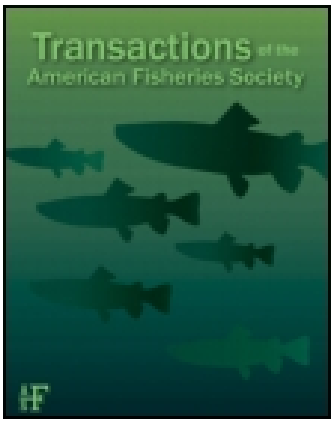

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Heritage Brook Trout in Northeastern USA: Genetic Variability within and among Populations

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Abstract.—Brook trout *Salvelinus fontinalis* from 21 unstocked waters, 3 naturalized lakes, and 4 hatcheries in New York and Pennsylvania were analyzed electrophoretically for allozyme expression. Thirty-two of the 68 loci examined were polymorphic. Average heterozygosity of samples from populations classified as wild–unstocked was 0.050 (range, 0.026–0.076). Differences ($P < 0.05$) occurred among the 21 wild–unstocked samples at 25 of 31 possible locus comparisons. All wild–unstocked samples were significantly different from each other and from hatchery samples ($P < 0.01$). A high fixation index ($F_{ST} = 0.375$) indicated that the wild–unstocked samples represented highly differentiated populations. A considerable portion of the gene diversity was found among major river basins (22.5%); the remainder was due to differences among minor river drainages within basins (10.0%) and among samples within minor drainages (5.0%). Cluster analysis of genetic distances organized samples into three main groups that were also associated by river basins. Management strategies for conserving the genetic variability of wild brook trout should focus on individual lake and stream populations within river basins as primary management units. Data indicated that naturalization had varying success in preserving the gene pools of the progenitor populations.

Wild brook trout *Salvelinus fontinalis* were once abundant throughout the coldwater lakes and streams of New York State; however, environmental perturbations such as deforestation, overfishing, hydroelectric power development, and acidic deposition have drastically reduced the number of wild populations. To compensate for the decline in wild fish, hatchery-reared brook trout have been stocked since the mid-1800s. Today, few brook trout populations remain that have not been recipients of past stocking. Less than 4% of the lakes and ponds in New York are thought to contain unstocked, wild populations of brook trout (Kretser et al. 1989).

The potential of stocked hatchery fish to compromise the genetic integrity and fitness of wild populations through interbreeding has been a concern in New York State. In past studies, domestic hatchery strains of brook trout exhibited lower growth, yield, survival, longevity, and natural reproduction than wild strains when both were stocked into the same waters (e.g., Webster and Flick 1981; Lachance and Magnan 1990). Presumably, the differences in performance were caused mainly by genetic differences, which often

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exist between hatchery and wild fish (e.g., Vuorinen 1984; Garcia-Marin et al. 1991). Genetic adaptations responsible for the superior performance of wild fish could be negatively affected by an influx of "foreign" genes from stocked hatchery fish (e.g., Bams 1976; Reisenbichler and McIntyre 1977). Therefore, New York has placed special emphasis on preserving the few unstocked brook trout populations that still exist in the state (Keller 1979). These rare populations are remnants of the original fish that colonized the area after deglaciation and are referred to in New York as "heritage" brook trout.

Information about the amount and distribution of genetic variability within and among populations is important for the development of rational conservation strategies (Ryman 1981). Salmonids in general have "a well-documented tendency . . . to evolve genetically discrete, ecologically specialized populations by natural selection over thousands of generations of adaptation to local environmental conditions" (Allendorf and Ryman 1987). Past studies suggest that brook trout are not an exception to this generalization. In the 1970s, significant differences were reported among brook trout populations located close to one another in Pennsylvania and Wisconsin (e.g., Eckroat 1971; Krueger and Menzel 1979). Analysis of brook trout from a broader geographic range identified even higher levels of genetic differentiation. For instance, the differences between wild brook trout in the northern and southern parts of the USA (e.g., New York and Tennessee) were large enough that the southern brook trout form was proposed as a separate subspecies (McGlade 1981; Stoneking et al. 1981). The genetic differences among New York's wild brook trout populations may also be large, reflecting founder effects associated with postglacial colonization and subsequcnt limited gene flow. On the other hand, unrecorded stocking of genetically homogenous, hatchery-raised brook trout may have reduced the differentiation among putative heritage populations. Therefore, populations most likely to be genetically "pure" and unaffected by past management practices should be identified genetically to help set priorities for conservation efforts.

Once heritage populations are identified, their preservation may be aided by naturalization in other waters. Naturalization, as used here, refers to the establishment of self-sustaining populations in new habitats void of the same species. Naturalized populations serve as reservoirs of genetic variability, sometimes termed "gene banks" (Hynes et al. 1981). In New York, for example, when eutrophication threatened the native brook trout in Tunis Lake, a naturalized population was established by directly transferring fish from Tunis Lake into Cables Lake (after chemical reclamation). Cables Lake now serves as a source of Tunis Lake strain brook trout that are stocked in other waters. Assessment of the success of naturalization in terms of gene pool replication requires genetic comparison of naturalized and progenitor populations.

The primary purpose of our study was to describe and compare the genetic variation within and among wild brook trout populations in New York State by use of allozyme electrophoresis. A hierarchy consisting of major river basins, minor river drainages, and individual populations was used to examine the distribution of gene diversity. In addition to the primary purpose, we compared wild populations with several hatchery stocks and compared one naturalized population with its progenitor.

Methods

Collections.—Twenty-four samples of brook trout were collected from lakes and streams in four major river basins: St. Lawrence, Hudson, Delaware, and Allegheny (Figure 1; Table 1). Sample sites for these collections were chosen on the basis of stocking information from the New York State Department of Environmental Conservation (NYDEC), the Adirondack Lake Survey Corp., fishing clubs, and lake owners. Four additional brook trout samples were obtained from three hatcheries.

Brook trout samples were classified into three categories as follows (see Table 1). (1) *Wild–unstocked* fish were sampled from lakes and streams for which there is no record of stocking; these fish were believed to be unaltered by interbreeding with hatchery fish. (2) *Hatchery* fish were sampled from strains that have been artificially propagated for at least 20 years (approximately seven generations). (3) *Naturalized* fish were sampled from lakes that had been reclaimed (i.e., had all fish removed) and stocked with a wild strain of brook trout.

Wild–unstocked samples were collected from 9 lakes and 12 streams. Most of these lakes were in remote areas difficult to reach and thus were unlikely to have experienced unauthorized stocking. Fish sampled from streams were typically above waterfalls that fish cannot ascend. Wild–unstocked samples contained several year-classes of fish with the exceptions of the Robinson River

