Effects of stocking on genetics of wild brook trout populations

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Department: Animal Ecology
Major: Fisheries Biology

Approved:

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Iowa State University
Ames, Iowa

1976

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ABSTRACT

The management of trout populations in streams often includes stocking of hatchery-raised fish to supplement natural reproduction. Due to artificial selective pressures, hatchery populations may vary genetically from wild fish. Through interbreeding or by otherwise altering natural selective forces, stocked fish can potentially affect the gene pools of wild populations. The present study was undertaken to evaluate the long term genetic influence of a 50+ year brook trout stocking program in Wisconsin.

Trout were collected from nine streams in Waupaca County and from the Osceola State Fish Hatchery. Blood plasma and whole eye samples were analyzed for esterase, transferrin (Tf) and lactate dehydrogenase (LDH) systems using starch and acrylamide gel electrophoresis. Esterase was monomorphic in all samples but Tf and LDH displayed genetic polymorphism.

Populations from Wisconsin streams exhibited a greater degree of polymorphism and a different allelic balance at the Tf and LDH-B loci than that reported for populations of eastern North America. The occurrence of several Tf AA phenotypes among wild caught fish was unusual because previous genetic studies indicated this combination to be lethal or semi-lethal. Only one field population sample was monomorphic at both the Tf and LDH-B loci. This result may reflect inbreeding and generally reduced genetic variability among the apparently small and isolated population.

The Osceola hatchery fish were genetically distinctive from the wild populations at the polymorphic loci. Genetic differences also occurred between various wild populations at one or both of these loci. Comparison

of LDH-B allelic frequencies among samples from three localities in one stream gave evidence for intrastream genetic variation.

Among wild populations, variation in Tf allelic frequencies appeared to have a geographic basis. Differences in natural selective pressures, associated with location within the drainage basin, may account for this genetic variation. At the LDH-B locus, wild populations which exhibited similar allelic frequencies also tended to share similar stocking histories. This correlation is not readily explainable on the basis of interbreeding between wild and hatchery stocks. It seems more likely that stocking in some way alters the natural selective processes affecting the LDH-B locus. This could be through direct interactions between native and hatchery fish or as a result of angling pressures related to stocking.

INTRODUCTION

The stocking of fish to supplement exploited natural populations has been a widespread practice since before the turn of the century. By 1870, brook trout, Atlantic salmon, American shad, whitefish, and lake trout were being cultured in the United States (Bowen, 1970). To counteract the effects of the rapid modernization of this period, stocking was often used as a management tool to rehabilitate fish populations. Because salmonids were particularly amenable to culture methods, hatchery stocking became an important method to manage trout waters. As a result, techniques have been perfected in raising trout throughout all life stages.

To assure of constant supplies of eggs for hatcheries, brood stock are often raised and kept. Genetically these fish usually are the product of selection for characters of growth, fecundity, disease resistance, and other attributes of interest to the hatchery manager (Wolf, 1953; Donaldson and Olsen, 1955; Buss, 1959; Ehlinger, 1964; Toney and Bowen, 1968). In addition, the development of closed production strains by inbreeding contributes to genetic alteration (Buss, 1959). Unintentional selection by the hatchery environment (e.g., concrete raceways, overcrowding, artificial feeding methods) also acts continually upon both brood stock and their progeny (Schuck, 1948; Calaprice, 1969). As a result the hatchery product being stocked into the wild often reflects these artificial selection forces (Vincent, 1960).

Three categories of trout stocking have evolved. The first is the introduction of a trout species to waters previously devoid of it. Where there is little competition such introductions have been highly successful

around the world (MacCrimmon and Marshall, 1968; MacCrimmon and Campbell, 1969; MacCrimmon, 1971). The second category may be defined as maintenance stocking. This is performed to compensate for a lack of spawning production. The stocked trout are intended to remain in the lake or stream for an extended period utilizing the water's productive capacity. The third type of stocking is to provide a "put and take" fishery. Legal-sized trout are released just prior to or during the open season to be harvested quickly with little utilization of the productive capacities of the habitat. In practice, stocking of trout for purposes other than introduction often results in fisheries with characteristics attributable to both maintenance and "put and take" stocking.

