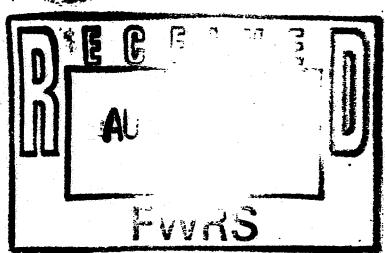


48Φ14Φ15Φ

HERITAGE BROOK TROUT IN WISCONSIN:  
IDENTIFICATION AND GENETIC  
VARIABILITY AMONG POPULATIONS



Study No. SSDS<sup>1</sup> (formerly RS423)

By Ed L. Avery

Period Covered: 1 July 1995-  
30 June 2000

Date Prepared: 1 March 1999

CONTENTS:

ABSTRACT . . . . .	1
PROBLEM DESCRIPTION . . . . .	2
STUDY OBJECTIVES . . . . .	3
STUDY DESIGN AND PROCEDURES . . . . .	4
PERFORMANCE ON SCHEDULED ACTIVITIES . . . . .	4
Activity 1 -- Select brook trout streams for sampling . . . . .	4
Activity 2 -- Sampling brook trout populations . . . . .	5
Activity 3 -- Genetic analyses of brook trout samples . . . . .	5
Activity 4 -- Compare analyses of this study with previous studies . . . . .	5
Activity 5 -- Analyze and write final report . . . . .	5
-- Genetic Contractor's Final Report (pp. 1-57) . . . . .	6
CONCLUSIONS . . . . .	6
ACKNOWLEDGEMENTS . . . . .	6
LITERATURE CITED . . . . .	7
APPROVALS . . . . .	8
TABLES 1-2 . . . . .	9
APPENDIX 1 . . . . .	11

ABSTRACT:

Genetic analyses of one hatchery brood stock and 17 wild brook trout populations (either no stocking history or stocking occurred  $\geq$  20 years ago) from Wisconsin were made to: (1) identify key genetic parameters for identification of heritage populations, (2) identify naturally occurring regional genetic differences, and (3) determine the genetic impacts of severely limited habitat and small populations. Sampled populations represented every major watershed and ecoregion of the state. An additional wild brook trout population from Iowa as well as the Iowa hatchery strain were also included in the genetic analyses. The most informative molecular genetic techniques for analysis of

<sup>1</sup> Funding for this study was provided in part through the Federal Aid in Fish Restoration Act.

brook trout were a combination of mtDNA RFLP and allozyme analyses. The two genetic techniques resulted in complementary, but slightly different data sets. Regional genetic structuring was evident and 7 genetic management regions were established in Wisconsin. Heritage populations (sources for propagation) and relict populations (preservation needed) were identified in each management region. Movement of brook trout between regions was not recommended as a management strategy. No evidence was found to indicate that stocking from outside sources has significantly impacted sampled populations. Additional genetic work was recommended, especially in the Lake Superior, St. Croix/Chippewa, Southwest, and L. Michigan regions, to help clarify intra- and inter-regional genetic relationships.

#### PROBLEM DESCRIPTION:

The greatest, and yet the most intangible, value of wild brook trout is their genetic resource value. Wild populations contain genetic resources which provide risk insurance against the catastrophic effects of environmental changes to which a population (or species) must adapt or perish (Soule 1980; Allendorf and Leary 1986; Shaffer 1987). The Endangered Species Act of 1973 states: "From the narrowest point of view, it is within the best interest of mankind to minimize the losses of genetic variation. The reason is simple: they are potential resources. They are keys to puzzles which we cannot solve, and may provide answers to questions which we have not yet learned to ask". Maintaining genetic diversity, i.e. variation within populations and divergence between populations, has become a tenet of modern conservation (White 1992a).

The brook trout (Salvelinus fontinalis) is the only stream-dwelling trout native to Wisconsin; however brown trout (Salmo trutta) and rainbow trout (Oncorhynchus kisutch), as well as brook trout, have been stocked in the state's inland waters since the late 1800's (Krueger and Menzel 1979). Brook trout were originally found throughout the state in suitable habitat, but environmental perturbations (e.g. deforestation, grazing, irrigation, careless use of agricultural chemicals, damming, overfishing, urbanization, introduction of exotics) have reduced the primary range to the east-central and northern portions of Wisconsin (Brasch et al. 1973).

Most hatchery-reared brook trout stocked in Wisconsin prior to 1973 came from the Osceola Hatchery, Osceola, Wisconsin and were referred to as the Osceola strain. These fish were acquired from a private hatchery (Rock Creek) located just north of St. Croix Falls, Wisconsin around the turn of the century and were thought to be of an indigenous strain (Lee Haas, Osceola Hatchery Supervisor, pers. comm.). The Osceola strain was destroyed in 1973 due to furunculosis or "red spot" disease. In 1973, a new brood stock called the St. Croix strain began at the St. Croix

Falls Hatchery, St. Croix Falls, Wisconsin from eggs acquired from Nashua, New Hampshire (Claggett and Dehring 1984). Brook trout stocked in inland waters since the mid-1970's have been St. Croix strain. Although stocking policies were modified in the late 1960's to discourage stocking into stretches of Class I streams, which by definition have adequate natural reproduction (Wis DNR 1980), stocking continues in Class II and tributary or other connecting waters.

A growing literature documents the negative impacts of stocking on the genetic integrity and long-term fitness of native salmonid stocks (Krueger and Menzel 1979; Allendorf 1991; Nehlsen et al. 1991; Hilborn 1992; White 1992a, 1992b; Yuskavitch 1999). In a comprehensive review of more than 40 papers examining releases of cultured salmonids into natural populations, Hindar et al. (1991) concluded that when effects were seen, they were always detrimental to the native stocks.

One of the greatest potential dangers to maintaining genetic integrity and fitness of wild brook trout populations is interbreeding with stocked fish. 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 (Webster and Flick 1981; Fraser 1981; Lachance and Magnan 1990;). Presumably, the differences in performance were caused mainly by genetic differences which exist between hatchery and wild fish (Vvorinen 1984; Garcia-Marin et al. 1991). Wide-spread stocking of genetically homogeneous hatchery-reared brook trout over the past century and subsequent interbreeding may have reduced differentiation and fitness among indigenous brook trout populations.

Currently, the status of genetically distinct wild brook trout stocks in Wisconsin is largely unknown. Krueger (1976) found evidence of distinct stocks both within and between watersheds in 9 streams of the Fox and Wolf River drainages in central Wisconsin. Callen (1983) also found evidence of distinct stocks within the Beef River watershed of the Mississippi drainage in west-central Wisconsin. The term "heritage" brook trout has been used to denote rare, unstocked, brook trout populations remaining in the northeastern United States (Perkins et al. 1993). By describing the patterns of genetic variation in Wisconsin brook trout populations examined in this study, indigenous brook trout populations most likely representing "heritage" stocks can be identified. This will help the Wisconsin Department of Natural Resources (DNR) set priorities for watershed management activities, e.g. land acquisition, inclusion in the Priority Watershed Program, habitat protection/enhancement activities, and restrictive angling and harvest regulations, that will preserve genetic diversity and protect endangered "heritage" stocks.

#### **STUDY OBJECTIVES:**

In 1993, the DNR initiated a "Wisconsin Trout Genetics Study" to clarify the genetic status of 14 domestic, naturalized, and wild populations of brook, brown, and rainbow trout (Larry Claggett, DNR, pers. comm.). Five wild brook trout populations and one domesticated brook trout population were sampled. The "Heritage Brook Trout Study" will expand upon the above effort and focus on wild brook trout. Specific objectives include:

1. Identify brook trout populations least likely to be impacted by propagation and stocking practices in both the unglaciated and glaciated regions of Wisconsin as well as in both the St. Lawrence River and Mississippi River watershed.
2. Select 12-16 wild brook trout populations not previously sampled and quantify genetic variability between them.
3. Identify spatial patterns of genetic variation to gain insight into historic fish distribution.
4. Compare results with those of other Wisconsin studies of domesticated brook trout strains (Krueger 1976; Callen 1983).
5. Transfer the results of this study to management personnel for use in development of management strategies to protect genetic diversity of indigenous brook trout populations, maintain biodiversity, restore heritage brook trout range, and develop several high quality heritage brook trout fisheries.

#### **STUDY DESIGN AND PROCEDURES:**

Fish managers from throughout Wisconsin were contacted to compile a list of brook trout streams that had either never been stocked (no records) or that had not been stocked for at least 20 years. All stocking records were checked to verify stocking histories. A sample of 30 brook trout from 12-16 streams were collected and sent to a genetics lab (Illinois Natural History Survey) contracted to fulfill Objectives 3, 4, and 5.

#### **PERFORMANCE ON SCHEDULED ACTIVITIES:**

##### **Activity 1 -- Select brook trout streams for sampling.**

Seventeen streams were selected for study from a composite list of 108 candidate streams (Table 1). Based upon access, stream size, the presence of natural fish barriers (waterfalls), and their stocking histories (or lack thereof), these 17 streams appeared to have the best chances of supporting brook trout populations genetically untainted by hatchery fish. Although numerous smaller streams represented as good, if not better,

choices, the streams selected were considered most likely to support brook trout populations large enough to withstand removal of 30 fish without undue genetic risks to their populations. Brook trout samples would be collected from only 12-16 streams (due to costs of genetic analyses). It was assumed that sufficient numbers of brook trout to facilitate sampling would not be found in one or more of the 17 streams selected.

**Activity 2 - Sampling brook trout populations.**

A sample of 30-35 brook trout were ultimately collected from 12 of the 17 streams sampled (Table 2). Brook trout were collected using either one or two 12v, DC, back-pack electrofishing units. At the request of the genetics laboratory, whenever possible brook trout  $\geq$  4.0 inches in length were selected to assure enough tissue for all analyses. Brook trout were collected from throughout 1 or 2 locations on each stream and a final sample selected that represented a cross-section of the length frequency observed. This was done to avoid taking the entire sample from related trout, i.e. siblings from one redd. Length frequencies of all sacrificed brook trout are provided in Appendix 1.

Brook trout were anesthetized with MS-222 and individually wrapped in foil. A label designating the date, stream, location, species and total length was included in the foil with each trout. Once labelled and wrapped in foil, individual fish were immediately placed in a cooler of ice. When all individuals from each stream had been processed, they were removed from the ice, placed in a heavy duty (5mm) white plastic trash-compactor bag with a second label containing date, stream, location, species, and number of fish. The white plastic bag was labelled on the outside with the same information using a permanent black marker pen. The white bag containing the fish was returned to the cooler, covered with ice, and transported to the vehicle. At the vehicle, the labelled white bag was transferred to a second cooler containing dry ice and transported to a DNR field headquarters where they were placed in a deep freeze at -20 C. Frozen trout were then packed in dry ice and transported to the Illinois Natural History Survey in Champaign, Illinois, for genetics analyses.

Similar procedures were also used to sample and transport 30 domestic brook trout from the St. Croix Fish Hatchery in St. Croix Falls, Wisconsin to the Illinois Natural History Survey in Champaign, Illinois.

**Activity 3 - Genetic analyses of brook trout samples.**

**Activity 4 - Compare genetic analyses of this study with previous studies.**

**Activity 5 - Analyze data and write final report.**

Robert D. Fields, with the Illinois Natural History Survey in

Champaign, Illinois, was contracted to conduct the genetic analyses of the brook trout samples, to compare his results to genetic analyses of brook trout from previous Wisconsin studies, and to submit a final report with management recommendations.

In addition to the brook trout samples received from the current study, brook trout samples from 5 additional Wisconsin streams, one Iowa stream, and a fish hatchery in Manchester, Iowa were consolidated into the genetic analyses and final report submitted by Mr. Fields to the DNR. The latter 7 brook trout populations were sampled in conjunction with the "Wisconsin Trout Genetics Study" headed by Larry Claggett (DNR). The "FINAL REPORT - Genetic Analysis of Wisconsin Brook Trout" authored by R.D.Fields and D.P. Philipp is attached.

The attached report and the accompanying text, etc. of the current report constitutes the final report for this study.

**GENETIC CONTRACTOR'S FINAL REPORT (pp. 1-57 attached).**

**CONCLUSIONS:**

Polymorphic allozyme loci and allele frequencies established significant variation among the Wisconsin brook trout populations examined. A phenogram based on a genetic distance matrix of 0.025 exhibited 6 geographic groupings of genetically similar populations. RFLP analysis of mtDNA found 7 distinct haplotypes or clonal lines. The two genetic analyses (allozyme electrophoresis and mtDNA RFLP) thus resulted in complementary, but slightly different data sets. Both analyses found regional genetic structuring that in the composite suggested 7 genetic management regions in Wisconsin. These regions were: Lake Superior, St.Croix/Chippewa, Southwest, South, L. Michigan, Northeast, and Upper Wisconsin River. The moving of wild individuals among streams and the stocking of domesticated strains should take into account the regional differences among populations. To preserve the majority of the observed genetic variation within the state, brook trout from each region should not be transferred to other regions. No evidence was found to indicate that stocking from outside sources has significantly impacted sampled populations. Genetic sampling of additional brook trout populations and the inclusion of ecological factors (e.g. habitat stability, thermal barriers) as well as stocking histories when selecting these additional populations is recommended to further understand and clarify inter-and intra-regional differences observed.

**ACKNOWLEDGEMENTS:**

I thank the many people who helped with field sampling, especially Kent Niermeyer, Larry Claggett, John Lyons, Russ Heizer, and Larry Smith. Larry Claggett provided considerable

assistance in drafting and facilitating the contract with the Illinois Natural History Survey. Funding for the genetic analyses phase of the study was provided by the DNR's Bureau of Fisheries Management and Habitat Protection.

LITERATURE CITED:

- Allendorf, F.W.  
1991. Ecological and genetic effects of fish introductions; synthesis and recommendations. *Can. J. Fish. Aquat. Sci.* 48(Suppl. 1):178-181.
- Allendorf, F.W. and R.F. Leary.  
1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conserv. Biol.* 2:170-184.
- Brasch, J., J. McFadden and S. Kmiotek.  
1973. Brook trout: Life history, ecology and management. Wisconsin Department of Natural Resources Publ. 226, 15 pp.
- Calen, K.  
1983. The genetic effect of stocking and population structure of brook trout (Salvelinus fontinalis) in the Beef River. Master's Thesis, Univ. Wisconsin-LaCrosse, 71 pp.
- Claggett, L.E., and T.R. Dehring.  
1984. Wisconsin salmonid strain catalog. Wisconsin Department of Natural Resources Administrative Report No. 19, 31 pp.
- Fraser, J.M.  
1981. Comparative survival and growth of planted wild, hybrid, and domestic strains of brook trout (Salvelinus fontinalis) in Ontario lakes. *Can. J. Fish. Aquat. Sci.*, 38:1672-1684.
- Garcia-Marin, J.L., P.E. Jorde, N. Ryman, R. Utter, and C. Pla.  
1991. Management implications of genetic differentiation between native and hatchery populations of brown trout (Salmo trutta) in Spain. *Aquaculture* 95:235-249.
- Hilborn, R.  
1992. Hatcheries and the future of salmon in the northwest. *Fisheries*, Vol 17(1):5-8.
- Hindar, K., N. Ryman, and F. Utter.  
1991. Genetic effects of cultured fish on natural fish populations. *Can. J. Fish. Aquat. Sci.* 48:945-957.
- Krueger, C.C.  
1976. Effects of stocking on genetics of wild brook trout populations. Master's Thesis, Iowa State University-Ames, 79 pp.
- Krueger, C.C. and B.W. Menzel.  
1979. Effects of stocking on genetics of wild brook trout populations. *Trans. Amer. Fish. Soc.* 108:277-287.
- Lachance, S., and P. Magnan.  
1990. Performance of domestic, hybrid, and wild strains of brook trout, (Salvelinus fontinalis), after stocking: the impact of intra- and interspecific competition. *Can. J.*

- Fish. and Aqua. Sci. 47:2278-2284.  
Nehlsen, W., J.E. Williams, and J.A. Lichatowich.  
1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Idaho, and Washington. Fisheries, Vol. 16(2):4-21.
- Perkins, D.L., C.C. Krueger, and B. May.  
1993. Heritage brook trout in northeastern USA: Genetic variability within and among populations. Trans. Amer. Fish. Soc. 122:515-532.
- Soule, M.E.(ed.).  
1986. Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, MA.
- Vuorinen, J.  
1984. Reduction of genetic variability in a hatchery stock of brown trout, (Salmo trutta). J. Fish Biol. 24:339-348.
- Webster, D.A., and W.A. Flick.  
1981. Performance of indigenous, exotic, and hybrid strains of brook trout (Salvelinus fontinalis) in waters of the Adirondack Mountains, New York. Can. J. of Fish. and Aqua. Sci. 38:1701-1707.
- White, R.J.  
1992a. Why wild fish matter: A biologist's view. Trout(Summer Issue):52-58.
- 
- 1992b. Why wild fish matter: Balancing ecological and aquacultural fishery management. Trout(Autumn Issue):16-33, 44-48.
- Yuskavitch, J.  
1999. Taking Stock of Stocking. Trout (Winter Issue):16-27.

APPROVALS:

Section Chief: \_\_\_\_\_ Date: \_\_\_\_\_

Table 1. Candidate streams selected for brook trout sampling in conjunction with the "heritage" brook trout study (? = could not verify presence or absence of previous stocking).

Stream	County	Stocking History	Major Drainage	Minor Drainage
Benson	Burnett	None	Mississippi	St. Croix
Grinsell	Richland	1974	Mississippi	Wisconsin
Keges (Geise's)	Door	?	St. Lawrence	L. Michigan
W. Br. Krok	Kewaunee	?	St. Lawrence	Twin
L. Onion	Bayfield	None	St. Lawrence	L. Superior
L. Scarboro	Kewaunee	1956	St. Lawrence	Kewaunee
Logan	Door	?	St. Lawrence	L. Michigan
Mason	Waukesha	?	Mississippi	Fox
Middle Br. Embarrass	Langlade	1973	St. Lawrence	Wolf/Fox
Mt. Pelee	Price	1940's	Mississippi	Flambeau/ Chippewa
Parfrey's Glen	Sauk	?	Mississippi	Wisconsin
Price	Sawyer	1940's	Mississippi	Flambeau/ Chippewa
Rosonow	Waukesha	?	Mississippi	Rock
Thompson	Bayfield	?	St. Lawrence	L. Superior
W.Fk. Shioc	Shawano	1951	St. Lawrence	Wolf/Fox
Whitcomb (trib.)	Waupaca	1969	St. Lawrence	Wolf/Fox
Wilson	Dunn	None	Mississippi	Red Cedar/ Chippewa

Table 2. Streams sampled and numbers of brook trout removed (<30 trout sampled were released).