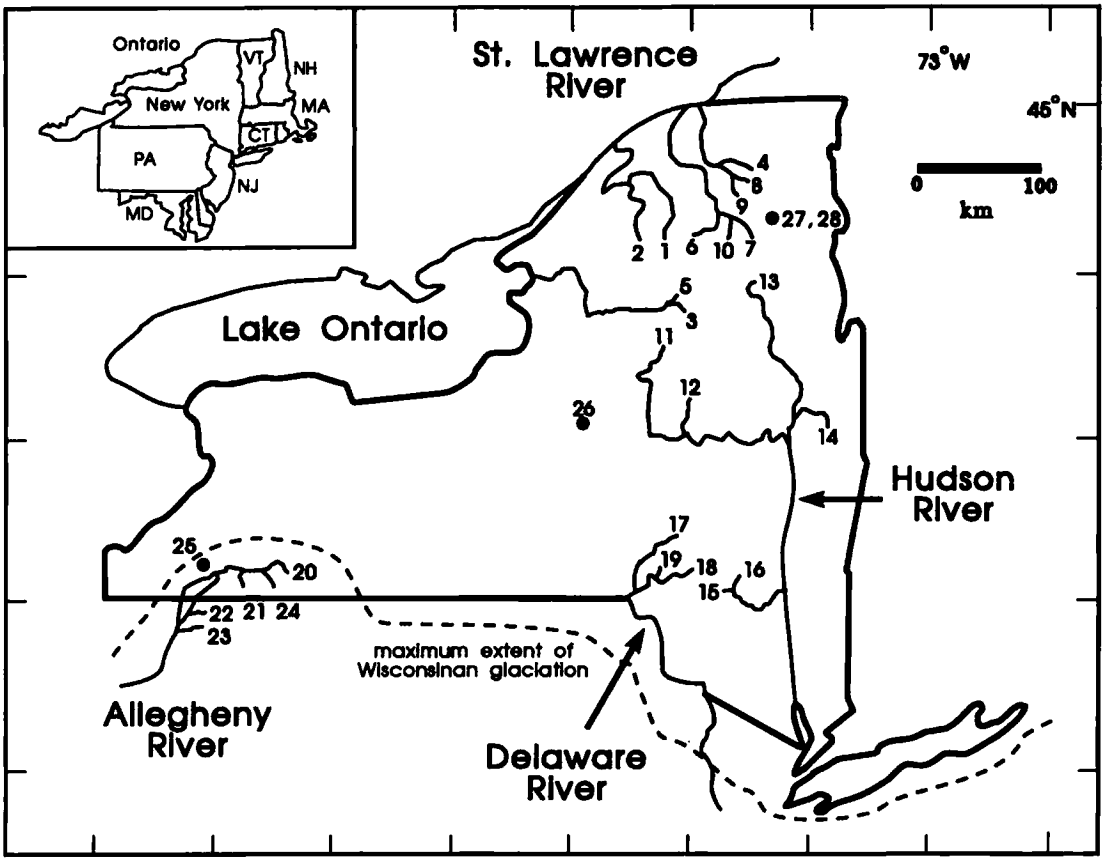

FIGURE 1.—Locations within New York and Pennsylvania (PA) where brook trout were sampled for allozyme analysis. Major drainages are shown; sample numbers are identified in Table 1. In the inset map, CT = Connecticut; MA = Massachusetts; MD = Maryland; NH = New Hampshire; NJ = New Jersey; VT = Vermont.

(sample 1), Lake Delaware (17), and Windfall Pond (9) samples. Samples from the Robinson River and Lake Delaware consisted solely of fry. In the analysis, the Lake Delaware sample was considered as wild–unstocked; however, Lake Delaware was stocked with 1,500 adult brook trout of unknown origin in both 1945 and 1947. The Windfall Pond sample consisted of 45 hatchery-reared fry that were the offspring of 23 females and a lesser number of males captured from Windfall Pond.

Although the wild–unstocked populations have no recorded stocking history (with the exception of Lake Delaware), the possibility still exists that they were contaminated with hatchery fish. Therefore, brook trout from several hatcheries were examined for genetic markers that might identify wild populations genetically altered by stocking.

Hatchery samples were obtained from the NYDEC fish hatcheries in Rome and Randolph and from Brandon Enterprises, Paul Smiths. The Rome strain (sample 26) was created in 1965 with fish from multiple sources. Brood stock at the Rome hatchery have been selected for resistance to furunculosis over the past 25 years. Brook trout propagated at the Randolph hatchery (25) originated from and have received periodic infusions from the Rome strain (see Appendix 1 for details). Fish from the Randolph and Rome hatcheries were compared to determine if differentiation occurred during a 7-year period after the Randolph stock was created from Rome fish.

Temiscamie (27) and Assinica (28) strain fish, obtained from the Brandon Enterprises hatchery, originated from populations in central Quebec (Flick 1977; Van Offelen et al. 1993). The Temiscamie strain was started with gametes collected in 1965 and 1967 from 40–60 adults from the Temiscamie River (Rupert River system). The Assinica strain was started in 1962 with gametes from

TABLE 1.—Geographic origin of brook trout sampled from New York and Pennsylvania. Samples were classified as wild–unstocked (W), hatchery (H), and naturalized (N). Sample abbreviations indicate geographic origin and classification.

Sample	Minor river drainage	Classification	Sample abbreviation	N
St. Lawrence River basin				
1 Robinson River	Oswegatchie	W	LAW-O1-W	46
2 Palmer Creek	Oswegatchie	W	LAW-O2-W	41
3 Horn Lake	Black	N	LAW-B1-N	45
4 Long Pond	Black	N	LAW-B2-N	47
5 Stink Lake	Black	W	LAW-B1-W	29
6 Charley Pond	Raquette	W	LAW-R1-W	19
7 Upper Preston Pond	Raquette	W	LAW-R2-W	46
8 Long Pond Outlet	St. Regis	W	LAW-S1-W	40
9 Windfall Pond	St. Regis	W	LAW-S2-W	45
10 Middle Anthony Pond	St. Regis	N	LAW-S1-N	47
Hudson River basin				
11 Jones Lake	Mohawk	W	HUD-M1-W	46
12 House Pond	Mohawk	W	HUD-M2-W	16
13 Nate Pond	Upper Hudson	W	HUD-U1-W	44
14 Lewis Hollow Brook	Upper Hudson	W	HUD-U2-W	45
15 Bear Hole Brook	Lower Hudson	W	HUD-L1-W	45
16 High Falls Brook	Lower Hudson	W	HUD-L2-W	45
Delaware River basin				
17 Lake Delaware	W. Br. Delaware	W	DEL-W1-W	48
18 Balsam Lake	Beaver Kill	W	DEL-K1-W	45
19 Russell Brook	Beaver Kill	W	DEL-K2-W	45
Allegheny River basin				
20 Twomile Creek	Direct tributary	W	ALG-x1-W	45
21 Christan's Run	Direct tributary	W	ALG-x2-W	45
22 Fourmile Creek	Direct tributary	W	ALG-x3-W	28
23 Hedgehog Run	Direct tributary	W	ALG-x4-W	42
24 Chipmunk Creek	Direct tributary	W	ALG-x5-W	45
Other				
25 Randolph hatchery	None	H	x-x1-H	39
26 Rome hatchery	None	H	x-x2-H	40
27 Temiscamie strain	None	H	x-x3-H	71
28 Assinica strain	None	H	x-x4-H	47

only seven adults collected near the outlet of Lake Assinica (Broadback River system).

To determine the effectiveness of naturalization as a method of gene pool preservation, one naturalized population (Middle Anthony Pond, 10) was compared with its progenitor population (Windfall Pond, 9). In addition, two populations naturalized with the same strain (Long Pond [4] and Horn Lake [3]) were compared with each other (the progenitor population no longer exists). Middle Anthony Pond was reclaimed and stocked several times over a 10-year period with progeny of fish from Windfall Pond (9; Appendix 1). Long Pond was reclaimed and stocked with Horn Lake strain fish in 1970. Horn Lake, after loss of its native population, was stocked in 1971 with Horn Lake strain fish and in 1974 with fish from Long Pond (Appendix 1).

Electrophoretic procedures and locus designations.—Whole trout were frozen in liquid nitrogen, transported to Cornell University, and stored at −80°C for 1–8 weeks before analysis. Horizontal starch gel electrophoresis was used to examine allozyme variation within white muscle, liver, and eye tissues (May et al. 1979; May 1992). Thirty-four specific enzymes and several other unknown proteins coded by 68 loci were examined for all samples (Table 2). Loci for the unknown proteins were identified based on electrophoretic band patterns that were consistent with Mendelian expectations. Enzyme nomenclature follows guidelines suggested by Shaklee et al. (1990). Allelic product mobilities reported by Stoneking et al. (1981) were the basis for our mobility designations.

