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Brook Genetics
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Genetic Variation in Natural Populations of Brook Trout
(*Salvelinus fontinalis*): Evidence Suggesting Subspecific Differences
Between Northern and Southern Populations*

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ABSTRACT

The existence of a taxonomically-distinct southern strain of brook trout (*Salvelinus fontinalis*) has not been properly investigated by genetic studies. To this end eight populations of wild brook trout, including three from Pennsylvania, two from New York, two from Tennessee and one from the Great Smoky Mountains National Park, North Carolina, were surveyed by starch gel electrophoresis for 39 loci. The frequency of observed heterozygotes was 0.081, which is higher than values reported for other wild salmonid populations. The mean genetic distance between northern and southern brook trout populations was 0.890. This, along with other evidence, is consistent with according subspecific status to southern brook trout, particularly those from the Great Smoky Mountains National Park.

INTRODUCTION

The brook trout, *Salvelinus fontinalis* (Mitchill), is ubiquitously distributed across North America from Georgia to Ontario and is the only salmonid native to the southeastern United States. Lennon (1967) suggested that the southern members of the species, particularly those residing in the isolated headwaters of streams in the Great Smoky Mountains National Park (GSMNP), constitute a separate subspecies or perhaps even species. However, such suggestions were based on a limited amount of morphological data and much speculation; no genetic data were available to support a separate taxonomic status for southern brook trout. Brussard and Nielsen (1977), using starch gel electrophoresis, surveyed 18 loci in six populations of wild brook trout, including one from GSMNP. They concluded that the GSMNP population was genetically distinct from the northern populations. Harris et al. (1978), in a more extensive study, surveyed 35 populations from the eastern United States, ranging from Georgia to New York, including nine populations from GSMNP. They also included one population each from Utah and Wisconsin. Based on their study of 29 loci they concluded that southern brook trout were not genetically distinct from northern brook trout.

Both of these studies, however, were plagued with difficulties in the genetic interpretation of the isozyme banding patterns. Brook trout are a tetraploid-derivative species and about 40% of the isozyme loci are still duplicated (May et al., 1980). The numerous multi-locus isozyme systems make it difficult, if not impossible, to accurately interpret the genetic basis of the electrophoretic variation without inheritance data. Neither of the above studies obtained inheritance data and thus the genetic interpretation of variation was based entirely on the banding patterns and fit

to Hardy-Weinberg proportions. Their errors of interpretation make the conclusions of these studies questionable.

For example, Harris et al. (1978) observed three separate, invariant zones of activity when staining liver extracts for glutamate dehydrogenase (GDH), sorbitol dehydrogenase (SDH), or xanthine dehydrogenase (XDH), and concluded that three loci code for each enzyme. However, inheritance results from this laboratory (unpublished data) reveal that in each case two of these zones are actually due to alcohol dehydrogenase and lactate dehydrogenase activity that stain incidentally with liver extracts. Thus Harris et al. overestimated the number of loci they examined.

For the past two years this laboratory has been involved with an extensive, intensive study of the inheritance of isozyme loci in salmonids, particularly *Savelinus* (May et al., 1979, 1980; Stoneking et al., 1979; Wright et al., in prep.). We have established the genetic bases of many isozyme systems in brook trout. During the course of these studies we screened a number of wild populations to detect additional variability for inheritance studies. Several of these populations were known to possess variants which were of interest to us (Harris, pers. commun.). This paper presents the results of our survey of allozyme variation in eight populations of wild brook trout. Knowledge gained from the previous inheritance studies enabled us to more accurately assess the taxonomic status of southern brook trout.