Stream	County	Location T.R.Sec.	Date Sampled	Brook Trout	Size Range (in)
Benson	Burnett	37N20W SE1/4 S4	5-29-96	30	3.8-7.4
Grinsell	Richland	12N01E NW1/4S11	10-06-95	30	4.3-9.5
Keges (Geise's)	Door	27N24E NW1/4S33	9-07-95	1	4.3
W. Br. Krok	Kewaunee	23N23E SE1/4S14	9-11-95	None	
L. Onion	Bayfield	50N04W SE1/4S31	10-12-95	35	2.6-7.8
L. Scarboro	Kewaunee	24N24E SW1/4S31	9-11-95	30	4.7-9.0
Logan	Door	29N27E SE1/4S28	9-07-95	1	11.8
Mason	Waukesha	08N18E SE1/4S6	9-06-95	30	4.5-5.7
Mid. Br. Embarrass	Langlade	30N11E NW1/4S35	9-12-95	30	5.1-9.4
Mt. Pelee	Price	38N01W SW1/4S16	Equipment Failure		
Parfrey's Glen	Sauk	11N07E SW1/4S23	10-06-95	30	4.2-9.3
Price	Sawyer	37N03WSE/ NE1/4S14	9-04-96	30	4.4-11.4
Rosonow	Waukesha	08N17E NE1/4S28	9-06-95	30	5.5-11.5
Thompson	Bayfield	48N05W SW1/4S1	10-12-96	None	
W. Fk. Shioc	Shawano	17N26E NW1/4S9	9-12-95	30	5.2-9.6
Whitcomb (trib.)	Waupaca	24N12E SE1/4S8	9-14-95	30	4.7-8.0
Wilson	Dunn	29N14W SE1/4S31	5-29-96	30	4.3-8.9

Appendix 1. Length frequencies of brook trout collected from 12 Wisconsin streams for genetic analyses.

Benson	Grinsell	L. Onion	L. Scarboro	Mason	M. Br. Embarrass	Parfrey's Glen	Price	Rosnow	W. Ek. Shioc	Whitcomb	Wilson
3.8	4.3	2.2	4.7	4.5	5.1	4.2	4.4	5.5	5.2	4.7	4.3
3.9	4.4	2.6	4.9	4.5	5.3	4.3	4.6	5.7	5.5	4.8	4.4
4.1	4.4	2.6	4.9	4.6	5.4	4.3	4.9	6.6	5.6	4.9	4.4
4.3	4.4	2.6	4.9	4.8	5.5	4.4	5.1	6.6	5.7	5.0	4.5
4.3	4.4	2.6	5.1	4.8	5.6	4.6	5.5	6.9	5.8	5.2	4.7
4.5	4.4	2.8	5.2	4.8	5.7	4.6	5.5	7.1	5.8	5.2	4.7
4.8	4.5	2.8	5.2	4.8	5.7	6.0	5.5	7.1	5.9	5.3	4.9
4.9	4.5	2.8	5.2	4.8	5.7	6.2	5.7	7.2	6.1	5.3	4.9
5.2	4.5	2.9	5.3	4.8	5.7	6.3	5.9	7.4	6.1	5.4	5.0
5.2	4.6	2.9	5.3	4.9	5.8	6.7	6.0	7.4	6.1	5.5	5.3
5.5	4.6	2.9	5.5	4.9	5.9	6.8	6.0	7.4	6.2	5.5	5.5
5.5	5.1	2.9	5.5	4.9	6.1	6.9	6.1	7.5	6.2	5.8	5.7
5.7	6.3	2.9	5.5	4.9	6.1	7.0	6.1	7.5	6.3	5.9	5.7
5.7	6.4	2.9	5.6	4.9	6.4	7.0	6.5	7.5	6.3	5.9	6.2
5.9	6.6	2.9	5.7	5.0	6.5	7.2	6.5	7.7	6.4	6.0	6.7
5.9	6.7	2.9	5.9	5.0	6.6	7.3	6.6	7.8	6.4	6.0	6.9
6.0	6.8	3.0	6.1	5.0	6.7	7.3	6.6	7.9	6.4	6.2	7.1
6.0	6.9	3.0	6.1	5.0	6.7	7.3	6.6	7.9	6.4	6.2	7.1
6.0	7.1	3.0	6.2	5.0	6.7	7.4	6.7	8.1	6.5	6.2	7.2
6.1	7.3	3.0	6.3	5.1	6.8	7.4	6.7	8.1	6.6	6.3	7.2
6.2	7.4	3.1	6.3	5.1	6.9	7.5	6.8	8.3	6.6	6.3	7.4
6.4	7.6	3.1	6.3	5.1	7.1	7.6	6.9	9.0	6.8	6.3	7.4
6.5	7.6	3.1	6.4	5.1	7.3	7.7	7.0	9.3	6.9	6.5	7.4
6.6	7.6	3.2	6.6	5.1	7.4	8.0	7.2	9.5	6.9	6.6	7.6
6.7	7.9	3.2	6.6	5.2	7.6	8.1	7.3	9.5	7.2	6.7	7.6
6.8	8.0	3.3	6.6	5.3	7.9	8.4	7.5	10.0	7.4	6.8	7.8

6.8	8.0	3.3	6.7	5.4	8.5	8.5	7.6	10.3	7.9	7.2	8.3
6.9	8.8	3.3	6.9	5.5	8.5	8.8	8.0	10.4	8.1	7.2	8.4
7.4	8.9	4.1	7.7	5.6	8.9	9.2	8.4	11.4	9.1	7.6	8.8
7.4	9.5	5.4	9.0	5.7	9.4	9.3	11.4	11.5	9.6	8.0	8.9

# ILLINOIS NATURAL HISTORY SURVEY

## GENETIC ANALYSIS OF WISCONSIN BROOK TROUT

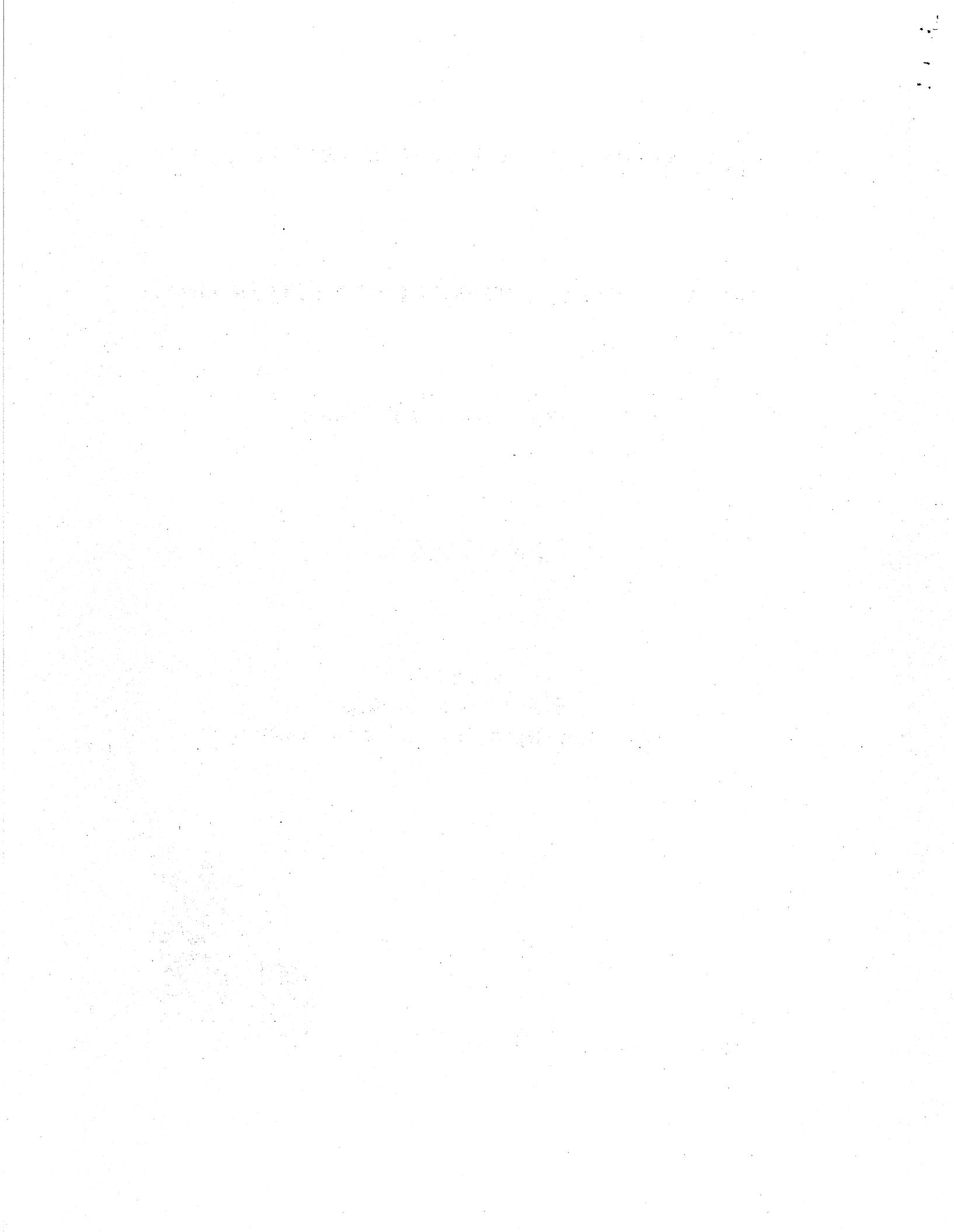
R. D. Fields and D. P. Philipp

### FINAL REPORT

Submitted to  
E. Avery and L. Claggett  
Wisconsin Department of Natural Resources



Aquatic Ecology Technical Report 98/2



## **Summary of Project Tasks for Brook Trout Portion of "Wisconsin Trout Genetics Study"**

**Task 1:** Muscle, eye, and liver of brook trout were run to assess tissue expression, identity of loci, and to optimize electrophoresis protocols.

**Tasks 2 and 3:** Literature was reviewed and compared to data for initial screenings. Three populations of wild fish (Lawrence, Eau Claire, and Soper) were screened to define polymorphic loci; to define approximate allele frequencies; and to clarify scoring of gels.

Samples from St. Croix hatchery strain were obtained well after the initial screenings; many of the steps had to be repeated to allow direct comparison of wild and hatchery fish, and to assure that all variants were detected. St. Croix specimens were screened simultaneously with wild fish using all scorable loci.

**Task 4:** The polymorphic loci identified above were used to analyze all individuals from 17 populations from Wisconsin and two from Iowa.

**Task 5:** DNA was isolated, quantified, and screened for all populations.

**Task 6:** Primers were designed and obtained, and optimization experiments were conducted for amplification of mitochondrial DNA genes. Amplification of five mtDNA regions was successful: D-loop and D-Loop/12S RNA; ND 5/6; ND 3/4; and ATPase 6.

**Task 7:** Restriction analyses of amplified mtDNA genes was conducted with individual hatchery (St. Croix) fish and wild samples (Eau Claire, Lepage, Soper, and Lawrence) to allow direct comparison. Nine restriction enzymes, with 4-base recognition sequences (*Hhal*, *Bst*UII, *aTaql*, *Hinf*I, *Hae*III, *Ddel*, *Dpn*II, *Msp*I, and *Rsa*I) were used to screen for polymorphic characters. Restriction digestion of the D-Loop/12S RNA region with *Ddel* and *Hinf*I resulted in distinctive polymorphic fragment patterns. (Also, the smaller

control region (1.3 kb) alone was amplified and digested with *HinfI*, *DdeI*, *AluI*, and *AseI*, to define the location of the newly identified polymorphic mtDNA characters.) Initial digests of both ND 5/6 and ND 3/4 resulted in monomorphic patterns. Restriction digests of ATPase 6 resulted either in a lack of restriction sites within the small (600 bp) gene, or in fragments too small to be observed clearly on gels.

**Task 8:** Polymorphic mtDNA characters (control region fragment digested with *HinfI* and *DdeI*) were used to analyze all individuals from 19 populations.

**Task 9:** Data were analyzed using appropriate methods.

**Task 10:** To allow comparison of data with a recent study of "coaster" brook trout (Burnham-Curtis 1996), and to potentially identify additional polymorphic characters among inland populations, the ND-2 mtDNA gene was amplified and digested with *PstI*, *SphI*, and *BanI*. Also, the smaller control region (1.3 kb) alone was amplified and digested with *AluI*, and *AseI*, to identify individuals with the polymorphic mtDNA characters that were useful in the "coaster" study.

**Task 11:** Data were summarized in an interim report (for all trout populations analyzed, including brown and rainbow trout) and presented to project coordinators in April 1996. The current Final Report represents the final analysis for all brook trout populations studied to date.

**Task 12:** Data have been presented at two professional meetings.

**Task 13:** Final genetic data analysis and management recommendations will be submitted for publication (manuscript preparation is ongoing).

**Abstract:** We have sought to identify key genetic parameters for identification of heritage brook trout in Wisconsin. Several factors are important in the identification and management of heritage populations. First, naturally occurring regional genetic differences must be identified. Second, the history and influence of past stocking practices must be identified and incorporated. Third, the genetic impacts of severely limited habitat and small populations must be determined. Our genetic analysis included one hatchery broodstock and seventeen wild populations (with no documented history of stocking) from Wisconsin. Populations represented every major watershed and ecoregion of the state. An additional wild population from Iowa was included, as was the Iowa hatchery strain. We used both mtDNA PCR-RFLP analysis and allozymes to genetically categorize populations. The two genetic techniques resulted in complementary, but slightly different data sets. Our results indicate regional genetic structuring; management (movement of individuals among streams and artificial culture) should take into account the regional differences among populations. For example, the genetically distinct populations in northwest Wisconsin should be managed separately from those in both the southwest "driftless" area, and in the central and eastern portions of the state. We have identified the most likely candidate populations to be used as putative heritage populations for use in reintroducing wild trout into appropriate habitats. Source populations should be from streams in close proximity, with little historical or genetic evidence of past stocking. No evidence was found to indicate that stocking from outside sources has significantly impacted sampled populations. Low variability within some wild populations indicates they have undergone severe reductions in population size, probably due to habitat decline or colonization by few individual fish. For example, populations in southeast Wisconsin appear to be small, relict populations in very limited habitat. The currently used hatchery strain can not be identified with specific genetic characters, however, the strain does possess distinctive protein alleles and haplotypes (at low frequencies) that could be useful in generation of genetically tagged fish for future studies of stocking impacts.

## **Introduction**

### *Importance of genetics in trout management*

A clear understanding of the genetics of wild and hatchery-propagated populations of brook trout is critical to many aspects of management. For example, stock structuring among native populations, resulting from genetic drift or local adaptation, is important to regional management and in defining appropriate locations for capture of wild broodstock, or for defining the limitations to be placed on artificial transfer of stocks.

Stock transfers may allow introgression of previously allopatric stocks that may muddle the distributional patterns of a species. Transfers may also lead to the loss of adaptive qualities through interbreeding of stocks that are adapted to particular habitats (Allendorf et al. 1980; Ryman 1981). There have been numerous recommendations against mixing of different stocks (Kapuscinski and Philipp 1988; Philipp 1991).

According to Dobzhansky (1948), advantageous gene combinations in specific environments are conserved, eventually forming coadapted gene complexes. Interbreeding between native populations and introduced stocks may disrupt these complexes, resulting in outbreeding depression (Templeton 1986; Hindar et al. 1991; Philipp and Whitt 1991). Because of the unpredictable effect of natural selection on these novel gene complexes, recovery of pre-introduction levels of fitness may be impossible (Allendorf and Leary 1988).

Effective management of brook trout demands accurate identification and characterization of wild populations. Brook trout are particularly susceptible to unforeseen genetic changes. For example, they are easily propagated in hatcheries,

which may lead to domestication or inbreeding. In the wild, brook trout are able to colonize and survive in small or marginal habitats, with the potential for reduced effective population size and inbreeding, or the loss of variability due to founding events.

The superiority of wild stocks over domesticated strains (or populations from distant sources) in performance and long-term survival is well documented (Flick and Webster, 1976; Reisenbichler and McIntyre, 1977; Lachance and Magnon, 1990; Hindar et al. 1991). Identification of native populations (as well as stock structure) is becoming a research priority in many situations (Stoneking et al. 1981, McCracken et al. 1993, and Perkins et al. 1993). The genetic variability present in any population (native or artificially-propagated) also may have a great impact on the long-term survival and adaptability of that population, thus the quantification and preservation of that variation is critical (Allendorf and Leary, 1988, and Liskauskas and Ferguson, 1991).

Plans for the ultimate re-introduction or recovery of native populations of brook trout should take into account all of the above genetic factors. Of utmost importance is the identification of appropriate populations to be used as source populations, and incorporation of means to monitor their progress over time. In fact, the ability to assess the efficacy and long-term impacts of any management plan involving movement or mixing of wild and/or hatchery stocks is dependent upon the identification of genetic markers that may be followed over a number of generations.

#### *Previous brook trout genetics studies in Wisconsin*

Kreuger and Menzel (1979) studied the genetic impacts of stocking on brook trout populations in one watershed of Wisconsin. In that study, it was shown that the

frequency of one allele (denoted the B2' allele of the LDH-B2 locus by Kreuger and Menzel, 1979) occurred at a relatively high frequency in the then-propagated Osceola strain; the frequency of the allele was also correlated with the degree of stocking that had occurred in sampled locations in the Wolf and Fox River drainages. A second locus used in that study, however, showed allele frequencies indicative of homogeneity among populations, regardless of stocking history.

Burnham-Curtis (1996) has surveyed coastal Lake Superior populations of brook trout from Wisconsin, Michigan, Minnesota, and Ontario, to determine whether there exists a definable mtDNA genotype associated with the anadromous "coaster" life history trait. That study revealed high within population variation, but relatively little differentiation among populations. In fact, one population known to be anadromous showed little differentiation from other coastal populations (Mary Burnham-Curtis, pers. comm.).

#### *History of brook trout strains and stocking in Wisconsin*

Little is known of the stocking histories, and the sources of fish used for stocking, for many streams in Wisconsin (Ed Avery, WDNR, pers. comm.) The original hatchery stock ("Osceola" strain) used for brook trout enhancement appears to have been derived in part from wild-caught fish, probably from native populations in northwest Wisconsin that was (or were) in close proximity to the Osceola hatchery (Wisconsin DNR hatchery record archives, Madison, WI.; Ed Avery and Larry Claggett, pers. comm.; and Kreuger and Menzel, 1979).

The Osceola strain was the hatchery stock that was used for enhancement stocking in the early 1900's; it may have also been supplemented with fish from outside

sources (Kreuger and Menzel, 1979). The Osceola strain was destroyed in the 1970's, and replaced with the currently propagated hatchery stock (St. Croix strain). The St. Croix strain was derived from fish obtained from the Nashua NFH, New Hampshire, and is derived (presumably) from the Paint Bank strain (Claggett and Dehring, 1984). Propagation and stocking records are fairly complete for the St. Croix strain, and populations chosen for the present study were presumably never impacted by stocking with St. Croix fish. Any stocking that may have occurred in study streams likely employed Osceola strain fish.