Statistical procedures.—Conformance of allelic frequencies to Hardy–Weinberg expectations within samples was assessed by the fixation index F_{IS} and log-likelihood G-test (Levene 1949; Nei

TABLE 2.—Tissue sources, locus designations, and electrophoretic buffers for brook trout proteins. Enzyme numbers are as recommended by IUBNC (1984). Tissues used were white muscle (M), liver, (L), and eye (E). Buffer systems used were those of (A) Ayala et al. (1973) as modified by May et al. (1979), (C) Clayton and Tretiak (1972) as modified by May et al. (1979), (M) Markert and Faulhaber (1965), (R) Ridgway et al. (1970), and (S-9, S-4) Selander et al. (1971; the S-9 tray buffer was adjusted to pH 8.0 and the S-9 tray buffer was diluted 1:19 for use as the gel buffer). "%SP" is the proportion of samples polymorphic at a given locus.

Enzyme or other protein	Enzyme number	Locus	Tissue	Buffer	%SP
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	M	R	91
		*sAAT-3**	E	R	9
		*sAAT-4**	L	R	91
Adenosine deaminase	3.5.4.4	*ADA-1**	E	C	0
		*ADA-2**	E	C	0
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	R	47
Adenylate kinase	2.7.4.3	*AK-1**	M	C	6
		*AK-2**	M	C	0
Creatine kinase	2.7.3.2	*CK-1**; *CK-2**	M	R	0
		*CK-3**; *CK-4**	E	M	0
Dihydrolipoamide dehydrogenase	1.8.1.4	*DDH-3**	M	C	9
Esterase[a]	3.1.1.1	*EST-1**; *EST-2**	M	R	0
Fructose-biphosphate aldolase	4.1.2.13	*FBALD-2**	E	A	0
Fructose biosphosphate	3.1.3.11	*FBP-4**	E	4	0
beta-*N*-Acetylgalactosaminidase	3.2.1.53	*bGALA-2**	L	R	72
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-4**	M	4	0
N-Acetyl-beta-glucosaminidase	3.2.1.30	*bGLUA**	E	R	0
Glycerate dehydrogenase	1.1.1.29	*GLYDH**	L	C	0
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	M	C, A	84
		*G3PDH-3**	M	C, A	19
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M	R	6
		*GPI-B1**	M	R	72
		*GPI-B2**	M	R	41
L-Iditol dehydrogenase	1.1.1.14	*sIDDH**	L	R	0
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*mIDHP-1**	M	4	3
		*mIDHP-2**	M	4	0
		*sIDHP-1**	E	A	94
		*sIDHP-2**	E	A	9
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	M	R	0
		*LDH-A2**	M	R	3
		*LDH-B1**	E	M, S-4	78
		*LDH-B2**	L, E	M	9
		*LDH-C**	E	M, S-4	0
alpha-Mannosidase	3.2.1.24	*aMAN**	L	M	0
Malate dehydrogenase	1.1.1.37	*sMDH-A1**; *sMDH-A2**	E	S-4, A	0
		*sMDH-B1,2**	M	S-4, A	75
Malic enzyme ($NADP^+$)	1.1.1.40	*mMEP-1**	M	C	63
		*mMEP-2**	M	C	6
		*sMEP-1**	M	C	6
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**			3
Methylumbelliferyl phosphatase	3.1.–.–	*MUP-1**; *MUP-2**	M, L	C	0
Octanol dehydrogenase	1.1.1.73	*ODH**	L	C	94
Dipeptidase[b]	3.4.–.–	*PEPA**	E	C	0
Peptidase-S[c]	3.4.–.–	*PEPS**	E	R	0
Proline dipeptidase[d]	3.4.13.9	*PEPD-1**; *PEPD-2**	L	M	0
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	R	3

TABLE 2.—Continued.

Enzyme or other protein	Enzyme number	Locus	Tissue	Buffer	%SP
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	M	C	0
		*PGK-2**	M	C, A	3
Phosphoglucomutase	5.4.2.2	*PGM-1**	M	C, A	3
		*PGM-2**	M	C	53
Purine-nucleoside phosphorylase	2.4.2.1	*PNP-2**	M	C	0
Inorganic pyrophosphatase	3.6.1.1	*PP-3**	M	S-9	0
General protein	No number	*PROT-1**; *PROT-2**; *PROT-3**; *PROT-4**	M	R	0
Superoxide dismutase	1.15.1.1	*sSOD**	L	R	0
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	M	C, A	9
		*TPI-2**	M	C, A	0
		*TPI-3**	M	C, A	3

[a] Variation at the *EST** locus was detected with the use of methylumbelliferyl butyrate.
[b] Glycyl-leucine substrate. [c] Leucyl-alanine substrate. [d] Phenyl-alanyl-proline or phenyl-proline substrate.

1977; Sokal and Rohlf 1981). Duplicated loci that share alleles (e.g., *sAAT-1,2**) were not examined for conformance to these expectations because the allelic variation observed cannot be assigned to a specific locus. Variation at duplicated loci was split equally between each locus for purposes of data analysis.

Genetic differences among samples were assessed with heterozygosity calculations, *G*-tests, and genetic distance coefficients (*D*). Observed and expected heterozygosities and their variance estimates were calculated as described by Nei and Roychoudhury (1974) and Nei (1977). Hereafter, mean expected heterozygosity per locus will be referred to simply as heterozygosity. Allele counts by locus were compared statistically by contingency table analysis with *G*-tests (Sokal and Rohlf 1981). The critical values used to reject the null hypothesis for the *G*-tests were increased (based on Sidak's multiplicative inequality) to account for the increase in type I error when multiple tests of the same hypothesis were made (Cooper 1968). We calculated *G*-values between all possible paired comparisons, among groups of populations associated with minor and major river drainages, and among all wild samples. Genetic distances (Nei 1972) were calculated with data from all loci and subjected to unweighted pair-group method cluster analysis (Sneath and Sokal 1973). With data from 39 loci, genetic distances were calculated between our samples and those from Stoneking et al. (1981). Cluster analysis of these genetic distances was performed to show the relationships among populations from a broad geographic range.

An analysis of the gene diversity (heterozygosity) was performed as described by Nei (1973) and detailed by Chakraborty (1980). The total gene diversity of wild populations (H_T) was divided into four components representing the average gene diversity within samples (H_S), among samples within minor river drainages (D_{SD}), among drainages within major river basins (D_{DB}), and among basins (D_{BT}):

$$H_T = H_S + D_{SD} + D_{DB} + D_{BT};$$
$$D_{SD} = H_D - H_S;$$
$$D_{DB} = H_B - H_D;$$
$$D_{BT} = H_T - H_B;$$

H_T = heterozygosity of the total population (i.e., the population formed by combining all samples into a single group; heterozygosity was calculated from the average allelic frequencies of all samples);

H_B = mean heterozygosity of major river basins (heterozygosity of a basin was calculated from the average allelic frequencies of all samples within that basin);

H_D = mean heterozygosity of minor river drainages (heterozygosity of a drainage was calculated from the average allelic frequencies of all samples within that drainage);

H_S = mean heterozygosity of all samples (i.e., subpopulations).

The four gene diversity components were each divided by H_T to assess their relative contribution to the total gene diversity. Analyses of the data were performed with "Genes in Populations," a

microcomputer program designed by B. May and C. C. Krueger and written in C by W. Eng, Cornell University.