MATERIALS AND METHODS

Sampling information

Wild brook trout were collected between October 1978 and July 1979 from the following eight localities: *New York*: Long Pond Outlet (Franklin Co.) and Slush Pond (Franklin Co.); *Pennsylvania*: East Branch of Big Run (Clinton Co.), John Summerson Branch (Clinton Co.), and Smays Run (Centre Co.); *Tennessee*: Blockstan Creek (Unicoi Co.) and Rocky Fork Creek (Unicoi Co.); *North Carolina*: Bunches Creek (Great Smoky Mountains National Park). All populations were sampled by electroshocking except the Slush Pond population, which was collected by gill net and angling. John Summerson Br., East Br. of Big Run, and Smays Run are tertiary tributaries of Kettle Creek, Bald Eagle Creek, and Moshannon Creek, respectively. These three streams flow into the West Branch of the Susquehanna River at approximately 40 mile intervals; the distances, coupled with the pollution of the Susquehanna, serve to completely isolate these three populations. Blockstan Cr., a tributary of Rocky Fork Cr., is isolated by a waterfall. Slush Pond was reclaimed in 1971 and stocked for several years in a row with approximately two thousand brook trout per year from Windfall Pond, N.Y., which has never been stocked. To the best of our knowledge all other localities have never been stocked; all maintain natural reproduction.

Electrophoresis

May et al. (1979) describe in detail the tissue extraction and horizontal starch gel electrophoretic techniques used in this study. The enzymes examined, their abbreviations, buffer systems, tissues, and number of loci scored are presented in Table 1. Staining recipes for all enzymes except AAT, LAP, and ODH followed those of Allendorf et al. (1977), except

that the substrate amounts we used were approximately a third of the suggested amounts. The staining procedure for AAT is described by May et al. (1979), for ODH by May et al. (1980), and for LAP by Ayala et al. (1972). Resolution of ADA, CPK, PGI, PMI, and 6PG was increased by using an agar overlay (Brewer, 1970).

Nomenclature

Allelic nomenclature follows that suggested by Allendorf and Utter (1979) and modified by May et al. (1979). Loci are numbered consecutively from most cathodal to most anodal; alleles are designated by the relative mobility of the protein product. For example, *LDH-2(50)* would refer to an allele of the second LDH locus whose protein product migrates half as far as the protein product of the arbitrarily designated 100 allele. A dash preceding the numerical designation indicates an allele whose protein product migrates cathodally, i.e. *ADH(-100)*. An allele whose protein product does not visibly migrate from the origin is designated 0, while a null allele (producing no detectable activity) is designated $\emptyset$. Where two duplicate loci share alleles producing electrophoretically-identical protein products, the two loci are set off by a comma, as with *MDH-3,4*.

RESULTS

Genetic Variation Within Populations

Eight populations of wild brook trout were scored for 39 loci; 20 loci were polymorphic (frequency of a variant allele greater than 0.05) in at least one population (Table 2). The 21 loci listed in Table 2 include *MDH-3,4*, a pair of duplicate loci that share electrophoretically-identical alleles. Although it is not possible to assign variation to a specific

locus of this duplicate pair, all of the variability we found was consistent with one monomorphic locus and one polymorphic locus. Thus we claim 20 polymorphic loci. We found rare variants at four loci: *ADA-1* and *CPK-3* in the Bunches Cr. population, frequencies of 0.01 and 0.02 respectively; *PGM-1* in the Long Pond Outlet population, frequency 0.05; and *LDH-1* in the Smays Run population, frequency 0.01. An additional 14 loci were monomorphic in all populations: *ADA-2*, *CMP*, *CPK-1*, *IDH-1*, *IDH-2*, *LAP*, *LDH-2*, *LDH-4*, *LDH-5*, *MDH-1*, *MDH-2*, *ME-3*, *SDH*, and *SOD*. May et al. (1979, 1980) and Stoneking et al. (1979) have described several isozyme systems in brook trout; we describe new systems below. Since no variability has ever been noted in brook trout for *ADA-2*, *CMP*, *LAP*, and *ME-3*, we conservatively assumed that a single locus was responsible for the expression of these zones of activity.

AAT - A variant of the eye-specific locus, *AAT-3*, was found in the population from the East Branch of Big Run. Heterozygotes displayed the three-banded pattern expected for this dimeric enzyme. Of the two *AAT* loci commonly expressed in the liver (variation described by May et al., 1980); one (*AAT-4*) was polymorphic with alleles 100, 133, and 170.

CPK - The Rocky Fork Cr., Blockstan Cr., and Bunches Cr. populations possessed an allele at the second muscle *CPK* locus that was not found in any of the other populations. The variation observed fits the model proposed by Utter et al. (1979) for muscle *CPK* variation in salmonids, where each allele produces two monomeric protein products. We observed additional variation at the eye-specific zone of *CPK* activity in the Bunches Cr. population, the first such variation reported in salmonids. The banding patterns indicated a single locus coding a dimeric enzyme; we have designated this locus *CPK-3*.