Beginning in the 1930's, fishery biologists began to question the effect of stocking programs on harvest (Cooper, 1970). Numerous projects to evaluate the stocking of trout were undertaken. Catch and survival of hatchery fish in the wild were found to be related to species, strain, number stocked, age, and time of planting (Needham and Slater, 1944; Schuck and Kingsbury, 1948; Cooper, 1952; Miller, 1954; Christenson et al., 1954; Mullan, 1956). Schuck (1948) and Cooper (1959) also suggested that selection for good hatchery performance had altered the genetic character and reduced the fitness of the trout.

Only recently have the effects of stocking on natural populations become a topic of interest. Public pressure for trout management sometimes results in stocking waters that have sufficient natural reproduction to keep wild populations near carrying capacity. Consequently just prior to opening day catchable trout are often stocked into such waters

providing a temporary "put and take" fishery during the first days of the season. Recently evidence has indicated that standing crops of natural stream populations of trout in Montana and Wisconsin were reduced by the stocking of cultured fish (Vincent, 1975; Thuemler, 1975). The cause was unknown but may be due to a behavioral interaction between the wild and domestic fish (Butler, 1975).

Concern has also been expressed that domesticated strains of trout may alter the gene pools of natural populations. Smith and Needham (1942) suggested that interbreeding between wild and hatchery fish could produce less fit offspring. Calaprice (1969, p. 385), theorizing on the long range effects of stocking on native gene pools, stated:

> It is conceivable that the continuous stocking of hatchery fish ill-adapted to natural environments might result in a continuous lowering of the fitness, and if continued, the extinction of local populations.

Especially with their long history of stocking, salmonid populations may be susceptible to such a lowering of fitness. Stocking may cause, at least temporarily, an alteration of biological interactions, which may, in time have a heritable effect on the managed populations. These effects may not be consistent with management goals.

The impact of cultured trout on native gene pools would depend on a variety of factors such as the genetic character, survival, and the number stocked relative to the receiving natural population size. Since hatchery populations are the product of a variety of artificial selection forces, they may vary genetically from wild populations. Comparisons of hatchery and wild populations behaviorally (Vincent, 1960), and electrophoretically (Wright and Atherton, 1970; Goldberg et al., 1971; Eckroat, 1971) lend

support to this idea. Survival and the number stocked are two related variables affecting the length and degree of interaction between hatchery stocks and wild populations. Field studies have demonstrated that survival varies between species. Hatchery brook trout in particular have high natural and fishing mortality in streams when compared to other cultured trout species (Cooper, 1952; Brynildson and Christenson, 1961; Onodera, 1962). Poor survival would shorten the time for interaction between hatchery and natural populations. High stocking rates, defined in terms relative to the receiving population size, however, would increase the opportunity for interaction before mortality. Stocking large numbers of fish may also increase the chance for survival of a few individuals to a time when the gene pool is particularly vulnerable to change (e.g., spawning time).

Historically, studies on the genetic effects of stocking on natural populations have utilized morphometric and meristic measurements. Hoyt (1974) used meristic characters to determine the effects of stocking varieties of northern smallmouth bass on populations of Neosho smallmouth bass. His evidence suggested that an alteration of values for meristic characters had occurred in populations where stocking had taken place and in the direction of the planted fish. Gard and Seegrist (1965) determined the effect of stocking hatchery rainbow and cutthroat trout on a native rainbow population by using morphometric measurements and meristic counts. Their results suggest a slight divergence from the native form, in the direction of the hatchery cutthroat but not toward the hatchery rainbow.