There are a number of reasons that reconstruction of stocking histories, based on current genetic data, would be difficult at best. First, the convoluted history of stocking and propagated strains (above) does not allow definition of "hatchery" or "wild" genotypes. In addition, some populations are present in extremely limited habitats, in which population bottlenecks are likely to have occurred, and continue to occur. Resultant disruptions of natural allele and haplotype frequencies make comparisons with other strains difficult. Founding populations, whether natural or hatchery-derived, are likely to also have been very small in some habitats. Finally, the levels of potential genetic impacts of stocking on genotype frequencies are likely quite variable, depending on the sizes of the original populations, movement and relative survival of stocked individuals, and their relative contribution to reproduction.

### **Objectives**

Several specific objectives were addressed in the current study. The first was to define the most useful and cost-effective molecular genetic techniques for assessing genetic stock structuring in the small geographic area of the study. Previous studies

have employed protein electrophoresis (Krueger and Menzel, 1979, McCracken et al. 1993, Perkins et al. 1993), mitochondrial DNA (mtDNA) sequencing (Bernatchez and Danzmann, 1991) or mtDNA RFLP analysis (Gyllensten and Wilson, 1987, Burnham-Curtis, 1986). More recently, microsatellite DNA has been shown to be useful in extremely small "microgeographic" studies (Angers et al. 1995). At the outset, it was suspected that a combination of two or more techniques would be most useful, because of potentially different inferences that may be made from population genetic data obtained with different techniques. For example, it has been shown that nuclear and mtDNA data may imply different levels of relatedness among the same populations (Danzmann and Ferguson). The potential disparity is likely intensified when examining small populations, in which founding events may allow maintenance of some portion of nuclear variation, but the loss of mtDNA variation.

The second objective was to define genetic differences between hatchery strains and wild populations. The currently propagated St. Croix strain was studied directly. Any information obtained for St. Croix fish may be useful in designing genetic tagging studies to monitor survival and natural reproduction of these fish when stocked into new habitats, and to determine the degree of introgression between hatchery and naturally-reproducing wild fish. Although Osceola strain fish were unavailable, it was hoped that genetic differences among existing populations could be used to infer a list of locations most likely to have been stocked (or founded) with the old Osceola strain fish. Such inference would be analogous to the conclusions drawn by Kreuger and Menzel (1979) in the Fox and Wolf River systems.

The third objective was to determine the genetic variability among wild populations to ascertain the extent of geographic stock structuring. Previous genetic

work on warmwater species in Wisconsin and Minnesota indicated distinct stock structuring within Wisconsin, based on extant and ancient watershed boundaries (Fields et al. 1997). Such information for brook trout would ultimately allow delineation of genetic management areas. An inherent assumption was that the majority of populations chosen for study were relatively unaffected by stocking, or that stocking histories could be used to explain the occurrence of apparently genetically anomalous populations. It was also assumed that most selected populations were of sufficient size to have maintained a majority of the genetic variation that originally existed.

A fourth, related objective, was to determine which geographic areas, and which specific populations, are most representative of the original "heritage" stock (or stocks) of brook trout in Wisconsin. Also, it was expected that data from the current study would be useful in identifying those populations (native or not) that have undergone severe reduction in numbers due to loss of habitat or overharvest. Such information is vital for planning of habitat restoration and population enhancement or restoration.

The final objective for this study was to identify healthy, wild, "heritage" populations that would be candidates for preservation, or for use in re-introductions. The underlying stock structure (if any) and the identification of potential source populations in each genetic management area would allow the establishment of brook trout populations in habitats from which the species had previously been extirpated. Such source populations could also be used to replace populations primarily composed of hatchery fish and their descendants. Populations most similar to the original, locally adapted stocks would give the best chance for long term survival and reproduction of these newly established populations.

## **Materials and Methods**

### *Sample Collection*

Specimens from Wisconsin populations were collected by personnel of Wisconsin DNR; additional populations from Iowa were provided by Iowa DNR. Population locations, stream length, and the number of specimens obtained are listed in Table 1, and their locations are shown in Figure 1. All samples were immediately placed on ice and frozen at -20°C as soon as possible. Samples were shipped to Illinois Natural History Survey, and eye, liver, and muscle tissues were extracted from all individuals and transferred to -80°C until the time of allozyme analysis and DNA isolation.

### *Allozyme Analysis*

Eye, liver, and muscle tissues were homogenized in 0.1M Tris-HCl (pH 7.0) and centrifuged at 22xg for 15 minutes at 4°C. We used three different buffer systems to electrophorese samples on vertical starch gels overnight (see Table 2). The starch gels were sliced and stained according to the methods described by Philipp et al. (1979). Histochemical staining for 33 enzymes revealed 62 loci that were screened for polymorphisms. Nomenclature follows the recommendations of Shaklee et al. (1990).

### *DNA Isolation*

DNA was isolated using a method described by Saghai-Marof et al. (1984) and modified by Fields et al. (1989). About 300mg of muscle was homogenized in a solution of 100mM Tris (pH 8.0), 10mM EDTA, 1.4M NaCl, 2.0% CTAB, and 0.2% 2-mercaptoethanol. The samples were then subjected to at least one phenol extraction

and at least two 24:1 chloroform: isoamyl alcohol extractions. DNA was precipitated with cold ethanol and allowed to dry. The DNA was then resuspended in a solution of 10mM Tris and 1mM EDTA. The DNA concentration and purity were quantified with a spectrophotometer ( $A_{260}$  and  $A_{280}$ ). Isolated DNA was stored at -20°C until the time of MtDNA RFLP analysis.

#### *MtDNA RFLP Analysis*

Genomic DNA was amplified by the use of PCR as described by Williams (1990), and modified according to Echt et al. (1992). Initial screening for amplification and polymorphism was conducted with five mtDNA regions: the region containing the NADH-dependent dehydrogenase gene subunits 3 and 4 (ND-3/4); the ND-5/6 region; the 12S rRNA-D-loop region; the ND-2 region; and ATPase 6. The primer sequences and the sizes of the DNA fragments amplified are shown in Table 3.

Each 25ml reaction tube contained 25ng of DNA, 80mM Tris-HCl (pH 9.0), 20mM  $(\text{NH}_4)_2\text{SO}_4$ , 100mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 1.5mM  $\text{MgCl}_2$ , 400nM of primer, and 1.0 unit of *Taq* polymerase (Perkin Elmer, Branchburg, NJ). A PTC-100 Programmable Thermal Controller (MJ Research, Inc., Cambridge, MA) was programmed with the following thermal cycling protocol for initial amplifications: 94°C for 2 minutes; 38 cycles at 94°C for 45 seconds, 41°C for 45 seconds, and 72°C for 90 seconds. Annealing temperature was increased to 50°C for population analyses with control region (D-Loop) primers.

Restriction analyses of amplified mtDNA genes was conducted with subsamples of hatchery (St. Croix) fish and wild populations (Eau Claire, Lawrence, Lepage, and Soper) to screen for variable restriction sites. Nine restriction enzymes, with 4-base

recognition sequences (*Hhal*, *BstUII*, *aTaql*, *HinfI*, *HaeIII*, *Ddel*, *DpnII*, *MspI*, and *Rsal*), were used to screen each mtDNA region for polymorphic characters.

To allow comparison with a concurrent study of "coaster" brook trout (Burnham-Curtis 1996), and to search for additional polymorphic characters among inland populations, the ND-2 mtDNA gene was amplified and digested with *PstI*, *SphI*, and *BanI*. Each of those restriction enzymes was previously identified as generating polymorphic sites in brook trout mtDNA by Bernatchez and Danzmann (1993) or Burnham-Curtis (1996). Also, the smaller control region (1.3 kb) alone was amplified and digested with *HinfI* I and *Ddel*, to localize the restriction site within either the 12S rRNA or D-Loop regions. The restriction enzymes *AluI* and *AseI* were also screened for their ability to identify a previously defined polymorphism (Bernatchez and Danzmann, 1993; and Burnham-Curtis 1996).

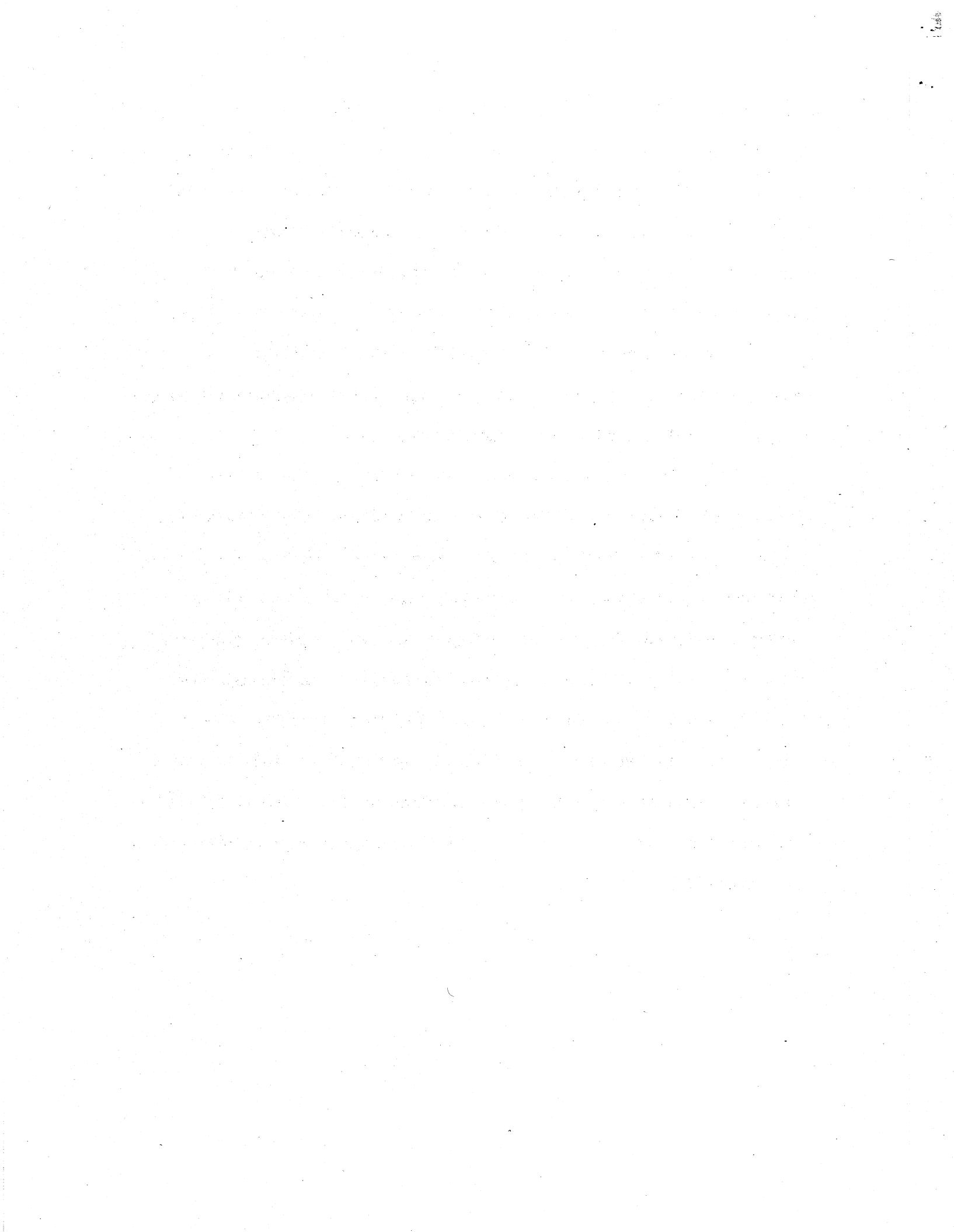
Amplification and restriction products were loaded on either a 2% agarose gel or a 1.5% agarose gel supplemented with 0.75% Synergel™ (Diversified Biotech, Boston, MA) and ethidium bromide. Gels were run at 3-5V/cm for 3-4 hours. DNA fragments were visualized by viewing on a UV transilluminator. Individuals were scored from Polaroid photographs for the number of bands amplified, and the approximate sizes of those bands (compared to a 100bp ladder).

#### *Data Analysis*

Protein electrophoretic data was analyzed using the computer package BIOSYS-I (Swofford and Selander 1981) to quantify allele frequencies, mean number of alleles per locus, percent polymorphic loci (0.95 criterion) and the mean heterozygosity ( $H$ ; direct count and expected from Hardy-Weinberg equilibrium) for each population.

Genotypic frequencies within populations were tested for deviation from Hardy-Weinberg equilibrium values using a chi-square goodness of fit test. Heterogeneity contingency tables were computed for each locus to test if the populations were drawn from a single homogeneous population. Population genetic differentiation was quantified using *F*-statistics (Wright 1978). Multilocus genetic relationships among populations were determined and genetic similarity/distance coefficients calculated (Cavalli-Sforza and Edwards 1967; Rogers 1972; Nei 1978). A set of three dendrograms of genetic distances was then generated with the unweighted pair group method (UPGMA) cluster analysis (Sneath and Sokal 1973).

MtDNA RFLP data were analyzed using the Restriction Enzyme Analysis Package (REAP; McElroy et al. 1991) and the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC; Rohlf 1989). The resulting nucleotide sequence divergence matrix (calculated from fragment sizes and haplotype frequencies) was entered into NTSYS-PC to generate a UPGMA phenogram of haplotype divergence among the populations. Population subdivision was assessed using the  $G_{ST}$  statistic (Nei 1973) and software designed by Alejandro Lynch (University of Papua, New Guinea).  $G_{ST}$  describes the relationship between within population diversity and total diversity of all populations combined. Nucleotide diversity (Nei and Tajima 1981) within populations and nucleotide divergence (Nei and Li 1979) among populations were also calculated using REAP.



## Results and Discussion

### *Identification of polymorphic allozyme loci and allele frequencies*

The polymorphic protein loci used in the study are listed in bold type in Table 2 (Relative mobilities are not reported here). Findings generally agree with those of previous workers (Kreuger and Menzel, 1979, McCracken et al 1993, and Perkins et al. 1993). The *LDH-B2\** locus is assumed to be the same as that described by Kreuger and Menzel (1979); the allele we have designated as "2" occurs at frequencies similar to their *B<sub>2</sub>'* allele. Our interpretation is also based on the phenotypic patterns described in Morrison (1970) and Wright and Atherton (1970).

We did not resolve the variation present at the *MEP* loci, previously reported by other authors (McCracken et al 1993, and Perkins et al. 1993), as resolution of this enzyme system was poor for some populations. The *G3PDH-1\** and *G3PDH-3\** loci revealed more variation than in previous studies; most authors have attributed observed phenotypic variation to one or the other of the two loci. Clear resolution of double homozygotes for the rare allele at each locus was observed here.

The allele frequencies for each locus are shown for each population in Table 4. Although mean within population variation over all loci ( $F_{IS} = 0.050$ ) was somewhat lower than has been previously reported for brook trout, it is apparent that the number is lowered significantly by the lack of variation at most loci in some populations (for example, Little Onion, Mason, and Rosonow). There is apparently significant variation among populations (mean  $F_{ST} = 0.195$ ). A substantial portion of the among population variation can be attributed to the *AAT-1,2\** isoloci and the *LDH-B2\**, and *IDH-B1\** loci.

### *Population structuring based on allozymes*

Figure 2 is a UPGMA phenogram based on genetic distance matrix of Nei (1978). Both Cavalli-Sforza and Edwards (1967) and Rogers (1972) genetic distance matrices resulted in similar clustering of populations (not shown). Genetically similar populations are generally from the same geographic areas (Figure 3; groupings are based on a genetic distance of 0.025) with some exceptions. The most distinct populations are clearly those for which populations are small, and in which genetic variability is most reduced (Little Onion, Mason, Rosonow, Parfrey's Glen, and Pine Creek, Iowa). For example, Little Onion is distinguishable from the other apparently reduced populations due to an unusually high frequency of the G3PDH-3 "1" allele. Genetic drift is expected to cause large frequency differences among multiple small populations, as observed here.

Northwestern populations from the Chippewa and St. Croix drainages (Wilson, Benson, and Big Brook) are very similar (compare Figures 2 and 3). The only exception is Price Creek. There are two possible explanations. First, based on the lack of mtDNA variation in this population (see mtDNA section below), the apparently anomalous affinity with other populations outside the watershed (Lepage, Soper, and Grinsell) would appear to have been caused by reductions in population size and resultant genetic drift. A second possibility is that the northwest region, and the Chippewa drainage in particular, was colonized from numerous sources or on multiple occasions. Similar inexplicable allele frequency similarities were seen between populations in this region and distant populations during a study of Wisconsin and Minnesota warmwater species Fields et al. 1997).

The Embarrass and Whitcomb populations are apparently distinct from other eastern populations, based on protein analysis alone; Lawrence, Eau Claire, Scarboro, and Shioe comprise a distinct group. Finally, a separate cluster of populations is comprised of Grinsell and Soper (both from the southwest), as well as Price (discussed earlier) and Lepage. Within this larger group are the nearly identical St. Croix, WI hatchery strain and the Iowa hatchery population (derived from the Wisconsin broodstock). The position of the two hatchery populations, ultimately derived from Nashua, New Hampshire, "Paint Bank" strain, among Midwest native populations shows the inherently high within population variation characteristic of brook trout, and the resultant difficulty in using protein analysis alone in determining genetic relationships clearly.

#### *RFLP Analysis of mtDNA*

Initial digests of both ND 5/6 and ND 3/4 resulted in monomorphic patterns. Restriction digests of ATPase 6 resulted in either a lack of restriction sites within the small (600-bp) gene, or in fragments too small to be observed clearly on gels. Amplification and digestion of two mtDNA regions (D-Loop/12S RNA and ND-2) resulted in polymorphic sites.

Digestion of the D-Loop/12S RNA region with enzymes *DdeI* and *HinfI* resulted in distinctive polymorphic fragment patterns. The variable sites are located within the control (D-Loop) region, based on separate amplification and digestion of the smaller control region (1.3 kb) alone. These polymorphic restriction sites have not been identified previously in brook trout, and these variable patterns may be distinctive to brook trout populations in Wisconsin (or in the upper Midwest). The most common

haplotype within this region of the mtDNA is modified either by lack of one restriction site for *Dde*I or an additional site for *Hinf*I. Restriction enzymes *Alu*I and *Ase*I were previously identified as revealing polymorphic restriction sites within the control region (D-Loop) of brook trout mtDNA, and the variant haplotypes were observed at low frequencies in some coastal Lake Superior stream populations by Burnham-Curtis (1996). However, a subsample of individuals from selected populations in the current study did not reveal the occurrence of the variant haplotype.

Amplification and digestion of the ND-2 mtDNA gene with restriction enzymes *Pst*I and *Ban*I resulted in variable fragment patterns among inland Wisconsin populations. Both *Pst*I and *Ban*I were identified previously as generating polymorphic sites in brook trout mtDNA (Bernatchez and Danzmann 1993, and Burnham-Curtis 1996). Those authors also report variable sites for the restriction enzyme *Sph*I, but no variable patterns were identified with *Sph*I among our populations.