IDH - A null allele was found at *IDH-4* in the populations from Blockstan Cr. and Rocky Fork Cr. Harris et al. (1978) reported Rocky Fork Cr. brook trout were fixed for a null allele at this locus.

PGM - May et al. (1980) describe three zones of PGM activity. A mobility-variant at the most cathodal zone in the Lond Pond Outlet population, the first such variation reported in salmonids, indicated a single locus coding a monomeric enzyme (*PGM-1*). Some individuals from this population possessed no *PGM-1* activity but otherwise normal PGM activity at the other two zones. A possible explanation is that a null allele might be segregating at either *PGM-1* itself, or at a gene regulating *PGM-1* activity. Variation at the middle zone of PGM activity in several populations also pointed to a single locus expressing a monomeric enzyme (*PGM-2*), consistent with the inheritance results of May et al. (1980). Variation at the most anodal zone of PGM activity was noted in several populations. The variation appeared to reflect a pair of duplicate loci but could not be accurately scored.

6PG - Variation was observed in the Bunches Cr. population but the number of loci could not be determined from the banding patterns alone. Brussard and Nielsen (1977) also noted *6PG* variation in brook trout from Bunches Cr. We assumed the most parsimonious explanation of a single locus to code for this dimeric enzyme in brook trout.

The phenotypic distribution observed at each polymorphic locus in every population fit that expected by Hardy-Weinberg equilibrium, except for the two muscle AAT loci (*AAT-1,2*). The *AAT-1,2* data will be presented in another paper and are not included in any of the analyses here. The frequency of observed heterozygotes for each population, averaged over all 39 loci, is given at the bottom of Table 2. These values were calculated by dividing

the observed number of heterozygotes by the total number of individuals typed at a particular locus, and then averaging across the 39 loci. The frequency of observed heterozygotes over all eight wild brook trout populations (mean heterozygosity) is 0.081 ± 0.029 .

Genetic differences among populations

Inspection of the gene frequency data given in Table 2 immediately reveals four loci (*CPK-2*, *ME-1*, *ODH*, and *PGI-2*) which show striking differences between the five northern populations (Long Pond Outlet, Slush Pond, East Branch of Big Run, John Summerson Br., and Smays Run) and the three southern populations (Blockstan Cr., Rocky Fork Cr., and Bunches Cr.). The gene frequency differences at these four loci are illustrated in Fig. 1. *ODH* is characterized by intermediate to high frequencies of the 100 allele in the northern populations and low frequencies in the southern populations; *ME-1* shows the opposite pattern. McGlade and MacCrimmon (1979) similarly found low to intermediate frequencies of the *ME-1*(100) allele among four Canadian brook trout populations. The southern populations possessed an allele at *PGI-2* at intermediate to high frequencies that was not found in any of the northern populations. All northern populations were fixed for *CPK-2*(100), while the southern populations were polymorphic or fixed for *CPK-2*(122).

Measures of genetic similarity and distance (Nei, 1972) were computed for all pairwise comparisons of the eight populations (Table 3). The mean genetic similarity among the five northern populations was 0.973 ± 0.015 ; among the three southern populations it was 0.955 ± 0.018 . The mean genetic similarity between northern and southern populations was 0.890 ± 0.032 . A cluster analysis of these similarities and distances was done using the

flexible strategy of Lance and Williams (1967); the resulting dendrogram is illustrated in Fig. 2. Populations from the same state cluster together at roughly equal distances, then all northern populations cluster together and all southern populations cluster together, and finally the northern and southern populations cluster together.

DISCUSSION

Several of the populations that we surveyed for allozyme variation were included in the previous electrophoretic studies by Brussard and Nielsen (1977) and Harris et al. (1978). Long Pond Outlet, Bunches Cr., and Windfall (the progenitor of Slush Pond) were studied by Brussard and Nielsen; however the nomenclature they used to identify loci and alleles precludes comparisons with our results beyond what we indicated in our description of variable enzyme systems. Harris et al. included populations from John Summerson Br., Rocky Fork Cr., and Bunches Cr. Our results are generally in agreement with their results for IDH, LDH, MDH, PGM, SOD, and one PGI locus (*PGI-3*), although we also found polymorphisms for *6PG* and *DIA* in the Bunches Cr. population. More significantly, their interpretation of the variability of *PGI-1* and *PGI-2* differed from ours, and was not consistent with the descriptions and drawings they offered. Their interpretation of the genetic basis of the *PGI-(1,2)* variation, coupled with their lack of data for *CPK-2*, *ME-1*, and *ODH* (the other loci showing the greatest differences between northern and southern populations), most likely accounts for their failure to find genetic differences between northern and southern populations of brook trout.

