P > 0.05$; Table 3).

Individual-Based Assignment Method

Number of candidate source populations.—We tested the probability of K populations in STRUCTURE to estimate the most likely number of candidate source populations given the individual microsatellite genotypes. The distribution of $P(K = X)$, with K varying from 1 to 12 populations, was bimodal with untransformed \log_e probabilities of $-11,902.9$, $-11,684.0$, $-11,546.0$, $-11,473.0$, $-11,397.8$, $-11,431.3$, $-11,388.0$, $-11,376.0$, $-11,401.0$, $-11,426.3$, $-11,514.0$, and $-11,816.0$. The corrected probabilities suggested a total of $K = 5$ or 8 candidate source populations within our sampling sites. Under systems of weak differentiation or population structure, the suggested conservative approach is to assume that the first mode in the distribution is most likely to be representative of the number of candidate source populations (Pritchard et al. 2000). Thus, the most likely number of candidate source populations given our data set was 5.

Description of candidate source populations.—Summary pie charts were prepared to illustrate the percentages of brook trout within sampling sites that were assigned to the five candidate source populations identified for the system (Figure 1). From these plots it was possible to infer which, if any, sampling sites were the major contributors for each candidate source population. Five of the 12 sampling sites (1MS, 2MS, 3MS, 6MS, and 3NW) had larger proportions (42–93%) of individuals per candidate source population, thereby becoming the most likely representative sites for each source population given our sampling (Table 4). Two of these populations, which (based on assignment success; Table 4) we refer to as the Southwest and Cains candidate source populations, consisted of individuals primarily inhabiting

TABLE 2.—Genetic data for brook trout from 12 sampling sites in the Miramichi River. Summary measures are as follows: N = sample size, A = number of alleles, H_E = expected heterozygosity, and F_{IS} = the correlation value of heterozygote deficiency. Significant deviations from Hardy–Weinberg equilibrium ($P < 0.05$) are denoted by asterisks.

Site	Summary measure	Locus					
		<i>Sfo-8</i>	<i>Sfo-12</i>	<i>Sfo-18</i>	<i>Sfo-23</i>	<i>MST-85</i>	<i>Ssa-197</i>
1MS	N	30	30	30	30	30	30
	A	11	5	5	14	7	5
	H_E	0.8	0.67	0.53	0.8	0.73	0.59
	F_{IS}	0.333	-0.039	0.179	0.256	0.728	-0.066
2MS*	N	30	30	30	30	30	30
	A	17	6	7	15	12	4
	H_E	0.93	0.76	0.72	0.91	0.86	0.67
	F_{IS}	0.067	-0.011	-0.015	0.233	-0.138	0.079
3MS	N	48	48	48	47	48	48
	A	19	6	8	24	17	4
	H_E	0.89	0.67	0.7	0.92	0.91	0.6
	F_{IS}	0.282	-0.057	-0.038	0.218	0.155	-0.357
4MS*	N	50	50	50	49	50	50
	A	25	8	9	25	18	7
	H_E	0.94	0.82	0.68	0.94	0.90	0.65
	F_{IS}	0.19	0.192	0.058	0.246	0.087	0.297
5MS*	N	50	50	50	50	50	50
	A	24	5	9	26	16	4
	H_E	0.94	0.75	0.73	0.94	0.87	0.56
	F_{IS}	0.385	-0.046	0.04	0.26	0.102	-0.251
6MS*	N	53	53	53	53	53	53
	A	19	7	9	26	18	5
	H_E	0.91	0.74	0.63	0.94	0.84	0.61
	F_{IS}	0.086	-0.122	-0.108	0.2	0.172	-0.107
1LS*	N	30	30	30	30	30	30
	A	15	6	6	19	13	5
	H_E	0.86	0.78	0.72	0.94	0.89	0.67
	F_{IS}	0.424	0.057	-0.214	0.332	0.032	0.26
2LS	N	30	30	30	30	30	30
	A	19	6	8	18	13	6
	H_E	0.92	0.75	0.75	0.93	0.90	0.63
	F_{IS}	0.246	0.026	-0.018	0.289	0.289	-0.283
1NW*	N	30	30	30	30	30	30
	A	16	7	8	20	14	3
	H_E	0.89	0.72	0.70	0.94	0.89	0.52
	F_{IS}	0.406	0.218	0.047	0.077	0.028	-0.214
2NW	N	30	30	30	30	30	30
	A	15	5	7	17	11	6
	H_E	0.90	0.72	0.43	0.92	0.82	0.42
	F_{IS}	0.148	-0.06	0.072	0.207	-0.016	-0.105
3NW	N	30	30	30	30	30	30
	A	19	6	9	16	10	4
	H_E	0.85	0.72	0.66	0.87	0.85	0.65
	F_{IS}	0.221	-0.159	0.037	0.197	-0.024	-0.084
MB*	N	30	30	30	30	30	30
	A	16	7	4	21	14	3
	H_E	0.91	0.72	0.60	0.91	0.84	0.54
	F_{IS}	0.305	0.125	0.283	0.161	0.253	-0.365