When the total composite restriction data for each region of the mtDNA genome are combined (both ND-2 and D-Loop), there are seven distinct haplotypes, or clonal lines, observed among sampled populations. The distribution of mitochondrial clonal types among populations is shown in Table 5. A phenogram of genetically clustered populations is shown in Figure 4, and the geographic distribution of the genetically similar populations is shown in Figure 5. There are clear indications of restricted population sizes, as well as geographic similarities among populations (regional genetic structuring) as was revealed by protein analysis,

The common (AAAA) haplotype is fixed within only three populations: Little Onion, Parfrey's Glen, and Pine Creek, Iowa (see Table 5). Those populations are clustered (Figure 4) with Rosonow, Price, and Mason. As was true for protein analysis,

the distinctiveness of these populations is most likely due (in most cases) to restricted population size and resultant loss of diversity. The only exception is Price Creek, which has maintained four haplotypes, albeit in low frequencies.

The two hatchery populations are similar, as expected, but are distinct from most native populations. Their distinctiveness is based on their relatively high frequencies of the AABA and AABB haplotypes in addition to the common AAAA haplotype. The AABA haplotype also occurs in most of the native populations. However, the hatchery populations possess the AABB haplotype at relatively high frequencies, compared to native populations. Only the native Rosonow, Scarboro, and Whitcomb Creek also possess this haplotype.

There are several possible explanations for the observed distribution patterns of the AABA and AABB haplotypes. The most likely explanation is that each of these haplotypes may have been present among eastern populations, from which the colonizing (founding) stocks arose. Both haplotypes would then be expected to appear in populations founded in Wisconsin. The AABA haplotype may simply have been present at a higher frequency within the founding stock. It is also possible that the AABB haplotype occurs at higher frequencies only among populations from farther east; this would explain its high frequency among fish derived from a strain (St. Croix hatchery stock) originally propagated in New Hampshire. The sporadic occurrence of the AABB haplotype among small, isolated populations in Wisconsin may be expected if this were true.

The occurrence of the AABB haplotype in a population, therefore, does not necessarily imply that the population was founded by, or supplemented with, a hatchery stock. The appearance of the haplotype in Scarboro, Whitcomb, and Rosonow could be

due to either undocumented stocking from an eastern population, or the remnants of low frequency genotypes in populations of reduced size. It is interesting that the few documented occurrences of the AABB haplotype are among populations from the eastern portion of the state. This would lend credence to the supposition that a colonizing stock arose in the east and founded populations through the Great Lakes region.

There is also a genetic grouping of populations from eastern Wisconsin: Lepage, Eau Claire, Whitcomb, Embarrass, and Lawrence populations are similar according to mtDNA analysis (see Table 5 and Figures 4 and 5). These are also the native populations most closely related (according to mtDNA data) to the hatchery populations from the Eastern U.S. This pattern is consistent with the pattern depicted by protein analysis (see section above).

Three populations from northwest Wisconsin (Big Brook, Benson, and Wilson) possess similar haplotype frequencies (Table 5), especially a high frequency of the ABAA haplotype. Grinsell (from the southwest) and Scarboro (in the Lake Michigan watershed) also possess this haplotype at high frequencies (Table 5). Inclusion of the latter two populations in the same genetic cluster is somewhat surprising. The Grinsell Creek population is lacking the mtDNA diversity seen in many other populations (only two haplotypes were observed), and the similarity to northwest populations may thus be due to a reduction in population or sampling error. Scarboro Creek, however, is nearly fixed for the ABAA haplotype. Which may indicate either a severe reduction in population size or the result of supplemental stocking.

It should be noted that, as in the case of allozyme analysis, the Price Creek population is apparently not genetically similar to the other northwest populations.

Allozyme analysis indicated a closer genetic relationship between Price and Grinsell. MtDNA indicates, however, that Grinsell is more closely related to the other northwest populations. The only way to resolve the boundary between the stocks in the northwest and southwest populations is to sample additional wild populations from the Chippewa watershed and from additional locations in the southwest (see future work, below).

The last two populations to consider (Soper and Shioc) are genetically distinct from all others studied, according to mtDNA results (see Figures 4 and 5). This is based on the very high frequencies of the BABA haplotype in each population, as well as a very reduced frequency of either of the two most common haplotypes (AAAA and AABA; see Table 5). It is expected that mtDNA should show the effects of population bottlenecks more readily than nuclear DNA genes, due to haploidy and maternal inheritance. If the original founding populations possessed large numbers of each mtDNA haplotype, it is expected that among a number of small populations, we would see some populations fixed for one haplotype, and other populations fixed (or nearly fixed) for alternate haplotypes, as seen in Soper and Shioc. The apparent reductions in population size at these locations, however, were probably not sufficient to reduce allozyme variation within these populations, hence the seeming disparate genetic relatedness groupings for these populations when allozyme and mtDNA data are compared.

## **Conclusions and Management Suggestions**

### ***Identification of appropriate genetic techniques***

The most informative molecular genetic techniques for analysis of brook trout in

Wisconsin were determined to be a combination of mtDNA RFLP and allozyme analyses. There are some clear discrepancies in the reported relatedness among populations when using the two techniques; the most accurate picture of relatedness, and the underlying mechanisms of divergence, are best inferred from a combination of the methods. This is not surprising, considering the expected disparity of impacts expected from reduced population sizes (that is, mtDNA is likely to be most influenced by population bottlenecks). A lack of concordance in inferred population differentiation, generated using nuclear markers versus mitochondrial DNA has also been demonstrated for other freshwater fish species (Ferguson et al. 1991, Toline and Baker 1995). Because mtDNA is inherited maternally, and is haploid, effects of population fluctuations such as extreme bottlenecks or founder events may have a greater impact on variability detected in mtDNA, compared to that in nuclear DNA (Takahata and Slatkin 1984).

For brook trout, which possess high genetic variation within populations, it may be necessary to use a combination of techniques to obtain the number of polymorphic characters capable of answering population genetic questions, as suggested by Carvalho and Hauser (1994) and demonstrated in arctic char (Tessier et al. 1995). A similar combination of techniques should be employed in future analyses of brook trout in Wisconsin. The large number of small, isolated populations and the high genetic variation present among all populations indicate that single methods, such as nuclear DNA microsatellites, may result in inconclusive data.

#### ***Ability to distinguish between native and hatchery populations***

It would be nearly impossible to reconstruct the genetic identity of the defunct

Osceola hatchery strain. The only published evidence of a genetic distinction between that strain and presumed native populations is that of Krueger and Menzel (1979). However, only two allozyme loci were employed in that study and native populations from only a single watershed were used for comparison. It is quite likely that small numbers of broodstock, and continued hatchery maintenance during the first half of the century contributed to significant temporal allele frequency fluctuations as well.

Krueger and Menzel (1979) also speculated that the interactions (and interbreeding) between hatchery and native fish may have been limited by ecological segregation of the two groups and the general lack of movement of stocked fish from stocking sites. A similar low level of introgression between hatchery and native populations of brook trout has been documented in Fundy National Park, New Brunswick (Jones et al. 1996).

In short, it is only possible to speculate on the genetic identity of the original Osceola strain and the potential impacts stocking may have had. One possible scenario is that the Osceola strain originated from a wild, locally adapted stock from the northwest portion of the state. (This is supported by speculation that commercial hatchery operators originally obtained brood stock from "a local pond"; see WDNR Hatchery Archives ca. 1930.) Based on the apparent remnant stock structuring observed in the present study, it is possible that these northwestern fish possessed a slightly different LDH-B2' allele frequency than that observed among wild fish in the Wolf/Fox system by Krueger and Menzel (1979). However, it is equally plausible to suggest that reputed supplementation of the Osceola stock from outside sources impacted those allele frequencies.

Identification of the St. Croix hatchery stock (relative to wild populations) is more

straightforward. Although genetic distinctions are possible based on frequency differences (see Figures 2 and 4), there are no clear, "fixed" differences identified in this study that allow definitive identification of individual fish as hatchery or native. The best use of the slight frequency differences of protein alleles and mtDNA haplotypes among native and hatchery populations will be in future genetic tagging studies. This conclusion is based on the assumption that all populations included in the present study are uncontaminated. For example, the rare, slow G3PDH-1\* and G3PDH-3\* alleles are found at low frequencies in the St. Croix and Iowa hatchery populations. Hatchery selection for either of these alleles could be used to generate an artificially genetically tagged population for monitoring of survival and reproduction of stocked hatchery fish. Similarly, selection of the AABB haplotype could generate a hatchery population with specific mtDNA markers.

Each of the polymorphic character types (mtDNA and allozymes) may find application in strain evaluation. MtDNA markers, because they are inherited only from the female parent, would only be useful to follow growth or survival of a single cohort. However, these markers would be of particular interest because of the ease of identifying variant individuals (due to high frequencies in some populations). Allozymes would be most appropriate for cases in which assessment of reproductive success is important.

#### ***Regional genetic structure among native populations in Wisconsin***

There is clearly sufficient variability present among the sampled populations to demonstrate that regional genetic structuring occurs within the state. The observed regional differences may have been intensified or diluted by previous stocking, but we

must work with the variation currently present, however it may have been derived. For example, the southeast populations are clearly distinct from surrounding populations. Whether this is due to population bottlenecks of wild populations that had previously possessed high genetic diversity or due to past hatchery influences is irrelevant; those populations should be considered unique and managed separately from all others.

The distinctiveness of northwest and southeast populations, and unclear patterns of relatedness among populations sampled from central and eastern sites (as evidenced by both analytical techniques) is consistent with the findings for warmwater species in Wisconsin (Fields et al. 1997). For most warmwater species, it was found that likely colonization routes from eastern and southern glacial refugia resulted in distinct geographic structuring, and predictable areas of genetic mixing. For example, for most species, the Fox and/or Rock River populations were quite distinct from those in the central and northwest portions of the state. Northeast populations were similar in some cases to populations in the northwest, but in other cases, similar to populations in the southern Lake Michigan drainages.

Seven suggested management regions and representative sampled populations will be discussed below. Figure 6A shows approximate boundaries of the seven suggested management units that would be most likely to preserve the majority of the observed genetic variation within the state. For comparison, Figure 6B shows one possible method of reducing the number of management units by combining all eastern and northeastern areas. Although this second management scenario is less conservative biologically, such a compromise may be necessary due to constraints of rearing facilities, manpower, and resources. In the following section, the specific management regions and populations will be considered relative to implications for

reintroductions and broodstock selection, as well as preservation of unique "relict" populations.

#### *Genetic management regions*

*Lake Superior Coastal Streams.* The only site sampled in this region was the Little Onion River, above a barrier to migration. Genetic evidence gained with both analytical techniques indicates this population has undergone severe reduction in numbers, with resultant loss of genetic diversity. This agrees with a limited population survey of coastal Lake Superior populations by Burnham-Curtis (1996), based on mtDNA analysis alone. However, other Lake Superior populations surveyed by Burnham-Curtis (1996) possessed haplotypes not identified among any inland populations in the current study. Managing Wisconsin coastal populations separately from all others should avoid introgression with migrating (or anadromous) populations from very distinct habitats (most notably the Nipigon strain). In addition, stocking of inland fish into coastal streams could adversely impact recovery of lake strains.

*Northwest – St. Croix and Chippewa Drainages.* The consistent similarity among three of the populations sampled from this region (Big Brook, Benson, and Wilson) and their distinctiveness from populations from other regions, based on both genetic methods, warrant separate management of all populations in this region.

Price Creek appears to be the genetic exception among populations in this region. As discussed previously, the genetic identity of Price Creek may have been impacted either by previous stocking, fluctuating population sizes, or both. As the stream is fairly short (3.8 miles; from Kniotek and Neremberg 1980), population size

may be expected to be reduced on occasion. Price Creek is also in contact with downstream Class II waters, which are most likely to have been stocked in the past. However, Wilson Creek, also in the Chippewa drainage, is also connected to Class II waters. Perhaps the combination of stocking and small native population size has resulted in a greater impact on genetic identity in Price, as compared to Wilson.

Benson Creek, despite its short length (1.4 miles) possesses reasonable genetic diversity and retains similarity to both Wilson and Big Brook. However, the downstream thermal barrier (St. Croix River) and the apparent stability of the habitat would seem to contribute to maintaining the genetic "purity" of the population (Frank Pratt, WDNR, pers. comm.).

The difficulty in predicting genetic identity of individual populations in this region demonstrates the importance of continued sampling, and the inclusion of ecological factors in determining the most likely "heritage" populations and the ecological barriers that may form the best management boundaries throughout the state. Although stream length and population size are important factors in determining the genetic health of a population, habitat stability is also important.

*Southwest – Lower Wisconsin River, and Mississippi River tributaries below the Chippewa River.* This region comprises a major portion of the "driftless" area, ecologically distinct from all other areas of Wisconsin. Brook trout populations tend to be disjunct, and limited to headwaters or to areas that were likely to have been stocked previously.

One of the populations from this region (Parfrey's Glen) shows obvious loss of genetic variation, and should be considered a "relict" population (see section below).

The small population size, as well as known environmental perturbations, have contributed to the loss of variation.

The relatedness of two populations (Soper and Grinsell) from the southwest area is unclear, both in their relatedness to each other, and to other populations in the state. Allozymes suggest that the two populations are similar, and also share similarities with Price Creek (northwest region). However, mtDNA haplotype frequencies for Soper Creek are distinct from most other populations surveyed. The connections between the Soper site and extensive Class II waters suggest the mtDNA haplotype frequencies may have been altered by stocking, but similar connections to Class II waters exist for Grinsell, and that population shows mtDNA similarities with eastern populations. Determination of a specific population in this area as most representative of the "heritage" stock is thus problematic. It is likely that similar inconsistencies exist between sampled sites and other populations in the region. This lack of an easily identifiable "genetic identity" for the region warrants separate management of populations in the region. In addition, the ecological distinctiveness of waters in this region likely have impacted local adaptation.

*Southeast – Upper Rock River.* All populations in this region are in small, isolated habitats, subject to thermal and flow perturbations. Brook trout are at the extreme limits of their natural range in these areas. Only Mason and Rosonow Creek were sampled from this region, and like the Pine Creek, Iowa population, show the genetic effects of extreme population bottlenecks, in their lack of overall diversity and high frequencies of otherwise rare alleles, as well as fixation for mtDNA haplotypes. The Rosonow Creek population also possesses the AABB haplotype, observed at high frequency in both

hatchery populations. This may indicate either artificial stocking from some eastern location, or an unexpected natural remnant of genetic diversity from the natural founding stock. Neither Mason nor Rosonow Creeks were included in previous surveys as containing brook trout (Kmiotek and Neremberg 1980) and other populations were previously maintained by stocking. It is impossible to determine the true origin of these populations, but if they are now maintained through natural reproduction, they should be considered as potential "relict" populations. In fact, all populations in this region should be treated in the same way.

*Lake Michigan – Lake Winnebago tributaries, Wolf, Fox, and Waupaca River systems.* This region includes some of the most extensive, contiguous, high-quality habitat for brook trout in the state. Native populations in this area are genetically distinct from populations in the northwest and, for the most part, from populations in the southwest.

Populations in this region should be managed separately from all others. Enhancement programs should be based on broodstock obtained and propagated (if necessary) from local habitats. The only sampled populations in this area that are of unclear ancestry are those obtained from the Scarboro and Shioc sites. Apparently, population fluctuation in the Shioc population has resulted in maintenance of nuclear diversity (as evidenced by relatedness to surrounding populations), but with a severe change in the mtDNA diversity (near fixation for an otherwise rare haplotype). Similarly, the disparate results were obtained for the Scarboro population when comparing the two methods.

If necessary due to budget constraints, it may be necessary to reduce the number of management units by combining the northeast and upper Wisconsin River

regions with the Lake Michigan region (compare Figures 6A and 6B). However, the importance of the populations in this region should be recognized, and source populations for reintroductions in this area should come from watersheds within this region (see discussion of specific "heritage" populations below).

*Upper Wisconsin River.* Only one sampling location was included from this region, and separation of this area as a separate management unit is based primarily on protection of surrounding regions, rather than observed genetic distinctiveness of the sole sampled population. The East Branch of the Eau Claire River was sampled in this region. Both genetic methods suggest similarities with eastern (Lake Michigan drainage) populations.

The fact that most of the stream is classified as Class II has some bearing; those streams are more likely to have been stocked than recognized Class I streams. Continued stocking in this area from other geographic regions may have minimal impacts on local streams, but taking fish out of this region is likely to have unpredictable results.

*Northeast – Menominee River and north shore Green Bay tributaries.* Only Lepage Creek was sampled from this region. Genetic results for this population are somewhat surprising. Allozyme data suggest frequencies are similar to those obtained for populations in the southwest, and mtDNA data are similar to those for other Lake Michigan tributary populations. The region should probably be considered as distinct from other Lake Michigan populations.

### ***Identification of heritage populations and sources for propagation***

Locations of suggested source populations (chosen from sample sites within suggested management regions) for reintroductions and propagation are shown in Figure 7 (based on the formation of seven management units shown in Figure 6A). Our identification of these sites has taken into account a number of factors. First, the populations used as sources of broodstock should be large enough to have retained a significant amount of the genetic diversity and variability that were likely to have been present originally. Those populations should also be of sufficient size that removal of broodstock would not significantly impact the population itself. For this reason, in some cases larger populations are suggested as "heritage" populations despite similarities among all populations in the region.

A second consideration in the designation of "heritage" populations is that they should not have been stocked. It should be noted that the populations sampled for the current study were chosen based on the assumption that they were least likely to have been stocked in the past. However, undocumented stocking and/or population fluctuations have clearly impacted the genetic survey. Most genetically anomalous populations have been explained based on these two (for the most part) unpredictable events. No genetically anomalous populations were identified that could be traced to specific outside stocking sources. To some extent, genetic purity may be inferred from genetic similarity among some or all of the presumably native populations in the area.

Another factor in designation of "heritage" populations is the assumption that source populations should be obtained from as close (geographically) as possible to the sites intended for reintroduction or enhancement. By definition, each genetic management region should possess at least one potential "heritage" population. We

have attempted to define specific populations, among those sampled, which will result in the most conservative means of preserving statewide genetic diversity. Finally, it is assumed that sufficient resources will be available to allow each source population to be maintained separately.

*Lake Superior Coastal Streams.* The only site sampled in this region was the Little Onion River, above a barrier to migration. Genetic evidence gained with both analytical techniques indicates this population has undergone severe reduction in numbers, with resultant loss of genetic diversity, making it a poor choice for any transfer or propagation programs. Other streams in the area should be surveyed for identification of appropriate source populations.

*Northwest Region.* Populations in the northwest region are clearly genetically distinct. The most likely candidate population for designation and use as a local "heritage" population, and for potential use in reintroductions, is Big Brook, based on its similarity to other local populations, as well as its size.

*Southwest Region.* In the southwest "driftless" area, there is an inexplicable divergence between the Soper and Grinsell sites. In such cases, a conservative, educated guess must be made, based on the general health of the population, as to whether a specific population warrants designation and use as a "heritage" population. Grinsell (*Melacthion*) has been designated here, based on the best likelihood for success in propagation. Whichever population is ultimately deemed to be the best candidate for propagation, fish from this region should not be transported to other areas.