In our survey of eight populations of wild brook trout for 39 loci we report mean heterozygosities ranging from 0.028-0.114, with a mean of 0.081 over all populations. This is somewhat higher than mean heterozygosities

calculated from previous studies of electrophoretic variation in brook trout. Wright and Atherton (1970) surveyed six wild and six hatchery brook trout populations; from their data we calculated mean heterozygosities of 0.053 ± 0.045 and 0.129 ± 0.024 respectively. However, these values are based on only six loci for two proteins (transferrin and LDH) that were known to be variable, thus biasing the estimates. From the data of Harris et al. (1978) we calculated a mean heterozygosity over all 35 populations of 0.017, ranging from 0.000-0.062. This low level of variability, in contrast to our estimate, is probably due to differences in interpretation of banding patterns and in the specific loci examined. A survey of four Canadian brook trout populations for 24 loci gave a mean heterozygosity of 0.075 ± 0.017 (McGlade and MacCrimmon, 1979), similar to the value we report.

The value we give of 0.081 for mean heterozygosity in brook trout populations is the highest reported for any wild salmonid species, although Busack et al. (1979) reported a mean heterozygosity of 0.095 ± 0.024 from their survey of 24 loci in four hatchery strains of rainbow trout (*Salmo gairdneri*). Allendorf and Utter (1979) give mean heterozygosities ranging from 0.000 to 0.063 for nine species of *Oncorhynchus* and *Salmo*, based on a minimum of 30 loci. Further, based on our study, brook trout on the average are more heterozygous than fishes in general (mean heterozygosity = 0.0513 ± 0.0338 ; Nevo, 1978).

Allendorf and Utter (1979) commented on the difficulties of explaining interspecies patterns of variation of mean heterozygosity among salmonids. Previous explanations centered around relative amounts of gene duplication, rates of evolutionary divergence, and effective population sizes. We make no attempt to relate our results regarding the relatively high mean heterozygosity of brook trout to any of these.

The pattern of variation of mean heterozygosities among brook trout populations is similarly difficult to explain. One might suppose that the increased isolation and smaller population size that characterize southern populations would result in less variability. Indeed, the two smallest mean heterozygosities belong to two southern populations: the Bunches Cr. population (0.028) and the Rocky Fork Cr. population (0.048). However, the third southern population, Blockstan Cr., has the highest mean heterozygosity (0.114). It could be that Blockstan Cr. has been stocked, resulting in a more heterogeneous population of fish. The Blockstan Cr. population is more similar to the northern populations than either the Bunches Cr. or Rocky Fork Cr. populations (Fig. 1 and Table 3), supporting the hypothesis that it has been stocked with brook trout of northern origin. However, Blockstan Cr. is a tributary of Rocky Fork Cr. and is less accessible, making this hypothesis less tenable. This point remains unresolved and can perhaps be best summarized as an example of the difficulty in attempting to explain such relationships based on just eight populations. It also illustrates the need (and difficulty) of guaranteeing the purity of wild brook trout populations used in studies such as this.

Lennon (1967) proposed that GSMNP brook trout, and perhaps all southern brook trout, were taxonomically distinct from their northern counterpart. He based this claim on GSMNP brook trout's lower fecundity, smaller and more numerous red spots on the sides, different relative sizes of body parts, and increased susceptibility to disease. He also cited records showing that hatchery-raised brook trout from GSMNP survived and reproduced when planted within GSMNP, but not when planted further north. Conversely, hatchery-reared northern brook trout have not survived when planted in GSMNP waters, despite repeated attempts. In contrast to Lennon's study, Harris et al.

(1978), in addition to their electrophoretic studies, studied 11 meristic and 15 morphometric characters and found no evidence of any taxonomic differences between northern and southern brook trout.