Results

Genetic Variability within Samples

Genotypic proportions deviated significantly from Hardy–Weinberg expectations in 13 of 268 tests (13 would be expected by chance alone at the 5% level). The deviations occurred at five different loci among 11 samples. The genotypic proportions at *sAAT-4** and *sIDHP-1** (the two most heterozygous loci) deviated from Hardy–Weinberg expectations in five and four samples, respectively.

Thirty-two of the 68 (47%) protein loci examined were polymorphic (frequency of an alternative allele exceeded 0). The average proportion of polymorphic loci over all samples was 19.6% (range, 8.8–26.5%, SE = 4.7; Appendix 2, Table A2.1). Forty-two alternative alleles were detected (Appendix 2, Table A2.2). Almost 50% of the wild populations (9 of 21) contained an allele found in no other wild population. No fixed allelic differences were detected among river basins; however, loci with distinctive allelic frequencies were present in the Allegheny (*ADH**, *GPI-B2**, *sIDHP-2**, *ODH**), St. Lawrence (*mMEP-1**, *PGM-2**), and Hudson and Delaware (*GPI-B1**) basins (Table A2.1). For example, *ADH*–205* was found in high frequencies only in samples from the Allegheny basin. Mean expected heterozygosity of wild–unstocked samples (H_S) based on 68 loci was 0.050 (range, 0.026–0.076, SE = 0.013; Table A2.1).

Genetic Variability among Samples

Genetic distances (D) between samples ranged from 0.001 to 0.094 (Table 3). Cluster analysis of genetic distances organized samples into three main groups that were also associated by river basins (i.e., Allegheny, St. Lawrence, and Hudson and Delaware rivers; Figure 2). Within the St. Lawrence basin, samples from the same minor river drainage grouped with each other. This organization by minor river drainages within a basin was not apparent in the Hudson–Delaware group.

Gene diversity analysis measured total heterozygosity over all wild–unstocked samples (H_T) at 0.080. Within-sample variation accounted for 62.5% of the total gene diversity. The remaining 37.5% was partitioned as follows: 5.0% was due to variation among samples within minor river drainages, 10.0% was due to variation among drainages within major river basins, and 22.5% was due to variation among major basins.

Based on G-statistics, significant differences existed among all samples and all groups of samples compared ($P < 0.01$). Among the 21 wild–unstocked samples, differences occurred at 25 of the 31 possible locus comparisons ($P < 0.05$). Differences were evident among major river basins, between samples within minor river drainages, and between all 756 possible paired sample comparisons ($P < 0.01$). All hatchery samples were significantly different from all wild–unstocked samples ($P < 0.05$); however, no alleles were found in any of the four hatchery samples that could be used as markers to identify stocked populations. Heterozygosities of the hatchery samples were within the range observed among the wild–unstocked samples (Table A2.1).

Naturalized Samples

Middle Anthony Pond versus Windfall Pond.—The Middle Anthony Pond sample (10) was quite similar to its progenitor population in Windfall Pond (9). The genetic distance between the samples (0.003) was the second lowest observed in this study (Table 3). However, four alleles detected in the sample from Middle Anthony (*bGALA-2*105*, *GPI-B2*39*, *LDH-A2*44*, and *mMEP-1*100* with frequencies of 0.01–0.17) were not detected in the sample from Windfall Pond. One allele detected in the Windfall sample, *sAAT-3*93* (frequency, 0.03), was not detected in the Middle Anthony sample.

Horn Lake and Long Pond.—Samples from Horn Lake (3) and Long Pond (4), which both contain naturalized Horn Lake strain fish, were not as similar to each other as expected ($D = 0.012$). Three alleles detected in the sample from Horn Lake, *sAAT-4*170*, *sIDHP-1*152*, and *sMEP-1*105* (with respective frequencies of 0.36, 0.28, and 0.07) were not detected in the sample from Long Pond. Three alleles detected in the sample from Long Pond, *sAAT-4*100*, *LDH-B1*72*, and *sMDH-B1,2*120* (0.26, 0.19, and 0.01) were not detected in the sample from Horn Lake (Table A2.1).

Discussion

Genetic Structure Based on Postglacial Colonization

The genetic variability of wild brook trout populations in New York State was organized on the basis of river basins. Cluster analysis of genetic distances identified three groups of genetically similar populations associated with the Allegheny, Hudson and Delaware, and St. Lawrence river basins (Figure 2). This organization may reflect col-

onization of river basins by genetically different groups of brook trout at different times. During the Wisconsinan glaciation, all of New York State was covered by ice except for the periphery of the Allegheny River basin (Prest 1970; Muller et al. 1986). One group of brook trout probably maintained itself in the Allegheny basin (Mississippi refugium) west of the Appalachian Mountains throughout the period of glaciation. As the glaciers retreated, southern and central New York became accessible to brook trout, via the Hudson and Delaware rivers, from the Atlantic refugium east of the Appalachian Mountains. Parts of New York north of the Adirondack Mountain peaks were probably not accessible to brook trout at this time because glaciers still covered much of this area. Several thousand years later, as the glaciers retreated further, the St. Lawrence River began to drain into the Atlantic Ocean. Brook trout could then have reached the northern Adirondack region and established the group of populations sampled in the St. Lawrence basin. Bailey and Smith (1981) also concluded, based on the brook trout's distributional pattern in North America, that populations must have existed in both the Mississippi and Atlantic refugia during the last glaciation.

The theory that brook trout colonized the St. Lawrence River basin from coastal populations of the Atlantic Ocean is supported by electrophoretic data from another study of brook trout populations in Canada and the USA (McGlade 1981). In that study, cluster analysis of genetic distances showed that brook trout samples from Quebec, Labrador, and New York (St. Lawrence basin) and from Prince Edward Island and Nova Scotia (northern drainages to the Atlantic Ocean) were similar to one another and formed a group separate from southern Appalachian populations. The similarity between samples from the St. Lawrence basin and northern drainages to the Atlantic Ocean suggests a common ancestry and colonization history.

Five brook trout samples did not group in the cluster analysis as might be expected based on the colonization-by-drainage theory. Two of 10 samples from the St. Lawrence basin (Charley Pond [6] and Upper Preston Pond [7]) were associated with the Hudson–Delaware group instead of with other St. Lawrence samples (Figure 2). Both of these samples were particularly similar to the sample from Nate Pond (13) in the headwaters of the Hudson River. Charley and Upper Preston ponds both drain into a valley traversed by a low divide

TABLE 3.—Nei's (1972) genetic distances (D) between brook trout samples from New York and Pennsylvania. Values are based on 68 allozyme loci. Column heads are sample numbers; sample names are given in Table 1.

Sample	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.003	0.035	0.017	0.023	0.024	0.028	0.020	0.027	0.020	0.027	0.035	0.025	0.041
2		0.048	0.026	0.033	0.027	0.028	0.022	0.031	0.024	0.027	0.038	0.023	0.034
3			0.012	0.011	0.031	0.042	0.029	0.030	0.027	0.027	0.028	0.038	0.060
4				0.012	0.028	0.035	0.020	0.022	0.017	0.016	0.032	0.033	0.053
5					0.026	0.036	0.015	0.016	0.014	0.015	0.021	0.029	0.054
6						0.008	0.013	0.024	0.014	0.033	0.020	0.003	0.015
7							0.023	0.036	0.027	0.042	0.034	0.012	0.018
8								0.003	0.001	0.020	0.012	0.011	0.023
9									0.003	0.024	0.019	0.025	0.035
10										0.019	0.016	0.015	0.028
11											0.028	0.030	0.043
12												0.016	0.027
13													0.011

(<7 m relief) between the Hudson and St. Lawrence basins. In some places, the two basins are connected by ponds that drain both ways (e.g., Shaw Pond in Essex County). Perhaps as the glaciers retreated, water levels were higher than they currently are and glacial ice or outwash north of the valley caused these waters to drain south into the Hudson River basin. Fish of southern origin could then have colonized ponds in this region, such as Charley and Upper Preston ponds. Later, the glacial blockages to the north may have disappeared and the drainages of some waters changed to the St. Lawrence basin.