the main southwest stem of the watershed. Individuals sampled from the middle stem of the watershed, the Renous River, were from two candidate source populations referred to as the Renous and Dungarvon populations. Finally, the fifth candidate source population consisted primarily of individuals sampled from the northwest stem of the watershed and hence were referred to as the North-west population.

Genetic Structure

Significant levels of genetic differentiation among these five candidate source populations were observed, values of θ ranging from 0.028 to 0.055 ($P < 0.001$; Table 5). These genetic relationships were further explored by multidimensional scaling (MDS) whereby the genetic differentiation between the five populations was visually observed (Figure 2). The Southwest population ex-

TABLE 3.—Temporal stability of four sampling sites where replicates were available over 2 years. The stability of allelic frequencies was first tested by comparing θ_{ST} values between years. Stability was also assessed by comparing the variance of the average probability of assignment to each population between years. A MANOVA was used to assess the sum of all effects among assignments between years. All statistically significant P -values are denoted by asterisks.

Site	θ_{ST}	P -value	Year	N	Statistic	Average probability of assignment to population					MANOVA P -value
						1	2	3	4	5	
6MS	0.028	<0.001*	1	23	Mean	0.091	0.047	0.756	0.062	0.044	0.233
				30	SD	0.138	0.041	0.219	0.065	0.038	
			2	30	Mean	0.042	0.042	0.824	0.051	0.041	
				30	SD	0.029	0.029	0.093	0.046	0.026	
5MS	0.022	0.006*	1	20	Mean	0.199	0.242	0.087	0.202	0.27	0.379
				20	SD	0.246	0.14	0.095	0.218	0.168	
			2	30	Mean	0.159	0.316	0.097	0.141	0.287	
				30	SD	0.212	0.179	0.149	0.159	0.154	
3MS	0.009	0.144	1	18	Mean	0.073	0.361	0.065	0.194	0.307	0.976
				18	SD	0.07	0.169	0.102	0.193	0.122	
			2	30	Mean	0.06	0.379	0.067	0.179	0.315	
				30	SD	0.062	0.111	0.073	0.138	0.091	
4MS	0.068	<0.001*	1	20	Mean	0.073	0.057	0.737	0.071	0.062	<0.001*
				20	SD	0.09	0.048	0.193	0.072	0.066	
			2	30	Mean	0.168	0.256	0.072	0.229	0.276	
				30	SD	0.208	0.111	0.055	0.117	0.118	

hibited more divergence than the other populations. This genetic structure was tested among groups by means of an AMOVA and by IBD predictions. First, a global AMOVA over the 12 sampling sites revealed that the overall variance of allelic frequencies contributing to genetic differentiation among samples was 3.98% ($P < 0.001$; Table 6). The possible existence of a genetic structure by hierarchical drainage pattern (i.e., sampling site groups among the Main Southwest Miramichi, Little Southwest Miramichi, and Northwest Miramichi rivers) was subsequently tested. These geographic groupings displayed extremely low variance (0.74%) in allelic frequencies, the group component of variance (F_{CT} ; $P = 0.097$) leading us to reject the possibility that drainage patterns contributed to genetic population structure among sampling sites. In contrast, when the individuals among sampling sites were grouped

according to their assignments to the five candidate source populations, the percentage of variance that explained the observed genetic distances among groups rose to 3.47% of the differences, significantly (F_{CT} ; $P < 0.001$) supporting this pattern of genetic structure. The possibility that this result can be explained by the relationship between the number of migrants and waterway distance was rejected because insignificant regressions provided no evidence for isolation by geographic distance in this system (Figure 3).