*Southeast (Upper Rock River)* As discussed above, all populations in the southeast region are in small, isolated habitats, subject to thermal and flow perturbations. Brook trout are at the extreme limits of their natural range in these areas. Neither of the sampled populations (Mason and Rosonow) would be expected to be of sufficient numbers to allow removal of fish for transfer or propagation. Stocking in this area should be designated as either reintroductions into habitats in which populations may be able to sustain themselves by natural reproduction, or as supplemental stockings. For the best chance for successful natural reproduction, stockings could be made from one of the other nearby regions (southwest or Lake Michigan tributaries). For put and take fisheries, the use of propagated hatchery fish would be warranted.

*Lake Michigan.* Based on similar considerations, either of the larger populations in the Lake Michigan region (Whitcomb and Embarrass) would make likely candidates for use in reintroductions. There is some discrepancy between mtDNA and allozyme data, relative to whether these two populations represent the original genetic identity of the region. The other likely representative population (Lawrence Creek) would appear to be too small to support significant withdrawal of breeding fish. Lawrence Creek was, however, suggested as one location in the Fox River system to have been impacted relatively little by the introduction of propagated Osceola strain fish (Kreuger and Menzel 1979).

*Northeast.* If the biologically conservative approach is used in establishing management regions (as depicted in Figure 6A), designation of a "heritage" or source population in

this region would be necessary. Lepage Creek is designated as a potential source population for reintroductions. This was the only population sampled in the region, and it does show some genetic differences compared to other populations. By default, it would best serve as the regional source for stocking. However, additional sampling and genetic analysis may help in identification of additional, larger streams more suited for use as sources for reintroductions. Nonetheless, Lepage Creek should be preserved as a "heritage" population, whether or not it is used for propagation.

*The upper Wisconsin River* is suggested here as a separate management region (Figure 6A), and the Eau Claire is suggested as the heritage population from which broodstock collection could be made. This is despite the observed genetic similarities between the East Branch Eau Claire River population and the nearby populations in the Lake Michigan drainage. Broodstock obtained here would best be used within the same watershed. Equally plausible source populations are available within the Lake Michigan watershed for stocking within that region, if constraints dictate fewer separate broodstocks. However, there are marked genetic differences between the lone sampled population from the upper Wisconsin River (Eau Claire) and those populations in the lower Wisconsin River watershed (Parfrey's Glen, Soper, and Grinsell). Therefore, a distinct stocking source, and separate management of the region should be viewed as maintenance of a "genetic buffer zone" between the important Lake Michigan region and the very distinct southwest.

### ***Relict populations***

Figure 8 highlights the location of several populations that, according to our results, are either greatly reduced in within-population variation, or are somewhat anomalous within their respective regions. Mason, Rosonow, Little Onion, Parfrey's Glen, and Pine Creek, Iowa, are clearly greatly diminished in population size. Shioc was included in this study because the history of the population was unknown. The near fixation for an otherwise rare mtDNA haplotype indicates a history distinct from all surrounding populations, though it is impossible to determine its history based on the genetic data alone.

All of the listed populations should be labeled, and managed, as "relict" populations. Each of these populations and the numerous other "relict" populations that are likely to exist within the state (see future work, below) warrant preservation, where possible. The survival and reproduction of each of these populations may be sufficient to sustain the populations, barring severe perturbations or introduction of disease. Whether they were initially founded by natural migration of wild fish or by undocumented introductions can never be determined, but many may represent portions of the original wild strain. However, none of these populations is worthy of transfer to other locations, and the general lack of genetic variation within each population prevents their consideration for hatchery propagation.

### ***Future Work***

If possible, the genetic work begun here should be continued. The methods are easily replicated, and the need for additional sampling sites is obvious. For example, the unclear relationship of Price Creek to surrounding populations indicates the need for

an additional site in the same area. There are also numerous additional small streams and beaver ponds in the northwest region that contain brook trout, but for which no genetic data exist. Future transfers, reintroductions, and propagation plans will depend on genetic characterization of many of those populations. Likewise, an additional isolated population from the driftless area may help clarify the relationship of Grinsell and Soper populations. It would also be informative to re-sample some of the Fox/Wolf populations used by Krueger and Menzel (1979). Finally, the coastal Lake Superior streams should be revisited to obtain a sample from a larger, more stable population, perhaps more indicative of the original founding stock, which may resemble either inland populations of lake-dwelling populations in Lake Superior itself.

## References Cited

- Allendorf, F. W., and R.F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2:170-184.
- Allendorf, F. W., D.M. Espeland, D.T. Scow, and S. Phelps. 1980. Coexistence of native and introduced rainbow trout in the Kootenai River drainage. *Proceedings of the Montana Academy of Sciences* 39:28-36.
- Anderson S, M.H. DeBruun, Coulson, I.E. Eperon, F. Sanger, and I.G. Young. 1982. Complete sequence of bovine mitochondrial DNA; conserved features of the mammalian mitochondrial genome. *Journal of Molecular Biology*, 156, 683-717.
- Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. *Journal of Fish Biology* 47 (Supp. A): 177-185.
- Bailey, R. M., and G.R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1539-1561.
- Bernatchez, L., and R.G. Danzmann. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchell). *Mol. Biol. Evol.* 10(5):1002-1014.
- Burnham-Curtis, M.K. 1996. Mitochondrial DNA variation among Lake Superior brook trout populations: summary of genetic analyses. Research Contract Completion Report, National Biological Service, Ann Arbor, Michigan.
- Carvalho, G.R., and L. Hauser. 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* 4:326-350.
- Cavalli-Sforza, L. L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 21:550-570.
- Claggett, L.E., and T.R. Dehring. 1984. Wisconsin salmonid strain catalog. Wisconsin DNR, Administrative Report No. 19.
- Cronin, M.A., W.J. Spearman, R.L. Wilmot, J.C. Patton, and J.W. Bickham. 1993. Mitochondrial DNA variation in chinook salmon (*Onchorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. *Can. J. Fish. Aquat. Sci.* 50 (4):708-715.
- Danzmann, R.G., P.E. Ihssen, and P.D. Hebert. 1991. Genetic discrimination of wild

- and hatchery populations of brook charr, *Salvelinus fontinalis* (Mitchill), in Ontario using mitochondrial DNA analysis. *Journal of Fish Biology* 39 (Supp. A):69-77.
- Echt, C.S., L.A. Erdahl, and T.J. McCoy. 1991. Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. *Genome* 35:84-87.
- Fields, R.D., K.R. Johnson, and G.H. Thorgaard. 1989. DNA fingerprints in rainbow trout detected by hybridization with DNA of bacteriophage M13. *Trans. Am. Fisheries Society* 118:78-81.
- Fields, R.D., M. Desjardins, J.M. Hudson, S.J. Johnson, T.W. Kassler, J.B. Ludden, J.V. Tranquilli, C.A. Toline, D.P. Philipp. 1997. Genetic analysis of game fish species in the upper Midwest; Final Report, Volumes 1-4. Illinois Natural History Technical Report 97/5.
- Flick, W.A., and D.A. Webster. 1976. Production of wild, domestic, and interstrain hybrids of brook trout in natural ponds. *J. Fish. Res. Bd. Can.* 33:1525-1539.
- Gyllensten, U., and A.C. Wilson. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes. In Ryman, N., and Utter, F. (eds.), *Population Genetics and Fishery Management*. University of Washington Press. Seattle, WA.
- Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48:945-957.
- Hutchings, J.A. and M.M. Ferguson. 1992. The independence of enzyme heterozygosity and life history traits in natural populations of *Salvelinus fontinalis* (brook trout). *Heredity* 69:496-502.
- IUBNC (International Union of Biochemistry, Nomenclature Committee). 1984. *Enzyme Nomenclature*. Academic Press. Orlando, FL.
- Jones, M.W., R.G. Danzmann, and D. Clay. 1997. Genetic relationships among populations of wild resident, and wild and hatchery anadromous brook charr. *Journal of Fish Biology* 51:29-40.
- Kapuscinski, A. R., and D.P. Philipp. 1988. Fisheries genetics: issues and priorities for research and policy development. *Fisheries* 13:4-10.
- Kmiotek, S., and J.R. Norenberg. 1980. Wisconsin Trout Streams. Wisconsin Department of Natural Resources, Publication 6-3600(80).
- Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals:

- amplification and sequencing with conserved primers. PNAS (USA). 86:6196-6200.
- Kreuger, C.C., and B.W. Menzel. 1979. Effects of stocking on genetics of wild brook trout populations. Transactions of the American Fisheries Society 108:277-287.
- Lachance, S., and P. Magnan. 1990. Performance of domestic, hybrid, and wild strains of brook trout after stocking: the impact of intra- and interspecific competition. CJFAS 47:2278-2284.
- Liskauskas, A.P., and M.M. Ferguson. 1991. Genetic variation and fitness: a test in a naturalized population of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 48:2152-2162.
- McCracken, G.F., C.R. Parker, and S.Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Transactions of the American Fisheries Society 122:533-542.
- McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1991. REAP: The restriction enzyme analysis package. Version 4.0. University of Maine. Orono, ME.
- Moritz, C., T.E. Dowling, and W.M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics 18:269-292.
- Morrison, W.J. 1970. Nonrandom segregation of two lactate dehydrogenase subunit loci in trout. Transactions of the American Fisheries Society 99:193-206.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the USA 70:3321-3323.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.
- Nei, M., and W-H. Li. 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA 76:5269-5273.
- Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.
- Park, L.K., M.A. Brainard, D.A. Dightman, and G.A. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). Molecular Marine Biology and Biotechnology. 2 (6):362-370.
- Perkins, D.L., C.C. Kreuger, and B. May. 1993. Heritage brook trout in northeastern

- USA: genetic variability within and among populations. *Transactions of the American Fisheries Society* 122:515-532.
- Philipp, D.P., W. Childers, and G.S. Whitt. 1979. Evolution of differential patterns of gene expression: a comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, *Micropterus salmoides salmoides*, and smallmouth bass *M. dolomieu*). *Journal of Experimental Zoology* 210:473-488.
- Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout. *J. Fish. Res. Bd. Can.* 34:123-128.
- Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. In *Studies of Genetics. VII. University of Texas Publication No. 7213.*
- Rohlf, F. J. 1989. *NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 1.50.* Exeter Publishing, Ltd. Setauket, New York.
- Ryman, N. 1981. Conservation of genetic resources: experiences from the brown trout (*Salmo trutta*). *Ecological Bulletin* 34:61-74.
- Saghai-Marof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosome location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014-8018.
- Shaklee, J.B., F.W. Allendorf, D.C. Morizot, and G.S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.* 119:2-15.
- Sneath, P. H. A., and R.R. Sokal. 1973. *Numerical Taxonomy.* W. H. Freeman. San Francisco, CA.
- Stoneking, M., D.J. Wagner, and A.C. Hildebrand. 1981. Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout. *Copeia* 1981(4): 810-819.
- Swofford, D.L., and R.B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Heredity* 72:281-283.
- Templeton, A.R. 1986. Coadaptation and outbreeding depression. In Soule, M.E., (ed.), *Conservation Biology, the Science of Scarcity and Diversity.* Sinauer, Sutherland, MA.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.

Nucleic Acids Research 22:6531-6535.

Wright, J.E., and L.M. Atherton. 1970. Polymorphisms for LDH and transferrin loci in brook trout populations. Transactions of the American Fisheries Society 99:179-192.

Wright, S. 1978. *Evolution and genetics of populations. Vol. 4. Variability within and among natural populations.* University of Chicago Press. Chicago, IL.

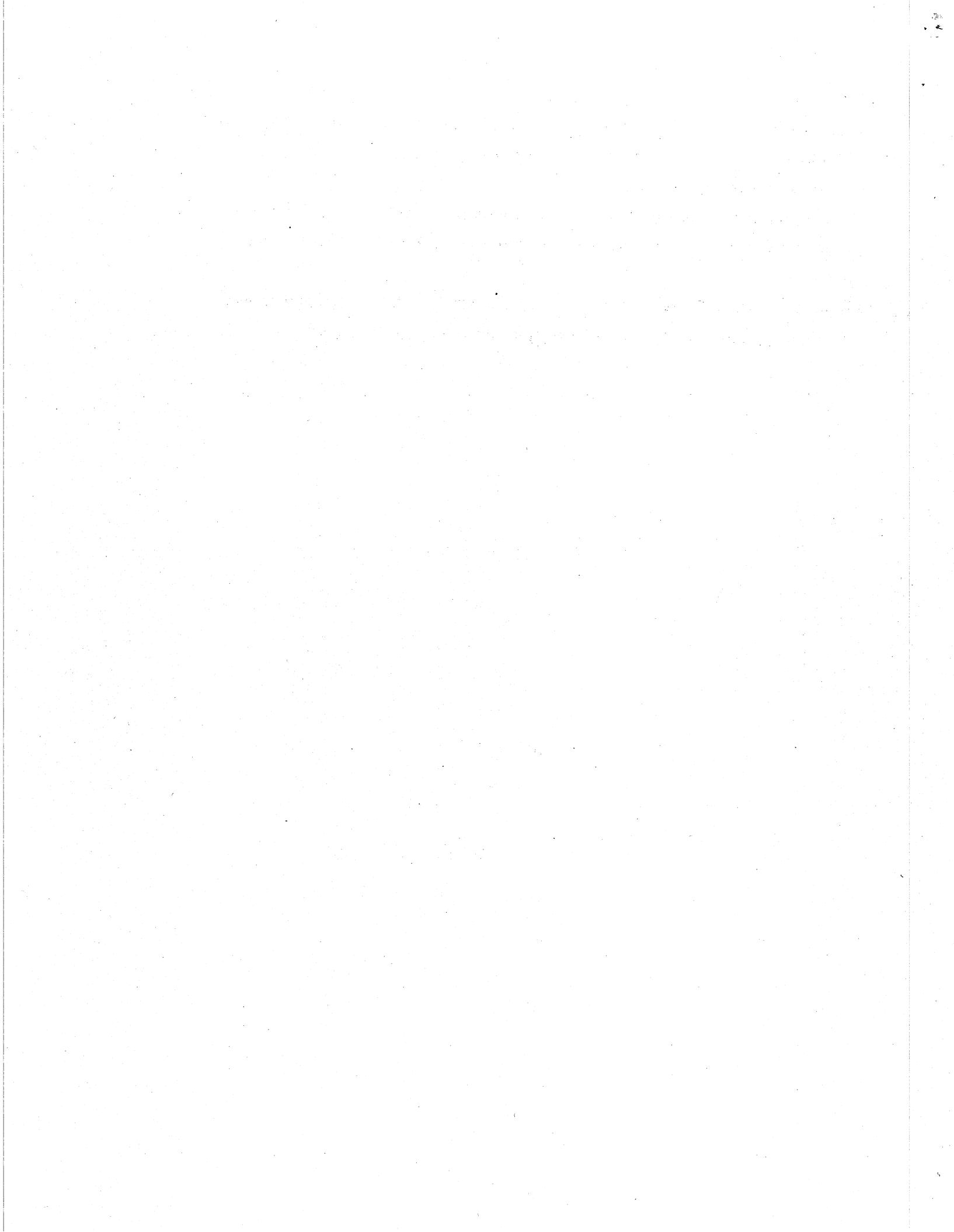


Table 1. Populations sampled, map abbreviation, county, watershed, and number of individuals. An asterisk (\*) indicates Class I streams with connections to extensive Class II waters; two streams are designated exclusively as "Class II". (See Kmiotek and Noremberg, 1980.)

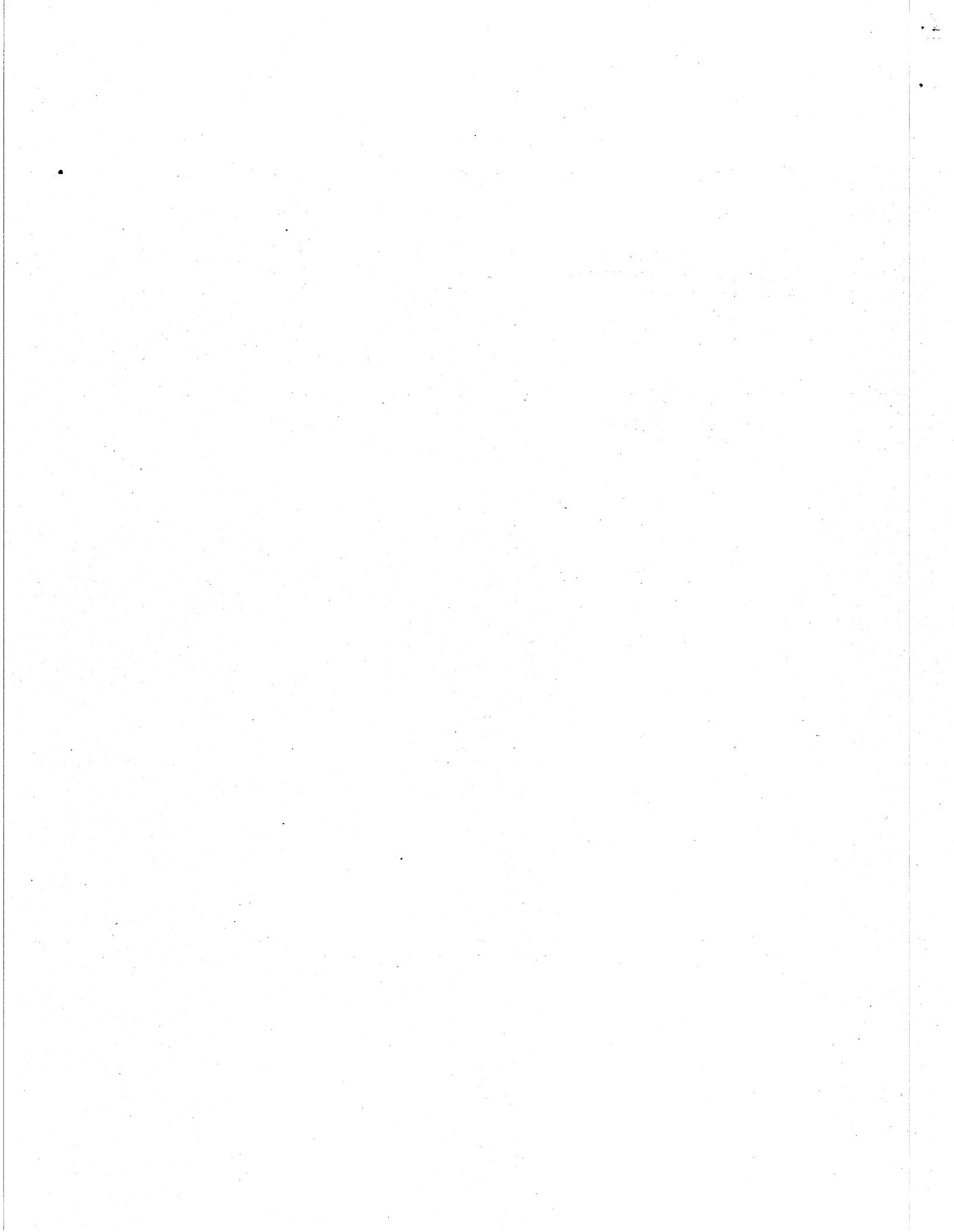
<u>Location</u>	<u>County</u>	<u>Watershed</u>	<u>Stream Length (mi)</u>	<u>N</u>
Price Creek (PR)	Sawyer	Flambeau River	3.8*	30
Wilson Creek (WI)	Dunn/St. Croix	Chippewa River	8.7 (Class II)	30
Big Brook (BB)	Bayfield	Chippewa River	6.5	25
Benson Creek (BE)	Burnett	St. Croix River	1.4	30
Rosonow Creek (RO)	Waukesha	Rock River	< 4.0	30
Mason Creek (MA)	Waukesha	Rock River	< 4.0	30
Eau Claire River (EC) (East Branch)	Langlade	Upper Wisconsin River	11.0 (Class II)	30
Parfrey's Glen (PG)	Sauk	Lower Wisconsin River	1.1	30
Soper Creek (SO)	Monroe	Lower Wisconsin River	6.5	30
Grinsell Creek (GR)	Richland	Lower Wisconsin River	2.5*	30
Little Onion (LO) (Above barrier)	Bayfield	Lake Superior	4.0	33
Shioc River (SH) (West Branch)	Shawano	Wolf River	0.5	30
Whitcomb (WH) (Tributary)	Waupaca	Wolf River (Lake Michigan)	14.3	30
Embarrass R. (ME) (Middle Branch)	Langlade/ Shawano	Wolf River (Lake Michigan)	22.4*	30
Little Scarboro Creek (SB)	Kewaunee	Lake Michigan	1.5	30
Lepage Creek (LP)	Florence	Menominee River (Lake Michigan)	4.5	30
Lawrence Creek (LA)	Adams/ Marquette	Fox River (Lake Michigan)	4.4	20
South Pine Creek, IA (PC)	Winneshiek	Mississippi River	0.5	23
St. Croix, WI (Hatchery) (SC)				30
Manchester, IA (Hatchery) (IH)				25

**Table 2.** Enzyme systems and putative loci screened for polymorphisms in brook trout. Buffer systems are described in the text. Tissues listed are white skeletal muscle (M); liver (L); or eye (E). (a): Loci identified as polymorphic during initial screenings, but not included in larger analysis due to poor resolution of genotypes. Loci in **bold** are included in population analyses.