Our results suggest definite genetic differences between the northern and southern populations surveyed. We found the average genetic similarity between northern and southern populations was less than that within northern and southern populations. The one population from GSMNP (Bunches Cr.) was fixed at *CPK-2* and *PGI-2* for alleles not found in any northern population. Furthermore, the Bunches Cr. population showed an unusual pattern of variation; of seven variable loci, four possessed alleles not found in any other population.

Are these genetic differences sufficiently pronounced that the southern brook trout merits taxonomic distinction? The answer to this question is not straightforward. Avise (1974) reported that similarities for a wide variety of organisms were usually above 0.90 between conspecific populations, and well below 0.80 between congeneric species. Comparisons between subspecies were usually in the range 0.80-0.90, with surprisingly little overlap between the various classifications. More recently, Avise and Smith (1977) examined 14 loci in populations of bluegill (*Lepomis*); they found an average genetic similarity of 0.977 ± 0.004 among populations of the same subspecies, and an average genetic similarity of 0.843 ± 0.013 between subspecies. Similarly, Buth and Burr (1978) reported average genetic similarities of 0.98 among populations and 0.84 among subspecies of the cyprinid genus *Camptostoma*. The similarities we report for within-northern (0.973) and within-southern (0.955) comparisons are well within the above range of over 0.90 for conspecific populations. On the other hand, our results suggest that the genetic differences between northern and southern populations of brook trout

(similarity = 0.890), are similar to those expected for subspecies.

We note that the average genetic similarity we report for comparisons between northern and southern populations is not statistically different from the average genetic similarities for within-northern and within-southern comparisons. However, the patterns of genetic variation we observed, and the results of the cluster analysis, support the hypothesis of subspecific differences between northern and southern brook trout. This conclusion is consistent with the observations of Lennon (1967) regarding morphology and reproductive traits. Certainly, before this hypothesis can be accepted, additional populations of wild brook trout, particularly from GSMNP, need to be screened to ascertain the applicability of our results to other populations. Granting subspecific status to GSMNP brook trout has important implications, in light of the threatening encroachment of rainbow trout and decline of brook trout in GSMNP waters (Lennon, 1967; Harris et al., 1978). It may not be sufficient (or possible) to restock GSMNP waters with brook trout from other localities; GSMNP brook trout may be taxonomically irreplaceable.

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Table 1. Enzymes and electrophoretic procedures used in this study.

Enzyme	Abbreviation	Tissue ¹	Number of loci scored	Buffer ² system
Aspartate aminotransferase	AAT	E L	1 1	RSL MF
Adenosine deaminase	ADA	L	2	MF
Alcohol dehydrogenase	ADH	L	1	RSL
Alphaglycerophosphate dehydrogenase	AGP	M	2	AHZV, CT
Cathodal muscle protein	CMP	M	1	RSL
Creatine phosphokinase	CPK	E M	1 2	MF RSL
Diaphorase	DIA	L,M	1	CT
Isocitrate dehydrogenase	IDH	E,L M	2 2	AHZV AHZV
Leucine aminopeptidase	LAP	E	1	AHZV
Lactate dehydrogenase	LDH	E L M	2 1 2	AHZV, MF RSL RSL
Malate dehydrogenase	MDH	E M	2 2	CT RSL
Malic enzyme	ME	M	3	CT
Octanol dehydrogenase	ODH	L	1	MF
Phosphoglucoisomerase	PGI	M	3	RSL
Phosphoglucomutase	PGM	M	2	CT
Phosphomannoisomerase	PMI	E	1	MF
Six-phosphogluconate dehydrogenase	6PG	M	1	AHZV
Sorbitol dehydrogenase	SDH	L	1	RSL
Superoxide dismutase	SOD	L	1	RSL

¹ E = eye, L = liver, M = muscle

² Buffers are from May et al. (1979).

Table 2. Gene frequencies of 21 loci and frequency of observed heterozygotes in 8 populations of brook trout. The number of individuals typed for a particular locus is in parenthesis.