Together, these results suggest that factors other than the geographic distances between populations are influencing the observed patterns of divergence among source populations. From the summary plots (Figure 1), it appears that tributaries closest to the mouth of the watershed (Miramichi Bay estuary) are also the most fragmented (when considering the degree of assignment to source pop-

TABLE 4.—Description of candidate source populations based on individual assignments.

Population	N	Characteristics of site with most source individuals				
		Individual probability of assignment (%)		Site	Population	Individuals within sample assigning to source (%)
		Average	Range			
1	96	61	27–95	1MS	Renous	90
2	83	44	24–57	3MS	Cains	42
3	97	69	29–96	6MS	Southwest	91
4	73	45	27–95	2MS	Dungarvon	93
5	92	40	26–56	3NW	Northwest	57

TABLE 5.—Pairwise estimates of genetic differentiation based on allelic variance (θ ; Weir and Cockerham 1984) for the five candidate source populations. Statistically significant comparisons ($P < 0.001$) are denoted by asterisks.

Population	1	2	3	4	5
1 (Renous)		0.042*	0.051*	0.037*	0.049*
2 (Cains)			0.044*	0.031*	0.028*
3 (Southwest)				0.044*	0.055*
4 (Dungarvon)					0.034*
5 (Northwest)					

ulations). The proximity of samples to the estuary, in turn, appears to have an impact on the degree of dispersal inferred from tributaries (Figure 2; Table 7). When the degree of successful assignment per tributary (as a measure of population fragmentation) was plotted against waterway distance to the estuary, a positive correlation emerged (Spearman's $R = 0.70$, $P = 0.011$; Figure 4).

Discussion

The main objective of this study was to assess brook trout genetic population structure in a large, open-river watershed. Brook trout inhabit virtually every area within the 14,000-km² Miramichi River watershed of central New Brunswick. The individual-based assignment method suggested that brook trout sampled across the 12 sites were representative of five candidate source populations. The genetic population structure among these five candidate source populations was significant, an AMOVA explaining 3.47% of the variance observed among populations, that is, more than five times the allelic variance observed among sampling sites under a geographic design. This result was strong evidence against the possibility that geographic factors alone contributed significantly to the observed patterns of genetic population structure for brook trout in the Miramichi River watershed.

The probability of individual assignments to these candidate source populations was asymmetrical among the 12 sites, indicating a pattern of

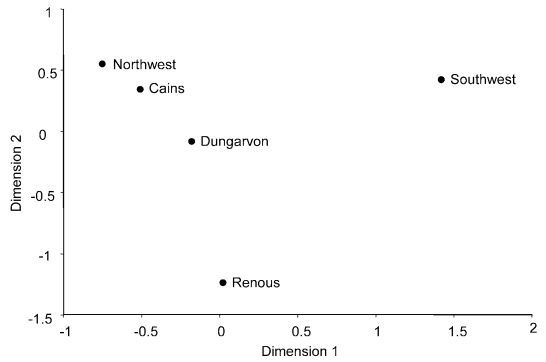


FIGURE 2.—Multidimensional scaling plot along two dimensions illustrating the degree of genetic differentiation between the candidate source populations as inferred from pairwise θ values (Weir and Cockerham 1984; see Table 5).

dispersal within the system at a level greater than that of the tributaries (Figure 1; Pritchard et al. 2000). Multidimensional scaling revealed that the Southwest population was the most divergent source population. This pattern was consistent with the assignment test, whereby individuals from 6MS had the highest probability of correct assignment (Table 4). This supports previous observations of a positive relationship between the level of F_{ST} and the probability of assignment success (Cornuet et al. 1999; Berry et al. 2004; Figure 2). The Northwest and Cains source populations were less differentiated genetically than the Renous and Dungarvon source populations, despite the close geographic distance between the latter two. This was the first evidence that an increase in geographic distance does not necessarily imply an increase in genetic divergence between populations within this system and is also consistent with the insignificant pattern of isolation by distance that we observed (Figure 3). Notably, the individuals assigned to the Renous and Dungarvon populations were found in all of the areas sampled (suggesting elevated levels of dispersal), a phenomenon that was only observed to a lesser degree for the South-

TABLE 6.—Percentages of the genetic variance among brook trout that are explained when structure is defined by tributaries, drainages, and source populations. Significant F_{ST} values ($P < 0.05$) are denoted by asterisks.