		RID/TC	M	P
		RID/TC	E	M
<b>sAAT-1,2*</b>	Aspartate aminotransferase			
<b>sAAT-3*</b>				
<b>sAAT-4*</b>		RID	L	M
<b>mAAT-1,2*</b>		RID	M/L	M
<b>ACP-1*</b>	Acid phosphatase	TC	L	M
<b>ADA-1*</b>	Adenosine deaminase	EBT	L	M
<b>ADA-2*</b>		EBT	L	M
<b>ADH-1*</b>	Alcohol dehydrogenase	TC	L	P
<b>SAH-1*</b>	Aconitate hydratase	TC	M	M
<b>SAH-2*</b>		TC	L	M
<b>AK-1*</b>	Adenylate kinase	TC	M	M
<b>AK-2*</b>		TC	M	M
<b>CAT-1*</b>	Catalase	TC	L	M
<b>CBP-1*</b>	Calcium binding protein	TC	M	M
<b>CK-A*</b>	Creatine kinase	TC	M	M
<b>CK-B*</b>		TC	E	M
<b>CK-C*</b>		TC	E	M
<b>EST-1*</b>	Esterase	EBT	L	M
<b>EST-2*</b>		EBT	L	M
<b>EST-3*</b>		EBT	L	M
<b>FBALD-1*</b>	Aldolase dehydrogenase	TC	E	M
<b>FBP-1*</b>	Fructose-bisphosphatase	TC	M	P (a)
<b>FH-1*</b>	Fumarase	TC	L	M
<b>GAPDH-1*</b>	Glyceraldehyde-3-phosphate dehydrogenase	TC	M/E	M
<b>GDH-1*</b>	Glucose dehydrogenase	TC	L	M
<b>G2DH-1</b>	Glycerate-2-dehydrogenase	TC	L	M
<b>G3PDH-1*</b>	Glycerol-3-phosphate dehydrogenase	TC	M	P
<b>G3PDH-2*</b>		TC	L	M
<b>G3PDH-3*</b>		TC	M	P
<b>G6PDH-1*</b>	Glucose-6-phosphate dehydrogenase	RID	L	M
<b>G6PDH-2*</b>		RID	L	M
<b>GK-1*</b>	Glucokinase	TC	L	M
<b>GPI-A*</b>	Glucose-6-phosphate isomerase	TC	M/E	M
<b>GPI-B1*</b>		TC	M/E	M
<b>GPI-B2*</b>		TC	M/E	P (a)
<b>mIDHP-1,2*</b>	Isocitrate dehydrogenase	TC	M	M
<b>sIDH-B1*</b>		TC	M	P
<b>sIDH-B2*</b>		TC	M	M
<b>sIDH-A*</b>		TC	L	M
<b>LDH-A*</b>	L-Lactate dehydrogenase	TC	M/E	M
<b>LDH-B1*</b>		TC	M/E	M
<b>LDH-B2*</b>		TC	M/E	P
<b>LDH-C*</b>		TC	E	M
<b>sMDH-A*</b>	Malate dehydrogenase	TC	M	M

Table 2. (Continued.)

sMDH-A*	Malate dehydrogenase	TC	M	M
sMDH-B1,B2*		TC	M	P
nMDH-M1*		TC	M	M
MEP-1*	Malic enzyme	EBT/T	M	P (a)
		C		
MPI-1*	Mannose-6-phosphate isomerase	RID/TC	L	M
PGDH-1*	Phosphogluconate dehydrogenase	TC	L	M
PGK-1*	Phosphoglycerate kinase	TC	L	P
PGM-A*	Phosphoglucomutase	TC	L	M
PROT-1*	General Muscle Proteins	TC	M	M
PROT-2*		TC	M	M
PROT-3*		TC	L	M
SDH-1*	Sorbitol dehydrogenase	TC	L	M
sSOD-1*	Superoxide dismutase	TC	E	M
TPI-1*	Triose-phosphate isomerase	EBT	L	P(b)
XDH-1*	Xanthine dehydrogenase			



**Table 3.** Amplified mtDNA regions, PCR primers, and sizes of amplified products.

Region	Primer sequences	Fragment Size (kb)	Reference
ATPase 6	5'-ATGAAACCTAAGCTTCTTCGACCAATT-3' 3'	0.6	A. Toline, unpublished
12S RNA/D-Loop	5'-ATAAAAAGGCTAAATTGTTTCGAT-3' 5'-TACCCCAAACTCCCAAAGCTA-3'	2.0	Kocher et al. (1993)
Control Region (D-Loop)	5'-AGGGGTGACGGGGGGGTGTGT-3' 5'-TTGGGGTTCTCGTATGACCG-3'	1.3	Cronin, et al. (1993)
ND-2	5'-AGAGCGGTGCGGTCTTGTAACCC-3' 5'-CTGAGGGCTTGAAGGCC-3'	1.1	Park et al. (1993); Burnham-Curtis, (1996)
ND-3/4	5'-AAGCTATCGGGCCCATACCC-3' 5'-GTACACGTCACTCCAATCA-3'	2.1	Cronin, et al. (1993); Park et al. (1993)
ND-5/6	5'-AGAATCACAACTCAATGTTT-3' 3'	2.3	Anderson et al. (1982)
	5'-GTTGAATGACAATGGGGTTCTTC-3'		

**Table 4.** Allele frequencies for polymorphic protein loci in brook trout.

<u>Locus</u>	<u>Allele</u>	<u>LAW</u>	<u>EC</u>	<u>STC</u>	<u>IHW</u>	<u>LP</u>	<u>BB</u>	<u>SOP</u>	<u>LO</u>	<u>BEN</u>
ADH-1*	1	0.925	0.833	0.983	0.983	1.000	1.000	0.828	1.000	1.000
	2	0.075	0.167	0.017	0.017	0.000	0.000	0.172	0.000	0.000
AAT-1*	1	1.000	0.907	0.967	0.883	1.000	0.983	0.966	1.000	1.000
	2	0.000	0.093	0.033	0.117	0.000	0.017	0.034	0.000	0.000
AAT-2*	1	0.350	0.389	0.417	0.433	0.935	0.600	0.740	0.517	0.967
	2	0.650	0.611	0.583	0.567	0.065	0.400	0.260	0.483	0.033
PGM-1*	1	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000
	2	0.875	0.950	0.850	0.917	1.000	0.967	0.800	0.914	1.000
	3	0.125	0.050	0.150	0.083	0.000	0.000	0.200	0.086	0.000
G3-1*	1	0.050	0.000	0.067	0.067	0.043	0.083	0.000	0.197	0.000
	2	0.950	1.000	0.933	0.933	0.957	0.917	1.000	0.803	1.000
G3-3*	1	0.000	0.000	0.033	0.117	0.000	0.000	0.000	0.000	0.000
	2	1.000	1.000	0.967	0.883	1.000	1.000	1.000	1.000	1.000
LDH-B2*	1	0.450	0.367	0.117	0.083	0.543	0.056	0.220	0.172	0.000
	2	0.075	0.050	0.017	0.017	0.457	0.019	0.000	0.086	0.000
	3	0.475	0.583	0.867	0.900	0.000	0.926	0.780	0.741	0.667
sIDHP-B1*	2	0.475	0.650	0.500	0.482	0.022	0.400	0.400	0.339	0.000
	3	0.200	0.067	0.161	0.268	0.022	0.317	0.460	0.357	0.375
	4	0.325	0.267	0.339	0.250	0.957	0.250	0.120	0.268	0.321
	5	0.000	0.017	0.000	0.000	0.000	0.033	0.020	0.036	0.286
										0.018
MD-B1*	1	0.000	0.100	0.033	0.017	0.000	0.067	0.000	0.000	0.000
	2	0.000	0.167	0.017	0.000	0.000	0.050	0.000	0.155	0.000
	3	1.000	0.733	0.950	0.983	1.000	0.883	1.000	0.845	1.000
MD-B2*	3	0.950	1.000	0.883	0.933	0.870	0.933	0.940	0.948	1.000
	4	0.050	0.000	0.117	0.067	0.130	0.067	0.060	0.052	0.000

**Table 4 (cont.).** Allele frequencies for polymorphic protein loci in brook trout.

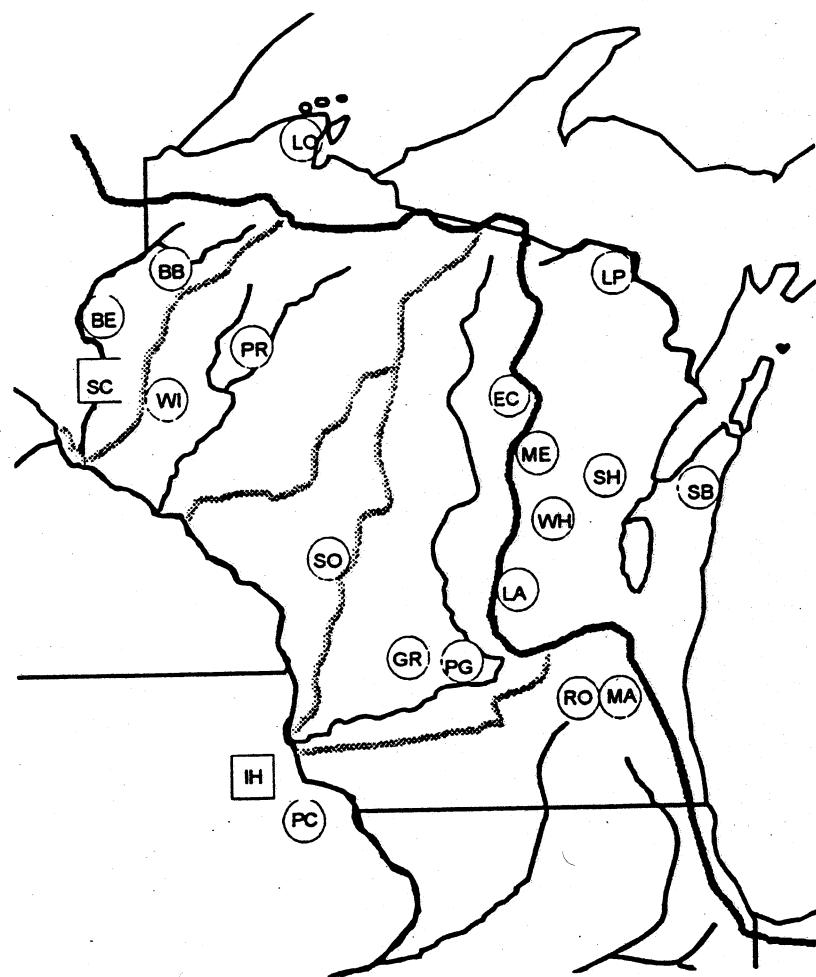
Locus	Allele	MBE	ROS	PQ	MAS	SHLOC	GRIN	WHIT	SCAR	PRICE	WILS
ADH-1*	1	0.717	0.850	1.000	0.917	0.850	0.845	1.000	0.950	0.967	0.867
	2	0.283	0.150	0.000	0.083	0.150	0.155	0.000	0.050	0.033	0.133
AAT-1*	1	0.883	1.000	1.000	0.917	0.950	0.883	0.850	0.917	1.000	0.000
	2	0.117	0.000	0.000	0.083	0.050	0.117	0.150	0.083	0.083	0.000
AAT-2*	1	0.400	0.600	1.000	0.867	0.183	0.567	0.283	0.333	0.467	0.867
	2	0.600	0.400	0.000	0.133	0.817	0.433	0.717	0.667	0.533	0.133
PGM-1*	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.900	0.883	1.000	0.700	0.883	0.717	0.867	0.733	0.733	0.717
	3	0.100	0.117	0.000	0.300	0.117	0.283	0.133	0.133	0.267	0.283
G3PDH-1*	1	0.017	0.000	0.133	0.167	0.017	0.000	0.017	0.017	0.067	0.000
	2	0.983	1.000	0.867	0.833	0.983	1.000	0.983	0.983	0.933	1.000
	3										
G3PDH-3*	1	0.017	0.000	0.117	0.150	0.000	0.033	0.000	0.000	0.000	0.000
	2	0.983	1.000	0.883	0.850	1.000	0.967	1.000	1.000	1.000	1.000
LDH-B2*	1	0.250	0.700	0.567	0.483	0.167	0.033	0.217	0.233	0.183	0.333
	2	0.067	0.000	0.000	0.050	0.000	0.000	0.150	0.033	0.017	0.033
	3	0.683	0.300	0.433	0.467	0.833	0.967	0.633	0.733	0.800	0.633
SIDHP-B1*	2	0.083	0.267	0.000	0.067	0.583	0.717	0.000	0.750	0.300	0.569
	3	0.267	0.067	0.017	0.033	0.000	0.150	0.567	0.017	0.133	0.362
	4	0.633	0.650	0.917	0.900	0.417	0.100	0.433	0.233	0.350	0.069
	5	0.017	0.017	0.067	0.000	0.000	0.033	0.000	0.000	0.217	0.000
MDH-B1*	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.050
	3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950
MDH-B2*	3	1.000	1.000	0.783	1.000	0.967	1.000	1.000	1.000	1.000	0.917
	4	0.000	0.000	0.217	0.000	0.033	0.000	0.000	0.000	0.050	0.083

**Table 5.** Frequency of each mitochondrial "clonal line". Order of letters indicates restriction patterns for D-Loop digested with Hinf I and Dde I, and ND-2 digested with Pst I and Ban I.

Population	<u>AAAA</u>	<u>ABAA</u>	<u>BAAA</u>	<u>AABA</u>	<u>AABB</u>	<u>ABBA</u>	<u>BABA</u>
St Croix Hatchery	16				6	6	
Iowa Hatchery	19			3	3		
South Pine Creek, Iowa	23						
Rosonow Creek	29				1		
Parfrey's Glen	30						
Little Onion River	33						
Mason Creek	27		2	1			
Price Creek	23	1	1	5			
Lepage Creek	15	6	1	9			
Lawrence Creek	4	3	10				
Grinsell Creek	16	14					
Big Brook	8	15		1		1	1
Scarboro Creek	2	25		1		1	
Benson Creek	1	24		2			
Whitcomb Creek	2	16		2			
Wilson Creek	6	17	4		6	1	2
E. Branch Eau Claire River	4	11	2		1	7	
Middle Branch Embarrass	3	11	3	1	1	11	
Soper Creek	5		1	8	1	13	
W. Branch Shioc River	4		1	3		22	

78

Figure 1. Brook trout sampling site locations. Squares signify hatchery populations, circles are wild populations. See Table 1 for site abbreviations.