Characters such as those used in the above studies may be both genetic and environmental expressions. The possible environmental influence on these characters (Barlow, 1961) reduces their sensitivity for detection of genetic change. A need exists, therefore, for inalterable, genetically determined, characteristics which may serve in making comparisons between populations. Protein characters as determined by biochemical analysis meet this need as tools of genetic research. Electrophoresis is particularly sensitive for detecting relatively minor differences between protein molecules. A protein solution may be separated through a gel medium in an electric field on the basis of molecular charge and size (Smith, 1968; Gordon, 1969). Protein bands may then be visualized through various staining procedures. Banding patterns in a gel are often the expression of codominant alleles which are inherited in Mendelian fashion. Electrophoretic techniques, combined with principles of Mendelian and population genetics, have been used successfully for identifying breeding units among numerous fishes (de Ligny, 1969). The technique provides data for statistical comparisons of phenotypic or allelic frequencies to describe temporal and spatial genetic relationships.

Several studies have provided evidence that the stocking of fish has altered the occurrence of electrophoretic protein patterns in natural populations. Northcote et al. (1970) believed that introgressive hybridization may have increased the frequency of the LDH-C^1 allele in a native rainbow trout population due to stocking of cutthroat trout. Møller (1970) suggested that the introduction of hatchery fish may have contributed to transferrin heterogeneity observed in Atlantic salmon populations. Eckroat (1971, 1973) used lens proteins in electrophoretic comparisons of

Pennsylvania brook trout populations. One population exhibited a high frequency of a rare allele not detected in other wild populations surveyed. The allele did however, occur in some hatchery populations. Eckroat believed that the location and nature of the population may have lent itself to planting of hatchery fish by a local sportsman's club. Yoshiyasu (1973) used starch gel electrophoresis to study the hemoglobins of salmon (Oncorhynchus spp.) in southwest Japan. O. rhodurus fry had been introduced thirty years previously into the Kuroko River, thought only to contain O. masou at the time. Presently, the fish meristically resemble O. rhodurus, however, the hemoglobin patterns showed individual differences resembling either O. masou or O. rhodurus.

In all of the literature cited above the research was not specifically designed to determine the genetic effects of stocking. Rather their results with reference to this question were byproducts of other goals being pursued.

The present research was conducted in an attempt to answer whether stocked fish could alter the genetic character of native trout populations. In theory, such alteration may result from the interbreeding of cultured fish with wild populations or from an interaction between the two populations, causing a change of fitness of certain genotypes in the wild population. The research focused on several objectives: 1) to survey and compare field populations and hatchery stocks of brook trout for genetic variability as expressed in terms of electrophoretically detectable differences in tissue proteins; 2) to determine the spatial relationships of breeding populations among wild trout based upon genetic characteristics

of the populations; and 3) to determine if stocking of domesticated strains of trout has had a genetic influence upon resident trout populations.

To achieve these goals a number of requirements in design had to be met. An indigenous trout was sought for which knowledge of the inheritance of electrophoretically determinable proteins existed. Such a fish would facilitate genetic characterization. To reduce variability between populations and achieve a spatial description of trout genetics, a search was made for a study area that contained a number of populations within one major drainage. The populations also had to possess a variety of stocking backgrounds with the source of stocked trout known and presently available for genetic characterization.

The Waupaca County area of Wisconsin is well-suited for this type of genetic research. Brook trout (Salvelinus fontinalis Mitchill) are native to the region's waters (MacCrimmon and Campbell, 1969). Inheritance for a number of proteins detectable by electrophoresis has been described for this species (Hoffman, 1966; Wright and Atherton, 1970; Eckroat and Wright, 1969).

Within the main drainage system (Wolf River) of the area, many streams occur which contain populations of brook trout. A spatial description of the genetics of wild populations was thus possible. This description included the genetic variation observed within streams, between streams, and variation as related to drainage position.