Locus	Allele	Long Pond Outlet	Slush Pond	East Branch of Big Run	John Summerson Br.	Smays Run	Blockstan Creek	Rocky Fork Creek	Bunches Creek
AAT-3		(48)	(33)	(27)	(45)	(33)	(23)	(31)	(44)
	93	-	-	.13	-	-	-	-	-
	100	1.00	1.00	.87	1.00	1.00	1.00	1.00	1.00
AAT-4		(48)	(33)	(27)	(43)	(33)	(22)	(31)	(44)
	100	.47	.92	.63	.34	.39	.96	.89	1.00
	133	.29	-	.13	.15	.46	-	-	-
	170	.24	.08	.24	.51	.15	.04	.11	-
ADH		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	0	.01	-	-	-	-	-	-	-
	-100	.99	1.00	.72	1.00	.97	1.00	1.00	1.00
	-205	-	-	.28	-	.03	-	-	-

Table 2. Continued.

Locus	Allele	Long Pond Outlet	Slush Pond	East Branch of Big Run	John Summerson Branch	Smays Run	Blockstan Creek	Rocky Fork Creek	Bunches Creek
<i>AGP-1</i>		(47)	(33)	(27)	(45)	(60)	(22)	(31)	(44)
	-32	.03	.26	.02	.08	-	.02	-	-
	0	-	-	-	.07	.11	-	-	-
	67	.63	.45	.22	.37	.34	.52	.79	1.00
	100	.34	.29	.76	.48	.55	.46	.21	-
<i>AGP-2</i>		(48)	(32)	(24)	(45)	(55)	(23)	(31)	(44)
	100	1.00	.84	.42	.58	.31	.98	.87	1.00
	111	-	.16	.58	.42	.69	.02	.13	-
<i>CPK-2</i>		(48)	(33)	(27)	(45)	(60)	(21)	(31)	(44)
	100	1.00	1.00	1.00	1.00	1.00	.62	.16	-
	122	-	-	-	-	-	.38	.84	1.00
<i>DIA</i>		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	89	-	-	-	-	-	-	-	.42
	100	1.00	.91	1.00	1.00	1.00	1.00	1.00	.58
	105	-	.09	-	-	-	-	-	-

Table 2. Continued.

Locus	Allele	Long Pond Outlet	Slush Pond	East Branch of Big Run	John Summerson Branch	Smays Run	Blockstan Creek	Rocky Fork Creek	Bunches Creek
IDH-3		(48)	(33)	(26)	(45)	(59)	(23)	(31)	(44)
	100	.45	.03	.50	.78	.84	.41	.87	1.00
	120	-	-	.27	.21	.15	.02	-	-
	140	.55	.97	.23	.01	.01	.57	.13	-
IDH-4		(48)	(33)	(26)	(45)	(59)	(17)	(31)	(44)
	Ø	-	-	-	-	-	.59	.97	-
	100	1.00	1.00	1.00	1.00	1.00	.41	.03	1.00
LDH-3		(48)	(33)	(27)	(45)	(60)	(23)	(30)	(44)
	72	.15	.46	-	.02	.02	-	.07	-
	86	.01	-	-	-	.02	-	-	-
	100	.84	.54	1.00	.98	.96	.46	.13	1.00
	117	-	-	-	-	-	.54	.80	-
MDH-3,4		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	100	.96	.91	1.00	1.00	1.00	.96	1.00	1.00
	120	.04	.09	-	-	-	.04	-	-

Table 2. Continued.

Locus	Allele	Long Pond Outlet	Slush Pond	East Branch of Big Run	John Summerson Branch	Smays Run	Blockstan Creek	Rocky Fork Creek	Bunches Creek
<i>ME-1</i>		(48)	(33)	(27)	(45)	(60)	(22)	(31)	(44)
	0	.83	.58	.63	.61	.82	.14	.05	-
	50	-	.01	.17	-	-	-	-	-
	100	.17	.41	.20	.39	.18	.86	.95	1.00
<i>ME-2</i>		(48)	(33)	(27)	(45)	(60)	(22)	(31)	(44)
	0	-	-	-	-	-	.18	-	-
	100	1.00	1.00	1.00	1.00	1.00	.82	1.00	1.00
<i>ODH</i>		(48)	(31)	(27)	(45)	(60)	(23)	(31)	(44)
	100	.62	.61	.93	1.00	.99	.28	-	-
	116	.38	.39	.07	-	.01	.72	1.00	1.00
<i>PGI-1</i>		(35)	(33)	(27)	(44)	(60)	(23)	(30)	(44)
	100	.86	.94	.89	.91	.87	1.00	1.00	1.00
	150	.14	.06	.11	.09	.13	-	-	-
<i>PGI-2</i>		(48)	(33)	(27)	(45)	(60)	(23)	(30)	(44)
	39	.04	.03	-	-	.01	-	-	-
	70	-	-	-	-	-	.56	.88	1.00
	100	.96	.97	1.00	.96	.99	.44	.12	-
	121	-	-	-	.04	-	-	-	-

Table 2. Continued.