Two populations from the Mohawk River drainage in the Hudson basin, Jones Lake (11) and House Pond (12), were not as similar, based on cluster analysis, to the other populations in the Hudson basin as might be expected (Figure 2). Brook trout from Jones Lake were most similar to a population in the Black River drainage (Stink Lake, 5). Muller et al. (1986) proposed that the upper parts of the Mohawk and Black rivers drained south into the Susquehanna River basin during the early stages of glacial retreat, which would explain why the Mohawk drainage populations were not similar to the Hudson basin populations. Alternatively, the Jones Lake and House Pond samples may have been affected by population bottlenecks, founder effects, or unrecorded stocking. House Pond (0.5 hectares) appeared to have few brook trout, and thus the population may have been particularly susceptible to large, random changes in allelic frequencies.

The fifth population that did not cluster as would be predicted, Chipmunk Creek (24), was quite similar to the Randolph hatchery fish ($D = 0.005$) and quite different from other populations within the Allegheny basin ($D = 0.031$–0.045). Records of the NYDEC indicate that Chipmunk Creek has never been stocked; however, Chipmunk Creek is only 30 km from the Randolph hatchery. This prompts speculation that anglers or others may have stocked Chipmunk Creek. When Chipmunk Creek is excluded from the gene diversity analysis, the differentiation within the Allegheny basin decreases 52% and the differentiation among major river basins increases 31%.

Genetic Differentiation among Populations

Wild brook trout populations exhibited a high level of genetic differentiation, which is typical of many nonanadromous salmonids (e.g., cutthroat trout *Oncorhynchus clarki* and brown trout *Salmo trutta;* Loudenslager and Gall 1980; Allendorf and

TABLE 3.—Extended.

Sample	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.037	0.043	0.036	0.044	0.036	0.084	0.068	0.066	0.067	0.024	0.020	0.033	0.017	0.034
2	0.042	0.040	0.030	0.042	0.032	0.079	0.063	0.064	0.065	0.019	0.014	0.024	0.016	0.027
3	0.051	0.065	0.052	0.060	0.055	0.091	0.071	0.067	0.074	0.041	0.046	0.066	0.033	0.049
4	0.049	0.055	0.046	0.055	0.051	0.094	0.070	0.071	0.073	0.028	0.026	0.042	0.018	0.036
5	0.038	0.053	0.046	0.053	0.046	0.081	0.068	0.063	0.067	0.037	0.035	0.057	0.022	0.043
6	0.010	0.021	0.011	0.018	0.009	0.060	0.049	0.043	0.049	0.014	0.015	0.024	0.014	0.013
7	0.022	0.030	0.018	0.025	0.016	0.073	0.060	0.055	0.054	0.019	0.020	0.030	0.012	0.009
8	0.022	0.028	0.021	0.028	0.019	0.066	0.058	0.051	0.059	0.018	0.012	0.024	0.009	0.026
9	0.036	0.042	0.036	0.041	0.034	0.083	0.074	0.065	0.076	0.029	0.023	0.037	0.013	0.040
10	0.023	0.032	0.027	0.033	0.025	0.074	0.063	0.057	0.065	0.022	0.016	0.029	0.010	0.028
11	0.054	0.053	0.040	0.052	0.044	0.076	0.055	0.057	0.065	0.026	0.025	0.042	0.021	0.033
12	0.024	0.025	0.024	0.020	0.021	0.064	0.057	0.050	0.061	0.028	0.026	0.040	0.028	0.039
13	0.012	0.018	0.006	0.016	0.005	0.057	0.047	0.043	0.051	0.011	0.010	0.017	0.015	0.013
14	0.027	0.019	0.005	0.016	0.007	0.060	0.050	0.045	0.055	0.012	0.013	0.015	0.021	0.020
15		0.018	0.020	0.020	0.015	0.068	0.067	0.057	0.059	0.032	0.028	0.037	0.032	0.030
16			0.017	0.008	0.012	0.054	0.051	0.042	0.050	0.020	0.021	0.030	0.031	0.037
17				0.015	0.006	0.053	0.046	0.042	0.050	0.010	0.010	0.011	0.023	0.018
18					0.009	0.058	0.053	0.043	0.054	0.020	0.022	0.032	0.030	0.034
19						0.051	0.045	0.038	0.047	0.011	0.014	0.019	0.021	0.021
20							0.009	0.006	0.008	0.045	0.061	0.077	0.071	0.072
21								0.006	0.010	0.031	0.049	0.065	0.056	0.055
22									0.009	0.031	0.048	0.066	0.052	0.055
23										0.038	0.054	0.071	0.057	0.056
24											0.005	0.012	0.011	0.018
25												0.004	0.010	0.018
26													0.022	0.024
27														0.016

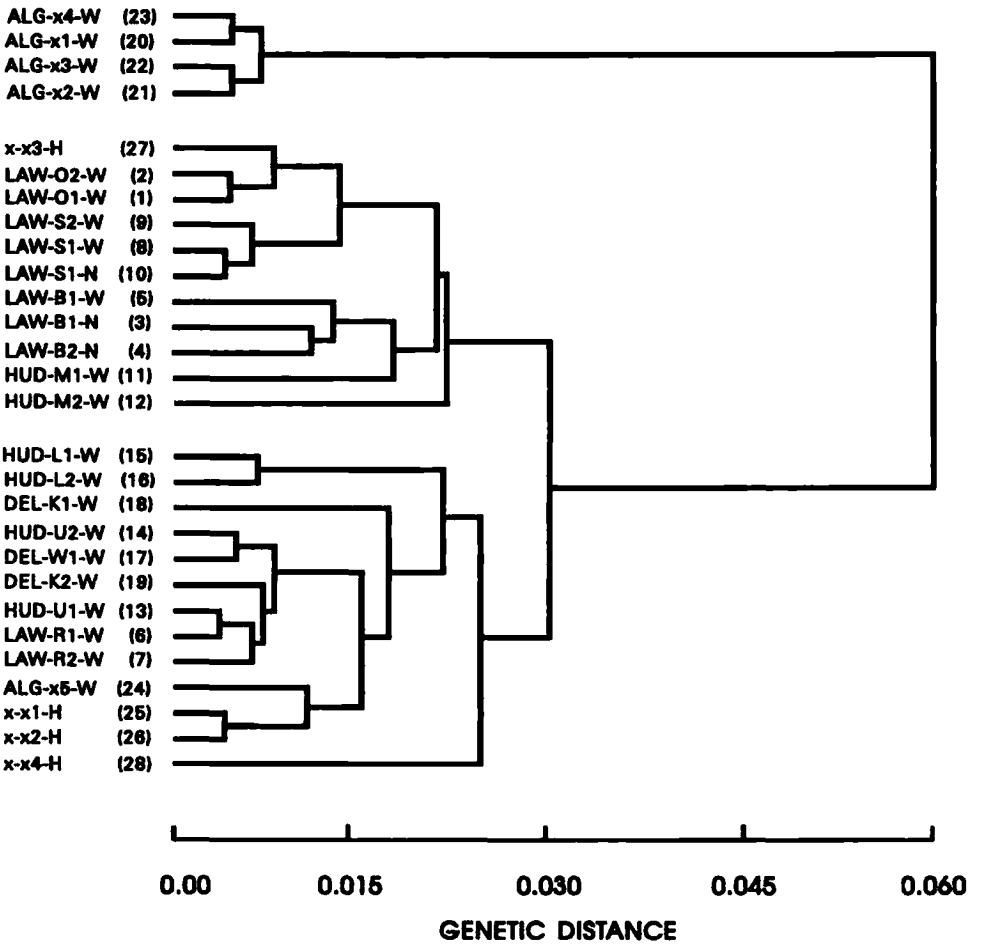

FIGURE 2.—Dendrogram generated by cluster analysis of Nei's (1972) genetic distance coefficients calculated between brook trout samples from New York and Pennsylvania with data from 68 loci. Sample abbreviations and identification numbers (in parentheses) are those given in Table 1. All samples were statistically different from each other.