The state of Wisconsin has for more than fifty years been engaged in the stocking of brook trout in streams. All the brook trout used for

stocking streams in the research area have originated from the Osceola State Fish Hatchery located in Osceola, Wisconsin. An inbred population primarily selected on the basis of color, shape and growth has been maintained at this hatchery. Comparison of the hatchery fish and field populations in terms of genetic variability was simplified by having stocked fish of one origin.

The stream populations of the area possessed a variety of stocking histories, ranging from no stocking to heavy stocking for 30+ years. As a result, hypotheses about the relationships between stocking history and the genetics of field populations were possible.

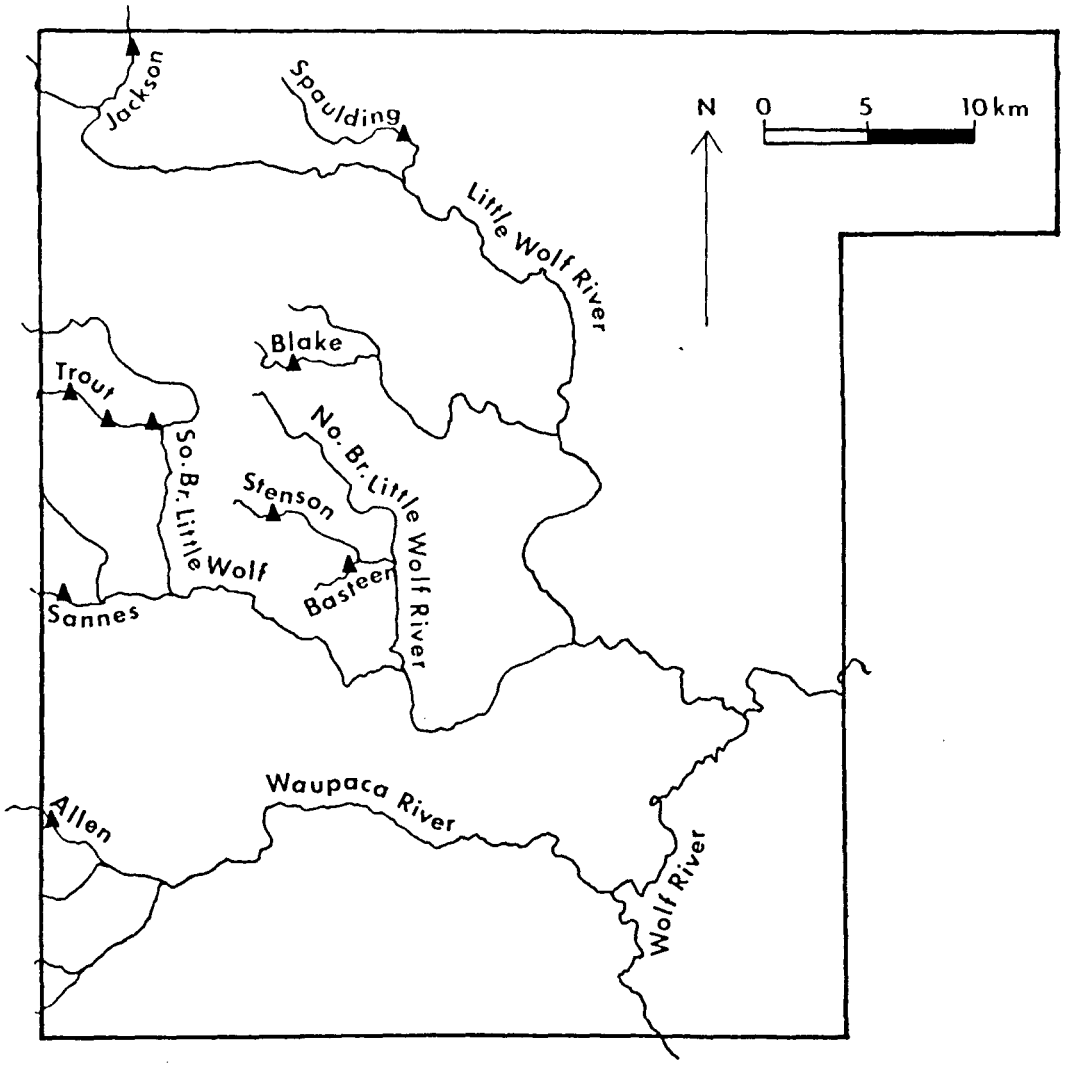
METHODS AND MATERIALS

Brook trout were collected from nine streams and the Osceola hatchery population in Wisconsin. All nine field collections were from the Lake Michigan-Green Bay drainage area. Eight of the field populations sampled are part of the Wolf River drainage in Waupaca County. Of these, seven were from the Little Wolf River drainage and one was from the Waupaca River drainage (Figure 1). One other field population was sampled in the vicinity of Waupaca County. This collection came from Lawrence Creek, Fox River drainage, Marquette Co.

The stream populations were sampled by electrofishing apparatus. A total of 405 brook trout were collected, including 51 fish from the Osceola hatchery. Typically, one sample of 30-40 trout was collected from each stream. To assure that sampled stream fish were not from recent hatchery plantings, only fish below the minimum size stocked (six inches) were collected. To facilitate a test for within-stream genetic variance, collections were made at three different locations in Trout Creek approximately 2 km apart. From field inspection, Trout Creek appeared to be the most likely to show within-stream variation due to its longer length in comparison to other streams sampled. Collections were made near the mouth, in the middle, and near its headwaters.

Selection of the streams was based on known trout stocking histories and the possibility of isolation from surrounding populations. Stocking information pertaining to the sampled streams was recorded from Wisconsin Department of Natural Resources records. Using this data, streams were selected to provide a variety of stocking background. Genetic isolation

Figure 1. Collecting sites (▲) in the Little Wolf and Waupaca River drainages