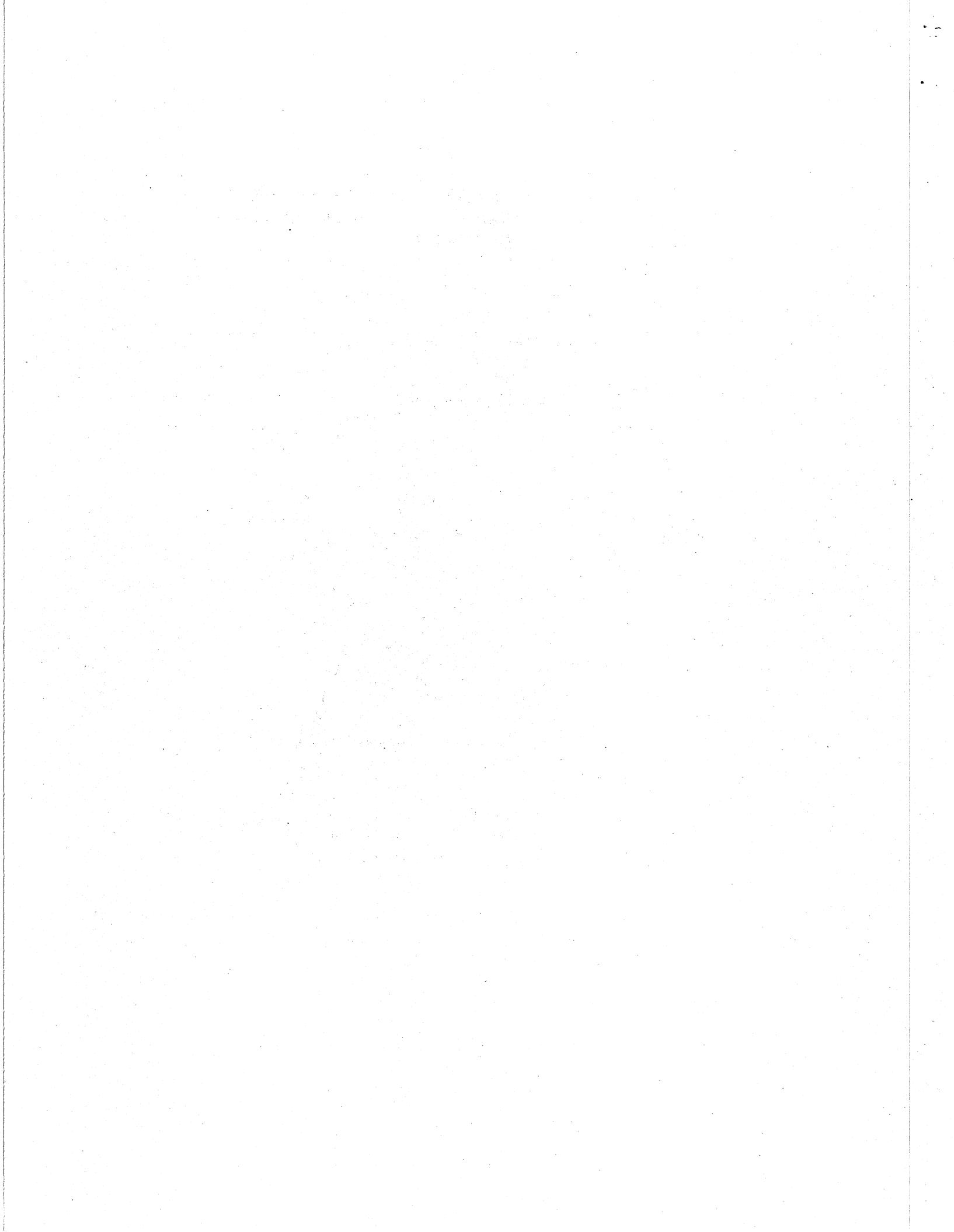
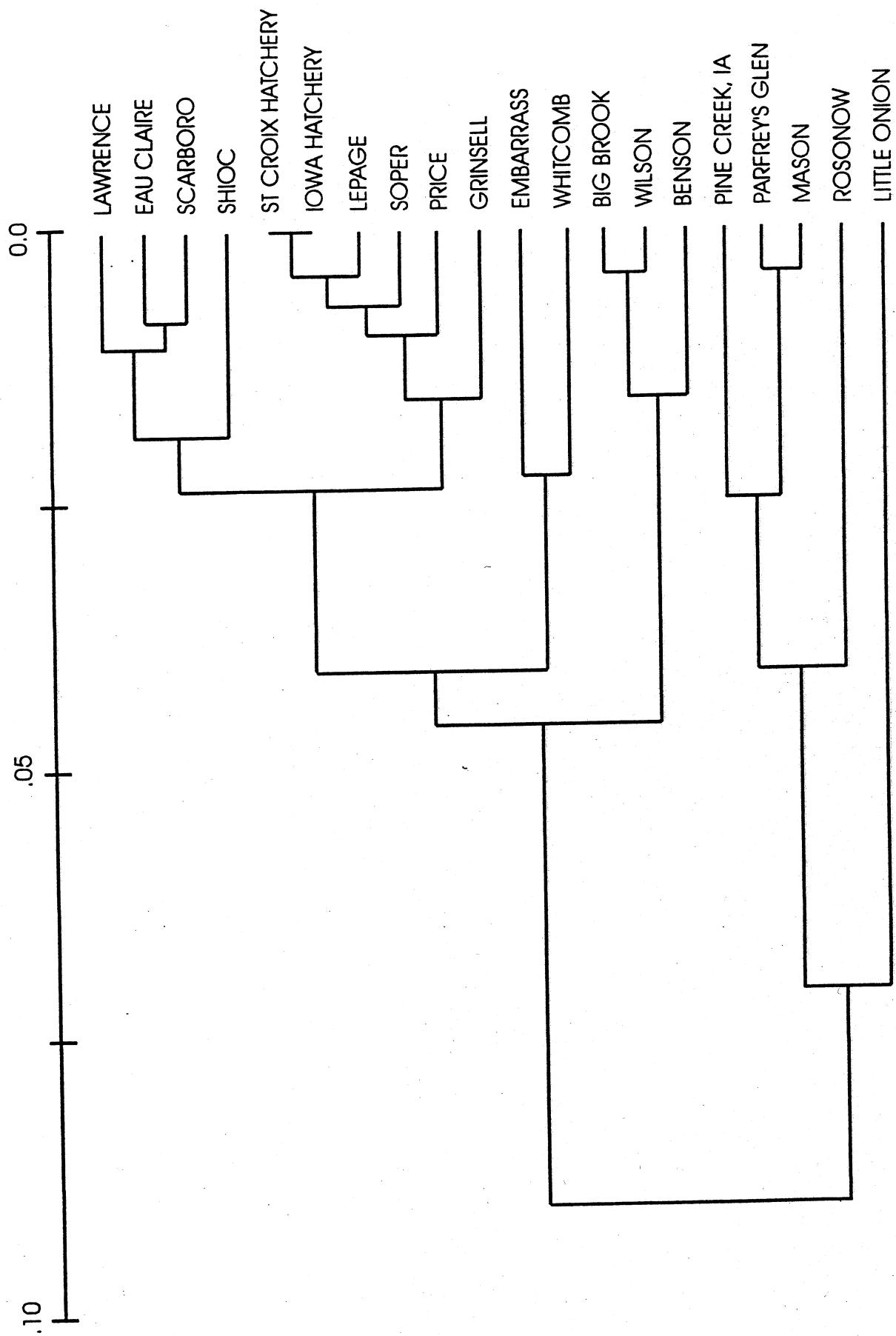


Figure 2. Dendrogram (Nei's genetic distance) for brook trout allozyme data.



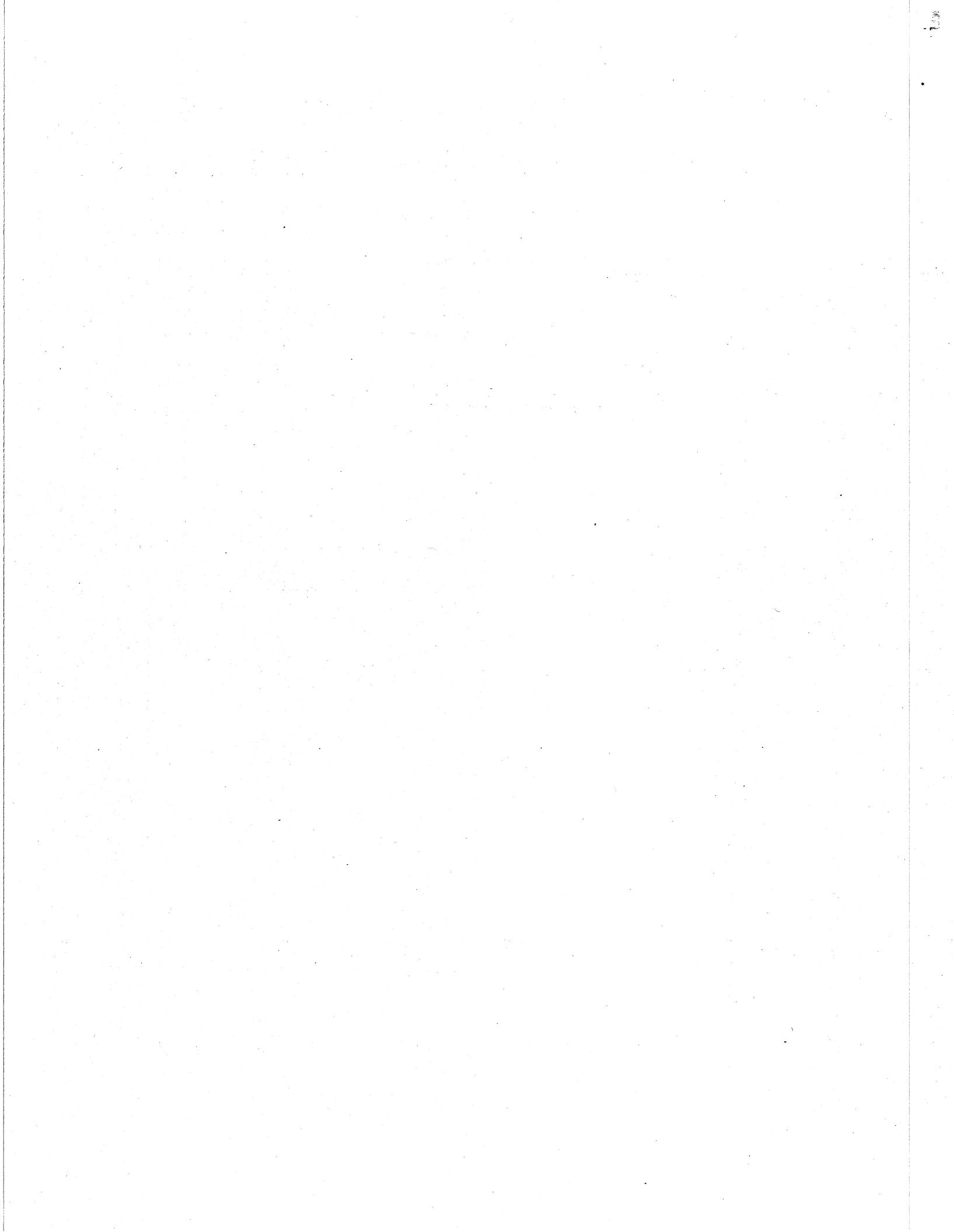
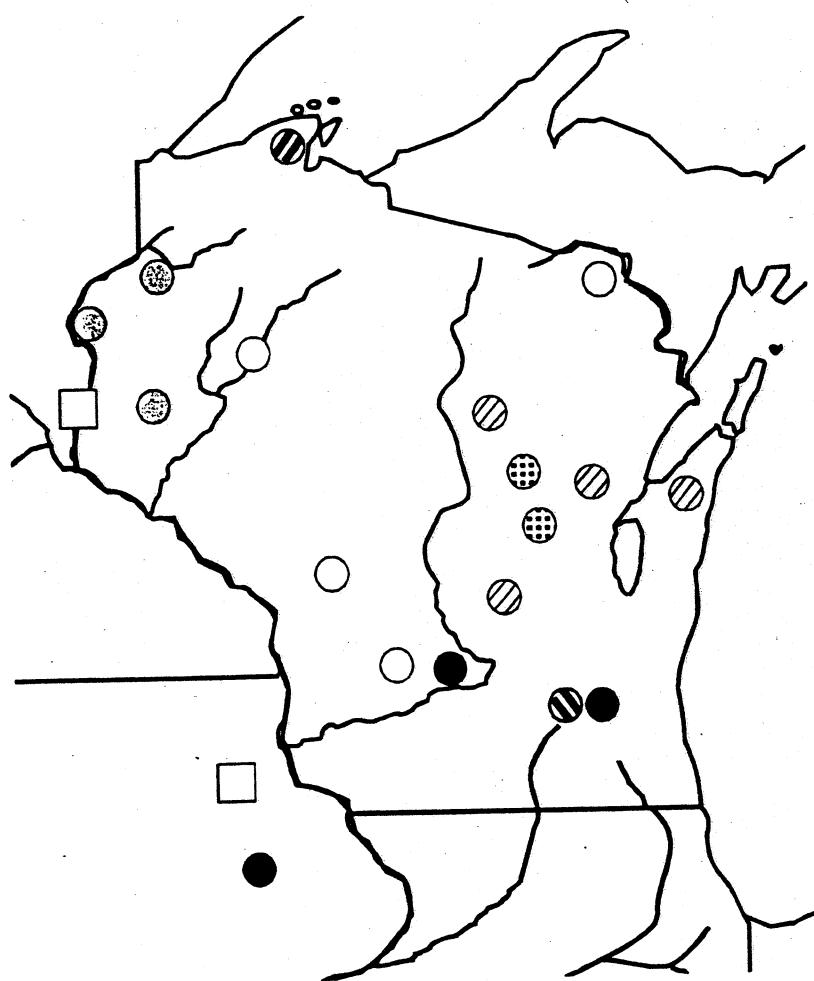


Figure 3. Conservative estimate of geographic distribution of genetically similar populations of brook trout, based on allozymes.



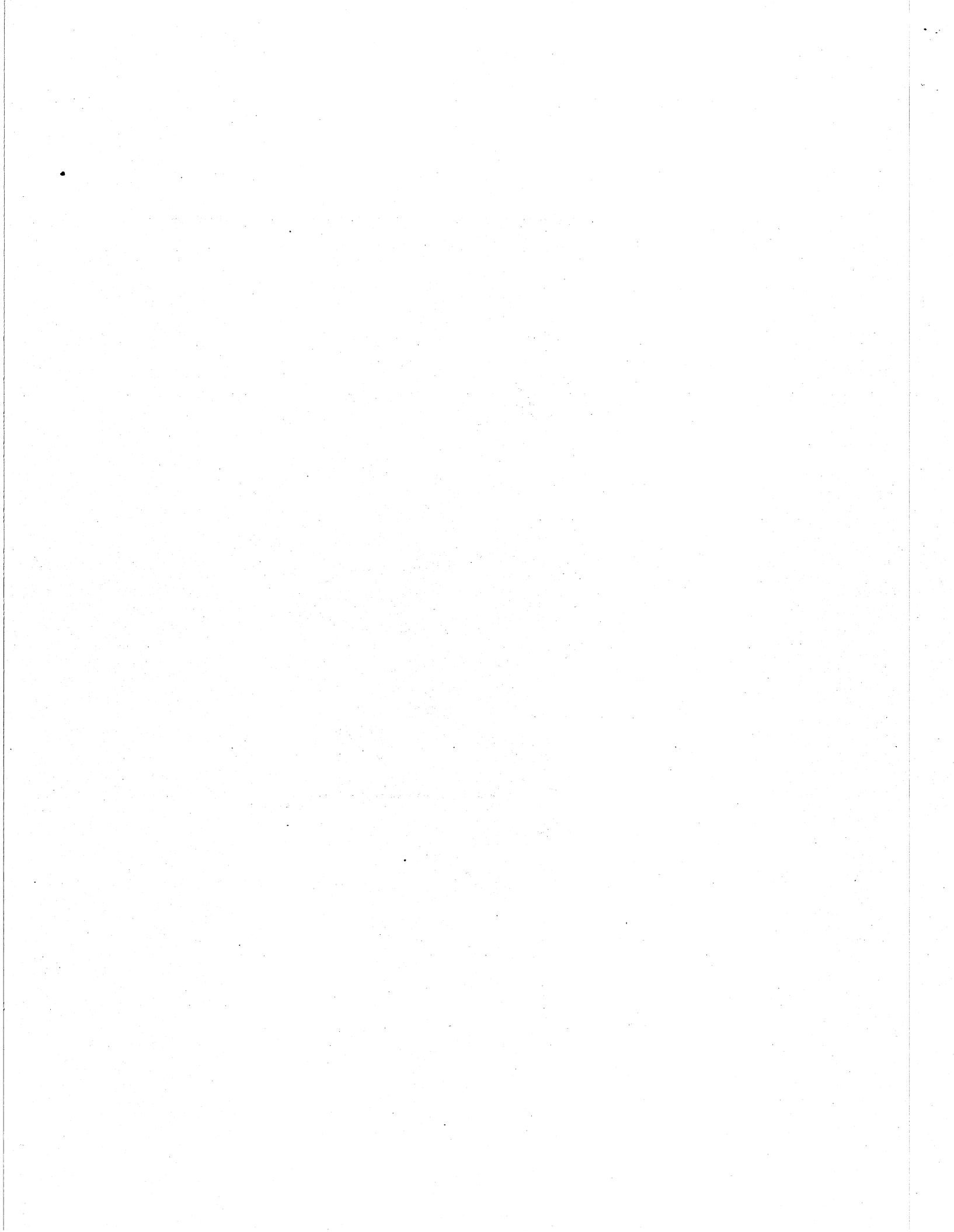


Figure 4. Phenogram based on MtDNA analysis.

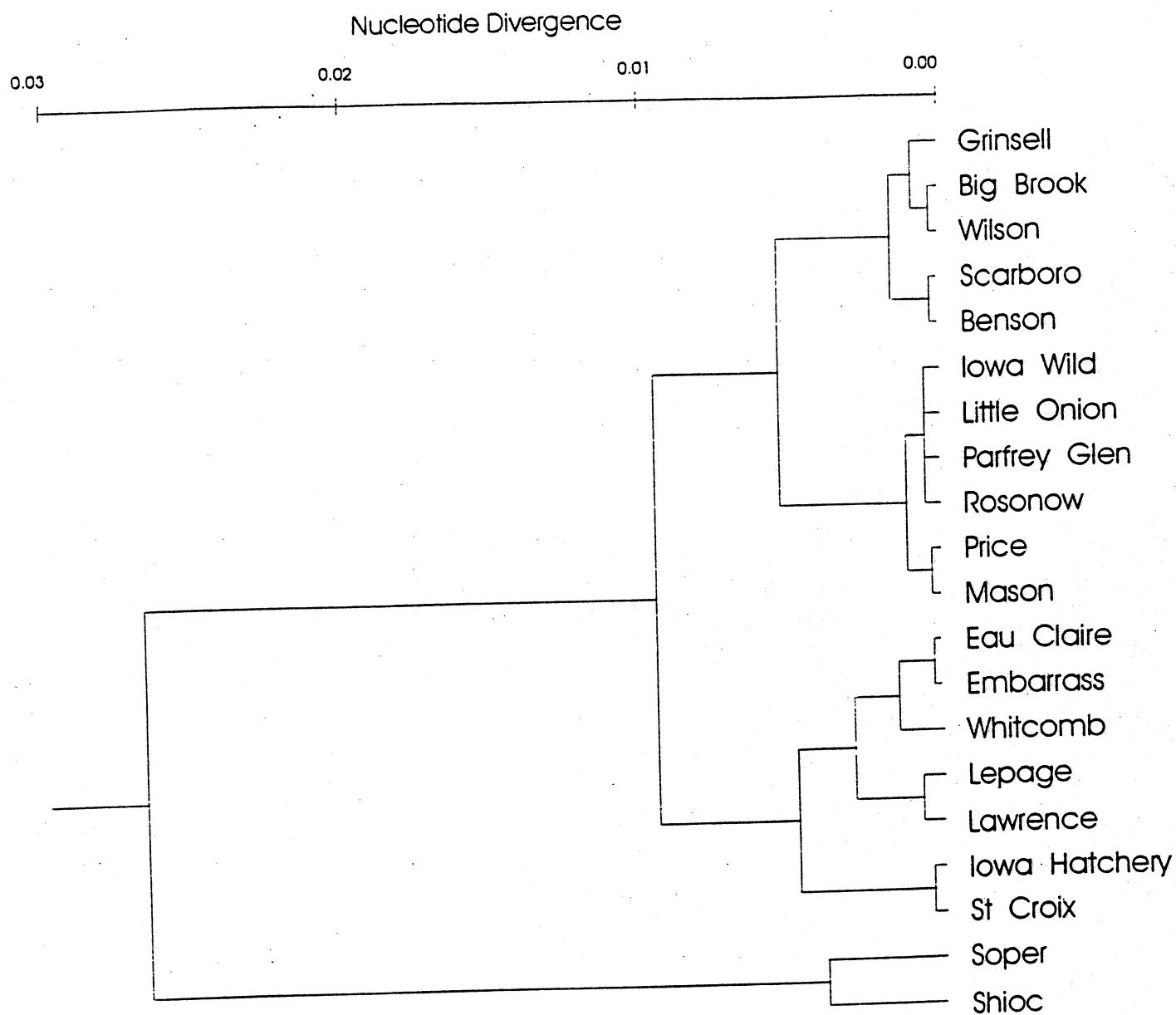




Figure 5. Conservative estimate of geographic distribution of genetically similar populations of brook trout, based on MtDNA analysis.