Leary 1988; Ryman 1983). The average brook trout population contained only 62.5% of the total genetic variation (i.e., $F_{ST} = 0.375$); most of the remaining variation was due to differences among major river basins. Differences among populations were even larger when data from brook trout in Pennsylvania, North Carolina, and Tennessee (Stoneking et al. 1981) were added to our analysis. The level of differentiation increased 24% ($F_{ST} = 0.464$, based on 39 loci), and cluster analysis identified groups of populations genetically distinct from the ones in New York's river basins (Figure 3). Analysis of brook trout from their entire native range (Georgia to northern Quebec) might reveal a level of differentiation comparable to that observed in Arctic char *Salvelinus alpinus* ($F_{ST} = 0.533$; data from Kornfield et al. 1981).

Gene Pool Preservation

The preservation of gene pools via naturalized populations can have varying degrees of success. Twenty years after naturalization, fish in Middle Anthony Pond (10) remained quite similar to their progenitors in Windfall Pond (9; $D = 0.003$). In contrast, the preservation of the Horn Lake gene pool was less successful. The genetic distance between the Horn Lake (3) and Long Pond (4) samples was 0.012, and each population had alleles in substantial frequencies (e.g., 0.36) not found in the other population. What caused the preservation of the Horn Lake gene pool to be less successful is not known. Many factors could have contributed to differences observed between the Horn Lake and Long Pond populations such as (a) in-

adequate number of founding adults used to create the naturalized populations (i.e., founders were not an accurate representation of the progenitor population), (b) genetic contamination via migration, stocking, or incomplete reclamation, (c) genetic drift in one or both populations, and (d) different forces of selection acting upon the two populations. The successful preservation of the Windfall Pond gene pool likely benefitted from multiple stockings of Windfall strain fish into Middle Anthony Pond that occurred over a 10-year period. This emphasizes the need for periodic gene flow from the progenitor to the naturalized population to prevent the accrual of genetic differences. As a general rule, several adults transferred each generation after naturalization should provide enough gene flow to maintain the similarity between the progenitor and naturalized populations (Allendorf and Phelps 1981; Lacey 1987).

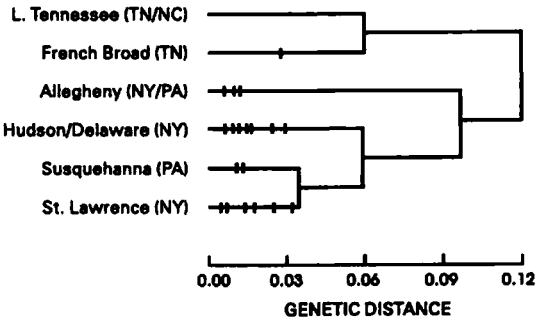

FIGURE 3.—Dendrogram generated by cluster analysis of Nei's (1972) genetic distance coefficients calculated between brook trout samples from the present study and those of Stoneking et al. (1981; data from 39 loci were used to calculate coefficients). States where samples were collected are given in parentheses after each river basin (NC = North Carolina; NY = New York; PA = Pennsylvania; TN = Tennessee). Hash marks represent cluster points of samples within a river drainage.

Management Implications

The geographical distribution of genetic variability observed among the wild, unstocked populations in this study indicates that remnants of New York's original brook trout exist. The pattern of gene diversity observed among the heritage populations apparently reflects colonization of different river basins by genetically distinct fish; populations within river basins then became differentiated due to geographic isolation. Significant genetic differences among all populations, even those within the same minor river drainage, suggest that individual heritage populations should be the primary ecological units on which management strategies are focused.

To protect the unique characteristics of heritage populations, future conservation goals should be to preserve as much of the gene diversity as possible. However, separate management of every lake and stream in New York suspected of containing a heritage population may not be feasible because of financial, legal, and sociocultural limitations. Preservation efforts may need to be directed to a subset of the heritage populations. At a minimum, this subset should maintain the genetic differentiation observed at two fundamental levels—among populations within river basins, and among river basins. To accomplish the former, *at least* two populations from each major river basin must be preserved (by definition, "among populations" requires more than one population). Special precautions should be taken to avoid gene flow into or among populations, because this could disrupt adaptations that have evolved due to the natural isolation of heritage populations. Heritage populations should be maintained at high effective population sizes (N_e) to minimize genetic losses due to stochastic events (Meffe and Vrijenhoek 1988).

The heritage populations represent an irreplaceable part of the brook trout resource in New York. The genetic variability of the heritage populations is important for the adaptive ability and long-term survival of the species. In addition, the superior performance of the wild strain brook trout for stocking (e.g., Webster and Flick 1981; Lachance and Magnan 1990) suggests that wild populations offer important reservoirs of genetic resources for fish management purposes.

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Appendixes follow

Appendix 1: Hatchery and Naturalized Samples of Brook Trout

Hatchery samples of brook trout were obtained from the NYDEC fish hatcheries in Rome and Randolph and from Brandon Enterprises, Paul Smiths. The Rome strain of brook trout was created with fish from multiple sources. Brook trout from at least 24 places in the USA were screened for resistance to furunculosis between 1952 and 1965 (Ehlinger 1964). Disease-resistant fish (or descendents thereof) were maintained at the Rome facility until 1965, at which time approximately 200 adult pairs were used to create the Rome strain. Which brook trout were represented among the adult pairs is not known. The Rome strain brood stock has continued to be selected for resistance to furunculosis since 1965.

Brook trout propagated at the Randolph hatchery from 1966 to 1976 were progeny of the Rome strain and were stocked throughout New York. In 1977, the Randolph hatchery was disinfected because of disease problems and gametes from the Rome facility were used to start a new Randolph brood stock. In 1978, additional fertilized eggs were transferred from Rome to Randolph, some of which may have been used to supplement the Randolph brood stock. From 1978 to 1984, the brood stock at Randolph was self-sustained and was not outbred or selected for furunculosis resistance. Starting in 1985, Randolph's brood stock were no longer progeny of fish from within the hatchery, but were progeny of fish from the Rome hatchery. Brook trout sampled from the Randolph and Rome hatcheries for our study were yearlings of the 1984 year-class.

Fish from the Randolph and Rome hatcheries were genetically similar to one another ($D = 0.004$) as would be expected based on their common origins. However, 10 alleles detected in the Randolph sample were not detected in the Rome sample. Eight of these 10 alleles had frequencies of less than 0.10 while the other two had frequencies of 0.13 and 0.29 (Appendix 2). All alleles detected in the Rome sample were detected in the Randolph fish. Genotypic proportions did not deviate from Hardy–Weinberg expectations in either sample.