Jackson
Spaulding
N
0
5
10 km
Little Wolf River
Blake
Trout
No. Br. Little Wolf River
So. Br. Little Wolf
Stenson
Basteer
Sannes
Waupaca River
Allen
Wolf River

of populations due to physical barriers such as waterfalls does not exist in these streams because of their low gradient character. Genetic isolation between populations may, however, result from mill pond dams which occur in the region. The trout streams of this area also often flow directly into warm water streams, which could result in a partial summer isolation of populations due to the intolerance of brook trout for warm water. The study streams either flowed into warm water rivers or had dams that would restrict the movement of brook trout from other waters into the areas sampled.

After capture, the fish were transported live in styrofoam coolers to Hartman Creek State Park, Waupaca County, using aerators and ice. The brook trout were than anesthetized with MS 222, and a blood sample was taken using cardiac puncture and heparinized capillary tubes. The blood was then centrifuged for one minute. Whole brook trout and blood samples were frozen and stored at -20°C.

Whole eyes, used for LDH determinations, were dissected from partially thawed trout just prior to electrophoresis. The eyes were homogenized in 0.5 ml glass distilled water in 10x75 mm glass tubes using a Teflon pestle powered by an electric motor. All steps in the grinding process were cooled by crushed ice baths.

A micro technique of polyacrylamide gel electrophoresis (Balsano and Rasch, 1974) was used to separate plasma transferrin and esterase. Seven and five-tenths percent acrylamide gels were prepared as described by Davis (1964). Electrophoresis of twelve 2 µl samples of trout plasma was conducted at 25 ma for approximately two hours at room temperature.

Horizontal starch gel electrophoresis of whole eye homogenate was used to distinguish lactate dehydrogenase (LDH) phenotypes. Starch gels were used because not all brook trout LDH phenotypes are resolvable in polyacrylamide gels (Wright and Atherton, 1970). Gel preparation has been described by Smithies (1955). Gels were prepared using 13% hydrolysed starch (43 grams per 330 ml buffer) in lucite gel molds (14x22x 0.6 cm). A tris-boric acid-EDTA buffer was used for both the gel and electrode chambers (Markert and Faulhaber, 1965). Up to twenty samples were applied to each gel using filter paper rectangles 4x6 mm. Electrophoresis was for 18 hours at 350 volts at 20-25 ma. The gels were placed inside a refrigerator during electrophoresis and cooled to 4°C.