Locus	Allele	Long Pond Outlet	Slush Pond	East Branch of Big Run	John Summerson Branch	Smays Run	Blockstan Creek	Rocky Fork Creek	Bunches Creek
<i>PGI-3</i>		(48)	(33)	(27)	(45)	(60)	(23)	(30)	(44)
	94	-	-	-	.06	-	-	-	-
	100	1.00	1.00	1.00	.81	.91	1.00	1.00	1.00
	106	-	-	-	.13	.09	-	-	-
<i>PGM-2</i>		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	0	.55	.24	.13	-	.05	-	-	-
	-100	.45	.76	.87	1.00	.95	1.00	1.00	1.00
<i>PMI</i>		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	100	1.00	1.00	1.00	.98	.98	1.00	1.00	.76
	104	-	-	-	-	.02	-	-	-
	106	-	-	-	.02	-	-	-	.24
<i>6PG</i>		(48)	(33)	(27)	(45)	(60)	(23)	(31)	(44)
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.92
	115	-	-	-	-	-	-	-	.08
Heterozygosity ¹		.094	.085	.107	.090	.078	.114	.048	.028

¹ Frequency of observed heterozygotes averaged over 39 loci, including 18 not listed above.

Table 3. Nei's index of genetic similarity (above diagonal) and distance (below diagonal) between eight populations of wild brook trout. Italics indicate comparisons between northern and southern populations.

	L.P.O.	S.P.	S.R.	J.S.B.	E.B.B.R.	Bl.C.	R.F.C.	Bu.C.
Long Pond Outlet	-	0.982	0.966	0.971	0.971	<i>0.938</i>	<i>0.872</i>	<i>0.884</i>
Slush Pond	0.018	-	0.948	0.956	0.964	<i>0.952</i>	<i>0.879</i>	<i>0.882</i>
Smays Run	0.035	0.053	-	0.993	0.991	<i>0.912</i>	<i>0.852</i>	<i>0.863</i>
John Summerson Branch	0.029	0.047	0.007	-	0.990	<i>0.926</i>	<i>0.866</i>	<i>0.878</i>
East Branch of Big Run	0.029	0.037	0.009	0.010	-	<i>0.926</i>	<i>0.856</i>	<i>0.865</i>
Blockstan Creek	<i>0.064</i>	<i>0.049</i>	<i>0.092</i>	<i>0.077</i>	<i>0.077</i>	-	0.976	0.942
Rocky Fork Creek	<i>0.137</i>	<i>0.129</i>	<i>0.160</i>	<i>0.144</i>	<i>0.155</i>	0.024	-	0.947
Bunches Creek	<i>0.123</i>	<i>0.126</i>	<i>0.147</i>	<i>0.130</i>	<i>0.145</i>	0.060	0.054	-

Fig. 1. Gene frequencies at four loci in wild brook trout, illustrating differences between northern and southern populations. The shaded portion of each circle represents the frequency of the 100 allele for *CPK-2*, *ME-1*, and *ODH*; for *PGI-2* it represents the frequency of the 70 allele.

Fig. 2. Dendrogram of genetic distance between eight wild brook trout populations. Two distinct clusters are evident, one consisting of the five northern populations, the other consisting of the three southern populations.

POPULATION

STATE

CPK-2

ME-1

ODH

PGI-2

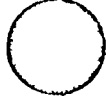
LONG POND OUTLET

NY



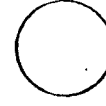
SLUSH POND

NY



SMAYS RUN

PA



JOHN SUMMERSON BR

PA



EAST BRANCH BIG RUN

PA



BLOCKSTAN CR

TN



ROCKY FORK CR

TN



BUNCHES CR

NC