Temiscamie and Assinica strain fish were obtained from the Brandon Enterprises hatchery in the Adirondack Mountains. As noted in the main text, these strains originated from wild Quebec populations in the 1960s; the Assinica strain has passed through a severe genetic bottleneck, starting as it did with gametes from seven adults. Brood stock of both strains have been maintained in Adirondack ponds by stripping gametes from adults, raising fertilized eggs in a hatchery, and then stocking age-0 fish back into the ponds. Natural reproduction does not occur in the brood-stock ponds.

Naturalized samples were collected from three ponds or lakes: Middle Anthony, Long, and Horn (Table 1). Middle Anthony Pond was reclaimed and then stocked in 1967 with progeny from 20 females and an unknown number of males collected from Windfall Pond (location of a wild–unstocked sample). Between 1971 and 1977, an additional 2,300 hatchery-reared fall fingerlings of the Windfall strain were stocked into Middle Anthony Pond.

The histories of the brook trout populations in Long Pond (not associated with Long Pond Outlet, sample 8; Table 1) and Horn Lake are complicated and involve a third lake, Canachagala. Canachagala Lake was reclaimed in 1965 and stocked in the spring of 1966 with approximately 10,000 brook trout fingerlings that were progeny of Horn Lake fish. In subsequent years, hatchery-reared fry derived from brook trout in Canachagala Lake were used to restock Canachagala Lake, to establish a naturalized population in Long Pond (1970), and to stock Horn Lake (1971). Horn Lake also received a direct transfer of 188 brook trout of various sizes from Long Pond in 1974. Horn Lake required stocking because low pH in the 1960s and 1970s resulted in poor brook trout reproduction. Whole-lake neutralization of Horn Lake with lime occurred in 1975, 1978, 1982, 1985, and 1989. Since 1975, natural reproduction of brook trout has been sufficient to maintain the population. A NYDEC survey of Horn Lake in 1975 indicated that 95–100% of the adult brook trout in the lake were from the 1971 and 1974 stockings. Therefore, the Horn Lake fish were considered a naturalized instead of a progenitor sample in the data analysis.

Appendix 2: Brook Trout Allele Frequencies

TABLE A2.1.—Allele frequencies, mean expected heterozygosity (H_S), and polymorphism percentages (P) for loci polymorphic in four or more samples of brook trout collected from lakes, streams, and hatcheries in New York and Pennsylvania. Sample abbreviations are defined in Table 1.

	Locus														
	*sAAT-1,2**			*sAAT-4**				*ADH**			*bGALA-2**		*G3PDH-1**		
Sample	**80*	**100*	**118*	**7*	**100*	**133*	**170*	*−205	*−100	**0*	**100*	**105*	**0*	**78*	**100*
1 LAW-O1-W		0.92	0.08		0.39	0.58	0.03		1.00		0.48	0.52	0.05	0.64	0.31
2 LAW-O2-W		0.90	0.10		0.46	0.43	0.11		1.00		0.56	0.44	0.01	0.33	0.66
3 LAW-B1-N		0.52	0.48			0.64	0.36		1.00		0.38	0.62	0.01	0.88	0.11
4 LAW-B2-N		0.61	0.39		0.26	0.74			1.00		0.28	0.72	0.21	0.68	0.11
5 LAW-B1-W		0.78	0.22		0.03	0.59	0.38		0.98	0.02	0.71	0.29		0.98	0.02
6 LAW-R1-W		0.78	0.22		0.39	0.50	0.11	0.03	0.97		0.89	0.11	0.05	0.53	0.42
7 LAW-R2-W		0.96	0.04		0.07	0.93		0.02	0.98		0.90	0.10		0.05	0.95
8 LAW-S1-W	0.01	0.89	0.10		0.59	0.19	0.22	0.03	0.97		0.95	0.05	0.05	0.64	0.31
9 LAW-S2-W	0.01	0.93	0.06		0.64	0.13	0.23		1.00		1.00		0.04	0.72	0.24
10 LAW-S1-N	0.01	0.82	0.17		0.63	0.31	0.06		1.00		0.97	0.03	0.12	0.76	0.12
11 HUD-M1-W		0.45	0.55	0.31	0.42	0.24	0.03		1.00		0.80	0.20	0.03	0.52	0.45
12 HUD-M2-W	0.03	0.84	0.13		0.59		0.41		1.00		0.93	0.07	0.13	0.62	0.25
13 HUD-U1-W		0.81	0.19		0.45	0.18	0.37		1.00		0.91	0.09	0.05	0.43	0.52
14 HUD-U2-W		0.95	0.05		1.00			0.13	0.87		1.00				1.00
15 HUD-L1-W		1.00			0.38	0.58	0.04		1.00		1.00		0.24	0.66	0.10
16 HUD-L2-W		1.00			1.00				0.51	0.49	0.86	0.14	0.57		0.43
17 DEL-W1-W		0.88	0.12		0.65	0.07	0.28	0.07	0.93		0.81	0.19	0.15	0.06	0.79
18 DEL-K1-W		0.99	0.01		1.00				1.00		1.00		0.42		0.58
19 DEL-K2-W		0.91	0.09		0.84		0.16		1.00		0.94	0.06	0.10	0.10	0.80
20 ALG-x1-W		0.59	0.41		0.66		0.34	1.00			1.00				1.00
21 ALG-x2-W	0.01	0.37	0.62		0.70		0.30	0.88	0.12		0.83	0.17			1.00
22 ALG-x3-W		0.54	0.46		0.73		0.27	0.82	0.18		1.00		0.05	0.05	0.90
23 ALG-x4-W		0.60	0.40		0.47	0.53		0.95	0.05		0.88	0.12		0.01	0.99
24 ALG-x5-W	0.03	0.69	0.28		0.83	0.07	0.10	0.23	0.77		0.54	0.46	0.08		0.92
25 x-x1-H	0.09	0.81	0.10		0.77	0.17	0.06		0.97	0.03	0.58	0.42	0.14	0.13	0.73
26 x-x2-H	0.17	0.80	0.03		0.87	0.05	0.08		1.00		0.44	0.56	0.15		0.85
27 x-x3-H		0.89	0.11		0.51	0.49			1.00		0.76	0.24		0.18	0.82
28 x-x4-H		0.60	0.40		0.13	0.81	0.06	0.04	0.96		1.00				1.00

TABLE A2.1.—Extended.