General protein banding was visible after staining the acrylamide gels in 0.1% (by weight) Coomassie Brilliant Blue R (Mann Research Labs, New York) in a 25.0% methanol, 7.0% acetic acid solution for one to two hours at room temperature. The gels were then rinsed in 7.0% acetic acid solution overnight to allow the protein pattern to be revealed. The transferrin (Tf) bands were clearly distinguishable in the general protein pattern. A technique staining specifically for Tf was also employed for positive identification (Menzel, 1970). This staining procedure involves the precipitation of the noniron binding protein fractions in the plasma due to their insolubility in rivanol (2-ethoxy-6, 9-diaminoacridine lactate; K and K Labs, Plainview, N.Y.). Five µl of 0.15% ferric ammonium citrate were added to 5 µl of plasma, before electrophoresis, to saturate the ferric sites on the molecules of transferrin. After mixing, 10 µl of 0.4% rivanol were added and stirred into the plasma solution. A 5 µl

sample of the resulting supernatant was then added to 0.05 ml large pore gel solution and subjected to electrophoresis. The gel was then stained as before in Coomassie Blue and the Tf bands were revealed.

Plasma esterase activity in acrylamide gels was detected by staining in a solution containing alpha-naphthyl acetate (1.0 ml of 1.0 g/100 ml acetone), 20.0 mg Diazo Blue B and 10 ml of tris-maleate buffer, pH 7.0 (Menzel, 1970). After incubating for several minutes in this mixture at room temperature, the gels were rinsed in water and the esterase phenotypes were visualized.

Lactate dehydrogenase (LDH) phenotypes were revealed by staining the starch gels in the method of Morrison and Wright (1966):

20 mg - p-nitro blue tetrazolium chloride

10 mg - phenazine methosulfate

30 mg - nicotinamide adenine dinucleotide

20 ml - dl-lactic acid lithium salt (0.5 M)

80 ml - tris-HCl buffer (0.1 M, pH 8.3)

The gels were incubated at 27°C for 4 hours. When staining was complete, the gels were fixed in a solution of methanol:H_2O:acetic acid (5:5:2). After becoming firm they were wrapped in clear plastic wrap.

Both polyacrylamide and starch gels were photographed using a twin lens reflex camera (Rolleiflex) equipped with a yellow filter and close up lens. Kodak Plus-X Pan Professional film in 2 1/4 x 2 1/4 inch negative size was used. Exposure for polyacrylamide gels was f8 at 1/30 second. Starch gel exposures varied at f5.6 or f8 at 1/15 second depending on the darkness of the gel. All gels were illuminated by a fluorescent light box.

Tests for statistical homogeneity in the phenotypic data utlized X^2 in contingency tables (Snedecor and Cochran, 1967, p. 250). This method is used in making a comparison of phenotypic frequencies between groups. Large X^2 values indicate differences in the frequencies. The p value expressed is the probability that the X^2 would be larger in another random sample, if the frequencies were the same between the groups. OMNITAB computer programs were written to perform the actual computations in homogeneity testing and Hardy-Weinberg equilibrium analysis (Appendix A and B). Cluster analyses of the unweighted pair group method (McCannon and Wenniger, 1970) were performed with the assistance of the Statistics Department, Iowa State University.

RESULTS

Stream Stocking Data

Records used for stocking characterizations of the streams spanned a 39 year period from 1937-1975. Year of stocking, species, size (catchable or fingerling), and number stocked were obtained for each stream. With this information, stocking pressure was expressed in a variety of ways such as total number and years stocked (Table 1). Sums of types of trout stocked, according to species and size, were divided by stream length (km) to yield the number stocked per kilometer. The recentness of stocking was also inspected for relationships with trout genetics.