Structure	N	Percentage of variance explained			
		Among populations (within groups)	P-value	Among groups	P-value
Tributaries	441	3.98*	<0.001		
Drainages	441	3.34*	<0.001	0.74	0.097
Source populations	441	1.31*	<0.001	3.47*	<0.001

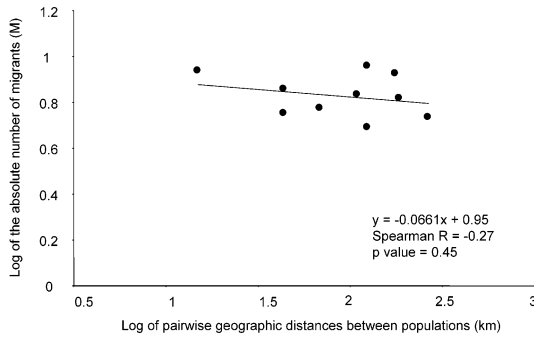


FIGURE 3.—Relationship between the number of migrants and isolation by geographic distance for the candidate source populations.

west, Cains, and Northwest populations. Individuals in the Southwest population, for example, appeared to exhibit much higher levels of fidelity to the upper main southwest stem, 89% of all individuals assigned to this candidate source population inhabiting this region (Table 7). These results are concordant with those of previous studies showing limited correlations for isolation by distance in salmonids (Ryman 1983; Moran et al. 1995; Hansen and Mensberg 1998; Youngson et al. 2003). However, the sampling sites closest to the mouth of the watershed were also the most fragmented, indicating a possible impact on the degree of dispersal inferred from sampling sites (Figure 2; Table 7). This correlation was significant ($P = 0.011$; Figure 4) and suggests that dispersal in brook trout coincides with other factors influencing structure.

The proximate causes for dispersal and their impact on brook trout genetic structure in the Miramichi River remain unknown, but several current hypotheses offer plausible explanations for our observations. First, anadromous trout are found throughout the Miramichi River, and this life history form frequently strays upon returning from its estuarine migration (Smith and Saunders 1958; Thorpe 1994). It has been estimated that 12–35%

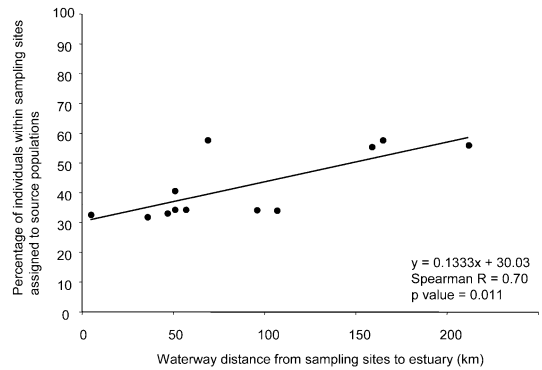


FIGURE 4.—Relationship between the percentage of individuals within a sampling site that were assigned to a particular source population and the waterway distance from the sampling site to the mouth of the estuary as a measure of population fragmentation with respect to proximity to the mouth ($N = 25$ pairwise comparisons; see Results and Table 7 for details).

of the brook trout within a river exhibit anadromy, while the remainder inhabit freshwater for their entire life cycle (Ryther 1997). Thus, it is possible that anadromous individuals provide a mechanism of dispersal among populations, effectively reducing the geographic patterns of divergence that may influence genetic structure. Under this hypothesis, population fragmentation in tributaries in close proximity to the estuary may be the result of straying induced by annual variations in river conditions (e.g., water temperatures and depths) or social interactions during estuarine residence (Curry et al. 2002). However, until we understand the genetic and ecological components interacting to maintain anadromy, comparative analyses of the relative contributions of each life history form will remain elusive.

There is also growing evidence that habitat selection is an important factor in the movement decisions of brook trout (Bélanger and Rodriguez 2002), yet the spatial and temporal scale within which these trout select habitat remains unknown.

TABLE 7.—Proportions of brook trout from the candidate source populations at the different sampling sites.