Sample	Locus													
	*G3PDH-3**		*GPI-B1**			*GPI-B2**		*sIDHP-1**				*LDH-B1**		
	*100	*111	*100	*135	*150	*39	*100	*100	*120	*140	*152	*72	*86	*100
1 LAW-O1-W	1.00		0.81		0.19	0.02	0.98	0.68	0.02	0.28	0.02	0.11	0.01	0.88
2 LAW-O2-W	1.00		1.00				1.00	0.61		0.33	0.06	0.26		0.74
3 LAW-B1-N	1.00		0.27		0.73		1.00	0.61	0.06	0.06	0.27			1.00
4 LAW-B2-N	1.00		0.52		0.48		1.00	0.67	0.15	0.18		0.19		0.81
5 LAW-B1-W	0.66	0.34	0.55		0.45		1.00	0.88		0.12				1.00
6 LAW-R1-W	1.00		0.71		0.29	0.08	0.92	0.11	0.05	0.84		0.05		0.95
7 LAW-R2-W	1.00		0.68		0.32		1.00	0.11	0.14	0.74	0.01			1.00
8 LAW-S1-W	0.96	0.04	0.69		0.31	0.03	0.97	0.33		0.67		0.13	0.01	0.86
9 LAW-S2-W	1.00		0.37		0.63		1.00	0.40		0.60		0.16		0.84
10 LAW-S1-N	1.00		0.59		0.42	0.05	0.95	0.35		0.65		0.17		0.83
11 HUD-M1-W	0.53	0.47	0.83		0.17		1.00	0.82		0.04	0.14	0.30		0.70
12 HUD-M2-W	1.00		1.00				1.00	0.28	0.47	0.25				1.00
13 HUD-U1-W	1.00		0.99		0.01		1.00	0.16	0.02	0.82		0.16	0.01	0.83
14 HUD-U2-W	1.00		0.87		0.13	0.10	0.90			0.84	0.16	0.41		0.59
15 HUD-L1-W	1.00		1.00				1.00		0.15	0.85		0.02		0.98
16 HUD-L2-W	1.00		1.00				1.00	0.10	0.46	0.44		0.02		0.98
17 DEL-W1-W	0.98	0.02	1.00				1.00	0.14	0.01	0.78	0.07	0.53		0.47
18 DEL-K1-W	1.00		0.88		0.12		1.00		0.74	0.26				1.00
19 DEL-K2-W	1.00		1.00				1.00	0.14		0.86				1.00
20 ALG-x1-W	1.00		1.00			0.90	0.10	1.00					0.24	0.76
21 ALG-x2-W	1.00		0.94		0.06	0.78	0.22	0.96		0.04		0.03	0.03	0.94
22 ALG-x3-W	1.00		0.72	0.07	0.21	0.72	0.28	0.96	0.04				0.05	0.95
23 ALG-x4-W	1.00		0.95	0.01	0.04	0.81	0.19	0.99		0.01			0.01	0.99
24 ALG-x5-W	1.00		0.77		0.23	0.06	0.94	0.46	0.02	0.52		0.21		0.79
25 x-x1-H	0.99	0.01	0.91		0.09	0.01	0.99	0.29	0.08	0.62	0.01	0.47		0.53
26 x-x2-H	1.00		1.00				1.00			1.00		0.74		0.26
27 x-x3-H	0.91	0.09	0.49		0.51		1.00	0.43		0.57			0.09	0.91
28 x-x4-H	1.00		0.91		0.09		1.00	0.04	0.11	0.85		0.27		0.73

TABLE A2.1.—Extended.

	Locus or statistic											
	*sMDH-B1,2**			*sMEP-1**			*ODH**		*PGM-2**			
Sample	**74*	**100*	**120*	**0*	**50*	**100*	**100*	**116*	**0*	**100*	H_S	*P*
1 LAW-O1-W		0.97	0.03	0.89		0.11	0.91	0.10	0.08	0.91	0.050	20.6
2 LAW-O2-W		0.95	0.05	1.00			0.89	0.11	0.01	0.99	0.052	19.1
3 LAW-B1-N		1.00		0.32		0.68	0.90	0.10	0.78	0.22	0.060	17.6
4 LAW-B2-N		0.99	0.01	0.91		0.09	0.99	0.01	0.51	0.49	0.062	19.1
5 LAW-B1-W		0.93	0.07	0.68		0.32	0.75	0.25	0.62	0.38	0.065	23.5
6 LAW-R1-W		0.97	0.03	0.22		0.78	0.82	0.18		1.00	0.057	23.5
7 LAW-R2-W		0.98	0.02	0.32		0.68	0.99	0.01		1.00	0.029	19.1
8 LAW-S1-W	0.01	0.98	0.01	0.81	0.01	0.18	0.60	0.40	0.47	0.53	0.061	25.0
9 LAW-S2-W		0.97	0.03	1.00			0.59	0.41	0.69	0.31	0.050	17.6
10 LAW-S1-N		0.92	0.08	0.88		0.12	0.66	0.34	0.46	0.54	0.066	23.5
11 HUD-M1-W		0.92	0.08	0.93		0.07	0.96	0.04	0.56	0.44	0.076	25.0
12 HUD-M2-W		1.00		0.28		0.72	0.66	0.34	0.81	0.19	0.051	14.7
13 HUD-U1-W		0.94	0.06	0.31		0.69	0.82	0.18	0.02	0.98	0.056	20.6
14 HUD-U2-W		0.96	0.04	0.17		0.83	0.98	0.02	0.17	0.83	0.039	20.6
15 HUD-L1-W		0.71	0.29	0.06		0.94	0.38	0.62		1.00	0.045	13.2
16 HUD-L2-W		0.79	0.21	0.15		0.85	0.52	0.48		1.00	0.050	14.7
17 DEL-W1-W		1.00		0.10		0.90	0.74	0.26	0.03	0.97	0.051	20.6
18 DEL-K1-W		1.00		0.01	0.05	0.94	0.63	0.37		1.00	0.026	11.8
19 DEL-K2-W		1.00		0.11		0.89	0.61	0.39		1.00	0.035	14.7
20 ALG-x1-W		1.00		0.02		0.98		1.00		1.00	0.029	8.8
21 ALG-x2-W		1.00		0.18		0.82	0.47	0.53		1.00	0.061	22.1
22 ALG-x3-W		0.98	0.02	0.04		0.96	0.35	0.65	0.02	0.98	0.059	22.1
23 ALG-x4-W	0.01	0.98	0.01	0.12		0.88	0.24	0.76		1.00	0.055	26.5
24 ALG-x5-W		0.96	0.04	0.51		0.49	0.79	0.21		1.00	0.074	25.0
25 x-x1-H	0.01	0.96	0.03	0.76		0.24	0.70	0.30	0.04	0.96	0.062	25.0
26 x-x2-H		0.99	0.01	0.71		0.29	0.74	0.26		1.00	0.040	13.2
27 x-x3-H		0.93	0.07	0.91		0.09	0.86	0.14	0.19	0.81	0.059	23.5
28 x-x4-H	0.21	0.79		0.55		0.45	1.00			1.00	0.054	17.6

TABLE A2.2.—Frequencies of alleles for loci polymorphic in three or fewer samples of brook trout collected from New York and Pennsylvania. Sample abbreviations are defined in Table 1.

Allele	Frequency	Sample
*sAAT-3*93*	0.03	LAW-S2-W
	0.37	ALG-x4-W
	0.02	ALG-x5-W
AK-1−125*	0.03	LAW-S1-W
	0.06	x-x1-H
*DDH-3*85*	0.03	LAW-B1-W
	0.15	DEL-W1-W
	0.08	DEL-K1-W
*GPI-A*106*	0.04	HUD-U2-W
	0.05	x-x3-H
*mIDHP-1*133*	0.11	HUD-L2-W
*sIDHP-2*null*	0.11	ALG-x2-W
	0.15	ALG-x3-W
	0.05	ALG-x4-W
*LDH-A2*44*	0.01	LAW-S1-N
*LDH-B2*150*	0.07	ALG-x3-W
*LDH-B2*240*	0.03	LAW-R1-W
	0.10	ALG-x4-W
*mMEP-2*60*	0.02	HUD-M1-W
	0.08	ALG-x4-W
*sMEP-1*105*	0.07	LAW-B1-N
	0.02	DEL-K2-W
*MPI*104*	0.02	HUD-M1-W
*PGDH*98*	0.27	ALG-x2-W
PGK-2−150*	0.13	ALG-x5-W
*PGM-1*109*	0.18	ALG-x2-W
*TPI-1*91*	0.10	LAW-O2-W
	0.14	x-x4-H
*TPI-1*183*	0.03	LAW-R1-W
*TPI-3*97*	0.30	ALG-x3-W