The streams sampled ranged from no stocking (Allen Creek) to 35 years of stocking (Blake Creek). Only five of the nine streams have received plants of catchable brook trout. All of the streams except Allen Creek have had fingerling or catchable brook trout stocking. Other trout species (predominantly brown trout) were also released into five of the nine streams (Basteen, Trout, Lawrence, Sannes and Blake creeks). Stream stocking rank, as determined by these parameters, shifted considerably depending on the measure used.

Electrophoretic Results

Genetic polymorphism was observed in two of the three protein systems examined. Plasma esterase proved to be monomorphic among the trout sampled, a finding which is consistent with reports on other brook trout populations (Nyman, 1967, 1972). A single banded pattern was observed for

Table 1. Stocking data and indices of field populations

Stream	Trout water (km)	Years of stocking	Catchable brook trout	Total brook trout[a]	Catchable brook trout per kilometer	Total brook trout per kilometer
Allen	1.9	0	0	0	0	0
Stenson	5.5	6	0	6900	0	1261
Basteen	1.3	8	0	3220	0	2496
Trout	7.7	12	0	11200	0	1449
Jackson	7.6	13	10769	45189	1423	5969
Lawrence	4.4	8	5403	53745	1222	12159
Spaulding	12.3	33	23756	102353	1928	8308
Sannes	3.5	29	11230	15030	3246	4344
Blake	8.1	35	20300	24550	2522	3050

[a]Includes catchables and fingerlings.

[b]Predominantly brown trout.

[c]Includes all species stocked.

Catchable other trout[b]	Total other trout[a]	Catchable other trout per kilometer	Total other trout per kilometer	Total catchable trout per kilometer[c]	Total trout per kilometer[c]
0	0	0	0	0	0
0	0	0	0	0	1261
770	34888	597	27045	597	29541
8870	22070	1147	2855	1147	4304
0	0	0	0	1423	5969
1857	26857	420	6076	1642	18236
0	0	0	0	1928	8308
660	1860	191	538	3436	4881
30230	64335	3755	7992	6277	11042

each fish. Esterase data, therefore, were not useful in describing differences between populations. Transferrin (Tf) and lactate dehydrogenase (LDH), however, displayed variability in banding patterns among individuals.

Transferrin polymorphism

Six transferrin banding patterns were observed (Figure 2). Identical banding patterns for hatchery brook trout Tf have been described by Hoffman (1966) and Hershberger (1970). Hoffman (1966) demonstrated through breeding experiments that brook trout transferrin banding is controlled by three alleles (A,B,C) at one locus. Her allelic designations have been followed here. The patterns observed are the phenotypic expressions of the six possible combinations of the three alleles. As shown in Figure 2, two protein staining fractions were produced by one allele (Tf^B or Tf^C). Hershberger (1970) treated brook trout transferrins with neuraminidase and demonstrated that this multiple banding is due to differing numbers of sialic acid residues attached to the protein molecule. The multiple banding pattern, therefore, is probably the result of molecular degradation due to electrophoresis.

Each brook trout was scored according to its transferrin phenotype. On the basis of the genetic hypothesis given above, allelic frequencies were calculated for each of the collections, and X^2 tests were performed to test for random union among gametes according to the Hardy-Weinberg Law (Table 2). A good fit with Hardy-Weinberg expectations was shown for all populations except Lawrence Creek. The excess BC phenotype in the Lawrence Creek sample chiefly contributed to the large X^2 value ($p>.05$).