Population	Site											
	6MS	5MS	4MS	3MS	2MS	1MS	2LS	1LS	3NW	2NW	1NW	MB
1 (Rénous)	3.1	9.4	5.2	5.2	1.0	28.1	4.2	4.2	4.2	16.7	11.5	7.3
2 (Cains)	0.0	19.3	4.8	24.1	1.2	1.2	9.6	8.4	7.2	3.6	8.4	12.0
3 (Southwest)	50.0	5.2	33.3	2.1	0.0	0.0	2.1	3.1	0.0	1.0	2.1	2.1
4 (Dungarvon)	2.7	9.6	5.5	16.4	38.4	1.4	4.1	5.5	4.1	4.1	6.8	1.4
5 (Northwest)	0.0	14.1	6.5	9.8	0.0	1.1	14.1	13.0	18.5	7.6	5.4	9.8
All	55.8	57.6	55.3	57.6	40.6	31.8	34.1	34.2	34.0	33.0	34.3	32.6

The evidence suggests that brook trout monitor habitat conditions within an open-river environment at a large spatial scale in order to gain access to optimal foraging habitats, even with temporally changing landscapes (Gowan and Fausch 2002). We observed that certain sampling sites dominated the contribution of individuals that were assigned to source populations (Table 4), yet the quality of habitat within these regions was not defined. However, sampling site 6MS from the Southwest population, where the highest level of fidelity was observed, is arguably the best brook trout habitat in the Miramichi River (private land with no exploitation). If habitat degradation is found to be prevalent in tributaries proximate to the estuary, this may also partially explain the level of population fragmentation observed for brook trout within these sampling sites. More studies are needed to address the mechanisms and potential adaptive responses of movement and dispersal (Gowan and Fausch 1996; Fausch and Young 1995; Riley and Fausch 1995), particularly in systems where anadromous and resident trout occur in sympatry (Curry et al. 2002).

Finally, our results, together with those of Castric et al. (2001), support the notion that geographic factors play only a minor role in determining the patterns of genetic structure among drainages. These results provide further support for the possibility that nonequilibrium conditions between drift and migration persist within this system. Unfortunately, theoretical descriptions of nonequilibrium population structure remain limited, but it would appear that the significant population structure observed among the candidate source populations is important to better understanding the relative influences of historical and ecological factors in shaping the genetic variation in young systems such as recently deglaciated areas (Schmidt 1986; Castric and Bernatchez 2003). Overall, the results leave open the possibility that asymmetric dispersal is more related to ecological dynamics (such as population size and better habitat in some areas of the Miramichi River) than to the "equilibrium" conditions that have been the traditional focus. Fraser et al. (2004) have recently provided evidence that such ecological dynamics have a much bigger influence on genetic structure in brook trout than previously realized.

Of course, these data do not strictly rule out the possibility that factors other than dispersal are responsible for the patterns of genetic population structure observed in this open-river environment. There is the obvious limitation of using only six

microsatellite loci and relatively small sample sizes to determine weakly differentiated structure among the candidate source populations. There is also the possibility that our locus-specific deviations from HWE reduced the efficiency within which population assignments were made (Pritchard et al. 2000). There is one particular source of concern with estimating the number of candidate source populations with the Bayesian model of STRUCTURE, namely, an inherent danger of overestimating the number of candidate source populations, especially when one group of individuals is significantly more divergent than the others. For this reason, the biological interpretation of candidate source population estimates may not be straightforward. We inferred population structure on the basis of small differences in $P(K = X)$ where a bimodal distribution was observed. If there had been no biological interpretations for the assignments, or if the assignments were roughly symmetrical to all populations and no individuals were strongly assigned, this would impose serious limitations on any conclusions. In our case, however, individual-based assignment appeared to provide a reasonably flexible ad hoc comparison among the sites sampled, as has been observed in similar studies (Berry et al. 2004).

These results suggest that the candidate brook trout source populations sampled exhibit more fidelity to the tributaries furthest from the estuary, but the degree of dispersal from these candidate source populations is differential and dependent on the region of the watershed. Brook trout sampled from tributaries may not always represent a distinct population in a watershed such as that of the Miramichi River. This has implications for the conservation of discrete candidate brook trout source populations. These results also suggest a potential bias when trying to elucidate genetic structure in river environments in which the potential for dispersal is elevated due to physical or behavioral factors (e.g., the lack of barriers or anadromy). The method of assigning individuals to candidate source populations based on genotypic information promises to provide an effective means to elucidate populations when caution is exercised.

Within the Miramichi River, it appears that factors other than historical (postglacial recolonization) and geographic (drainage subdivision) effects have influenced the patterns of genetic structure observed for brook trout. It will be important to elucidate the degree to which these source populations contribute to the overall state of brook trout

in the watershed. Once the mechanisms and proximate causes by which these trout move are better understood, the impact on patterns of genetic population structure will allow for better management practices and ultimately a better understanding of brook trout population biology.

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