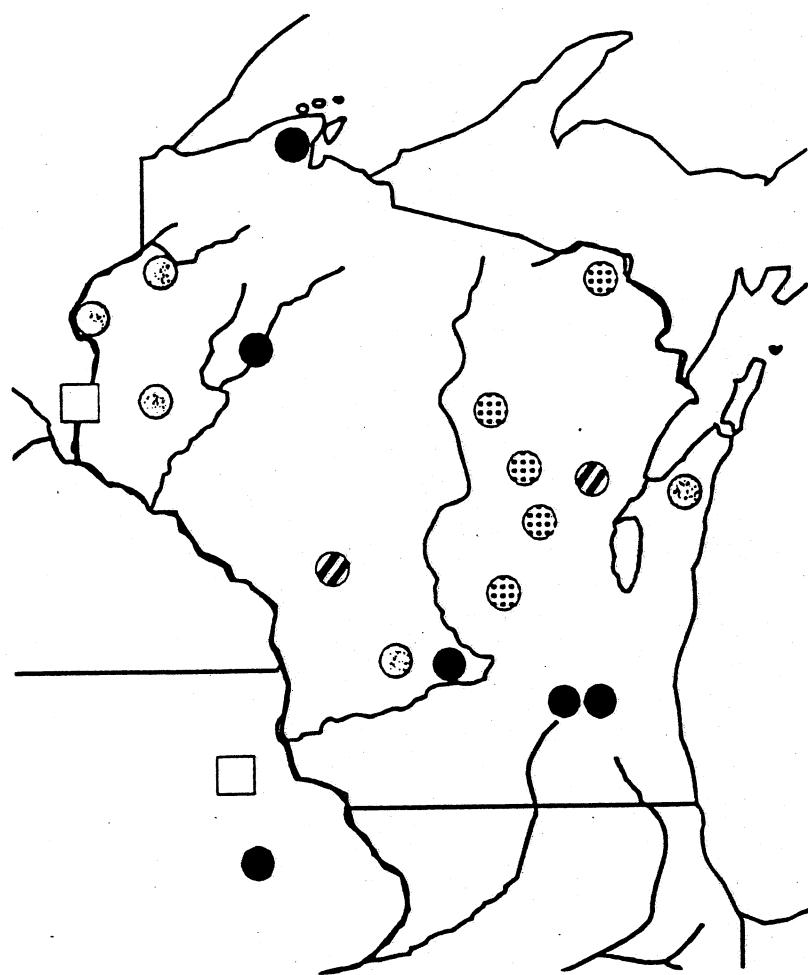
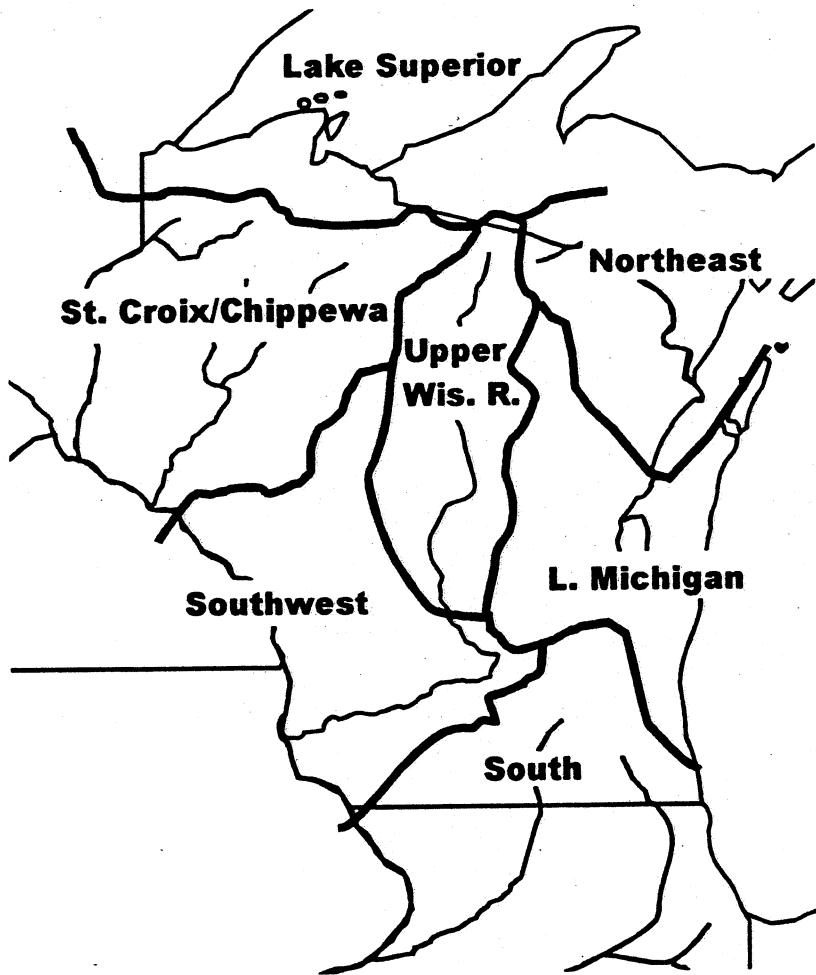




Figure 6A. Recommended genetic management zones for conservation of statewide genetic diversity of brook trout.



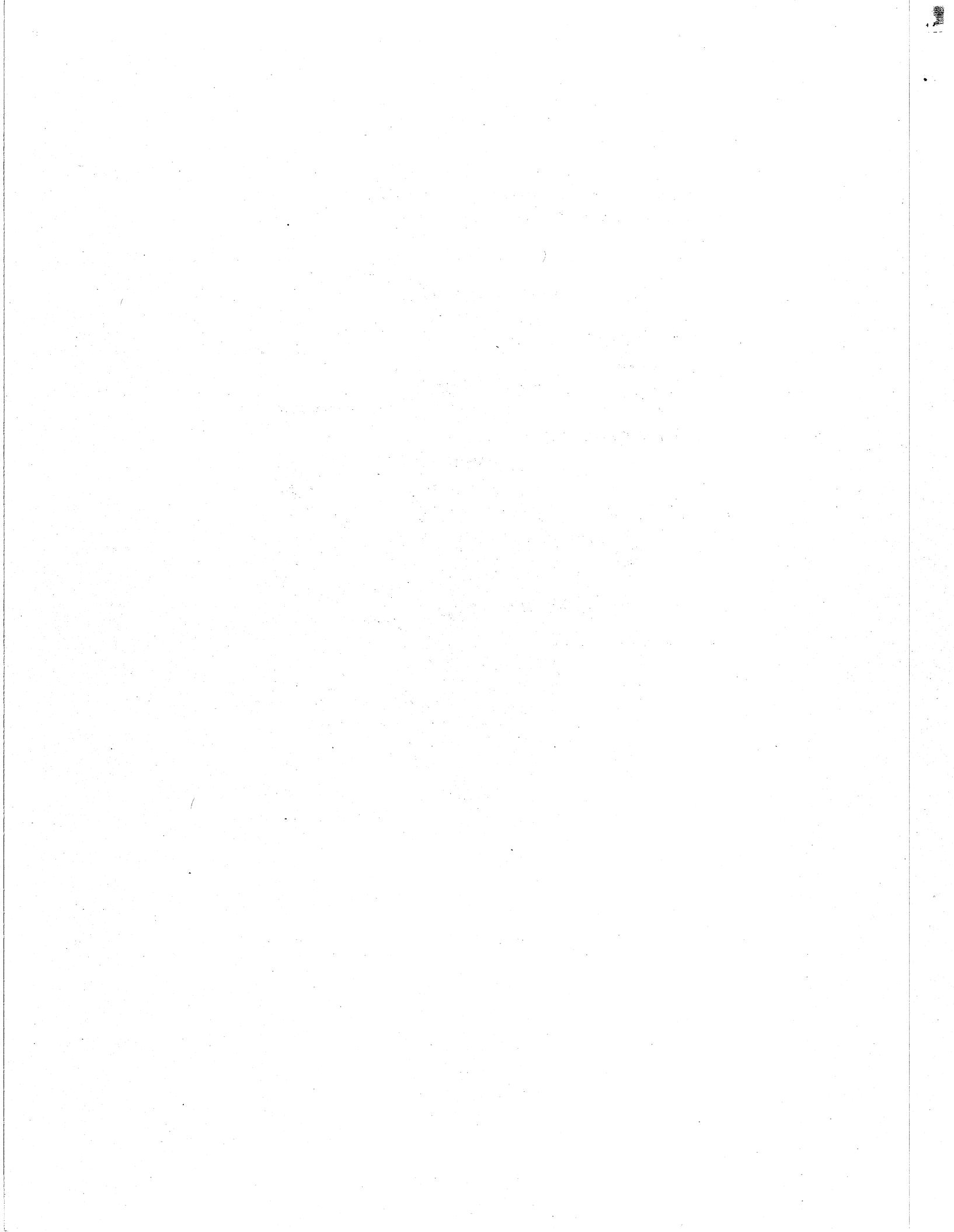


Figure 6B. Broad genetic management zones for conservation of the most significant portions of statewide genetic diversity of brook trout.

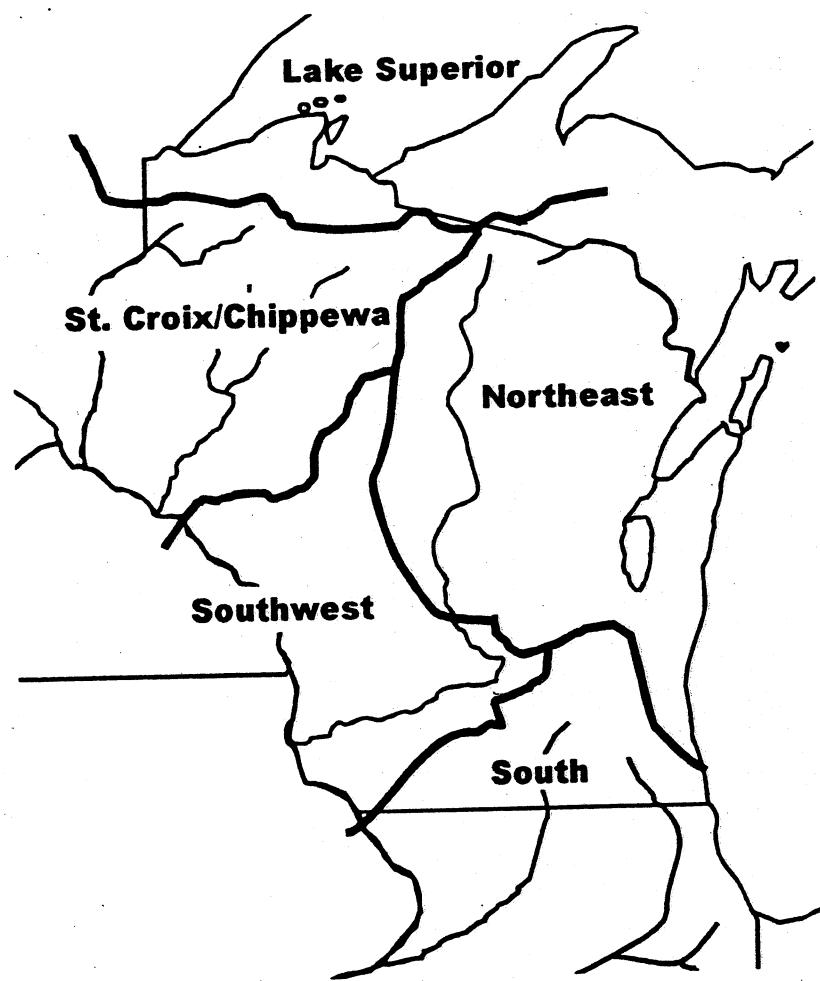
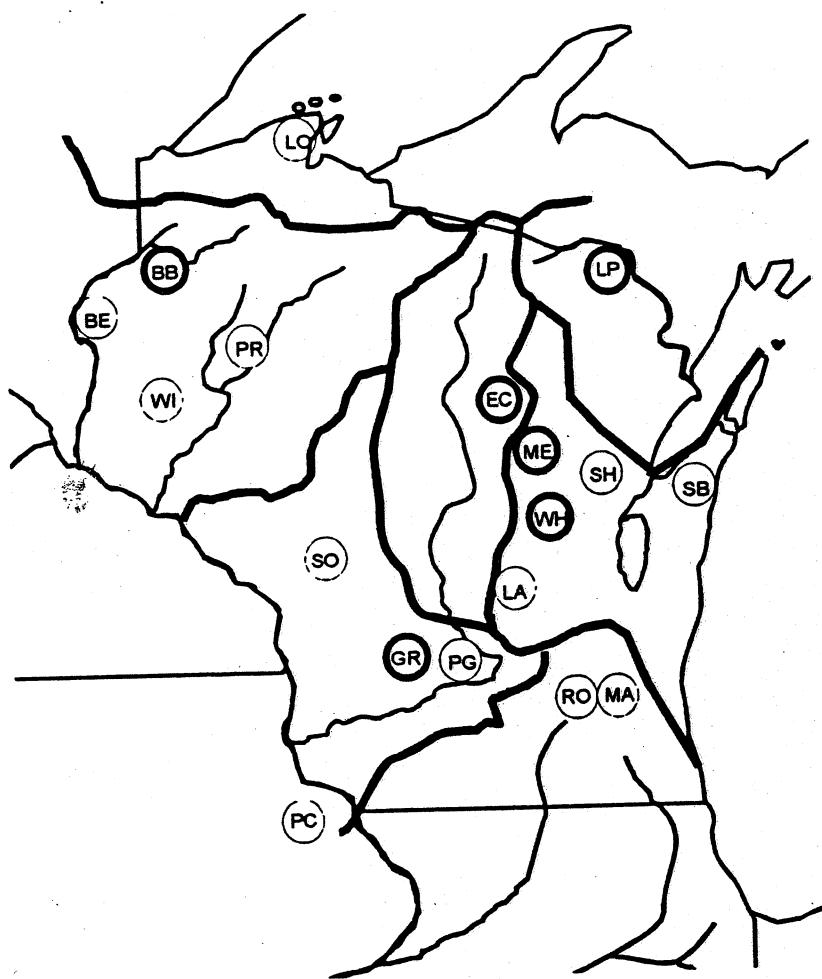




Figure 7. Possible sources (bold circles) for local reintroductions of brook trout.



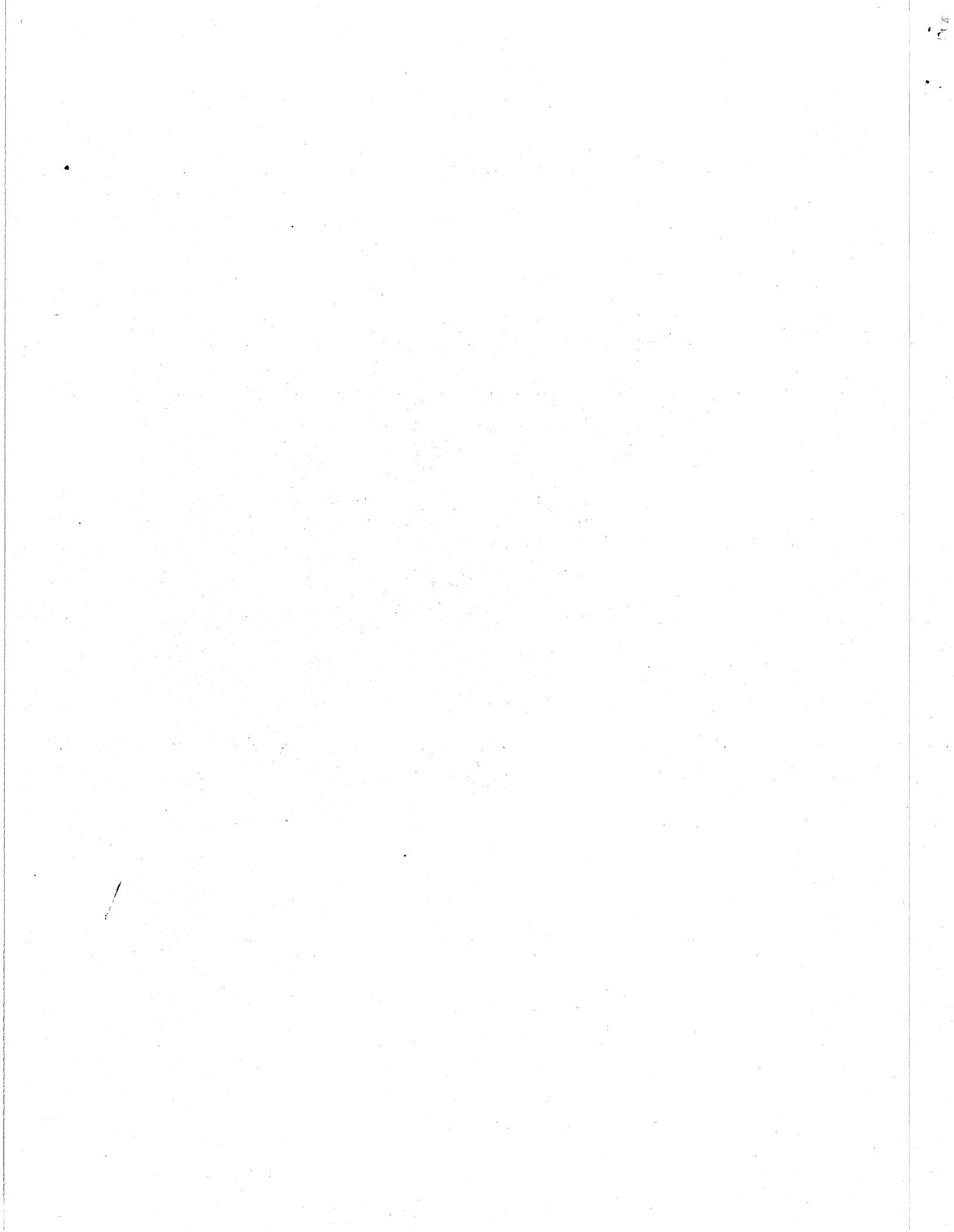


Figure 8. Distribution of "relict" populations of brook trout (bold circles).