Figure 2. Tf banding patterns and phenotypic designations

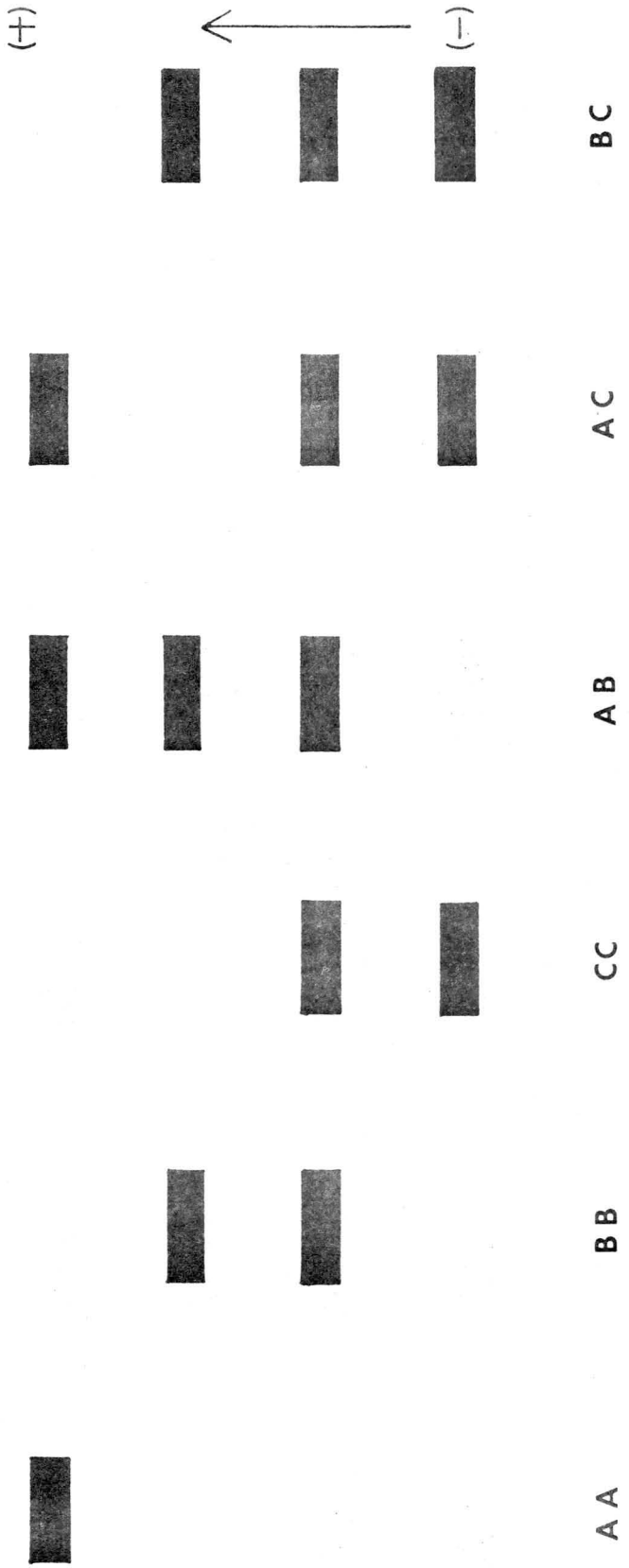

(+)
(−)
BC
AC
AB
CC
BB
AA

Table 2. Transferrin phenotypes and allelic frequencies with H-W equilibrium probability values

Population		Phenotypes							P of a larger X^2	Allelic frequency		
		AA	AB	AC	BB	BC	CC	Total		A	B	C
Allen	Observed	0	0	0	0	0	20	20	>.99	0	0	1.0
	Expected	0	0	0	0	0	20					
Stenson	Observed	0	4	2	19	11	0	36	>.40	.08	.74	.18
	Expected	.25	4.4	1.1	19.5	9.5	1.2					
Basteen	Observed	0	11	4	6	9	5	35	>.20	.21	.46	.33
	Expected	1.6	6.7	4.9	7.3	10.5	3.8					
Trout I	Observed	1	2	3	1	8	14	29	>.50	.12	.21	.67
	Expected	.42	1.4	4.7	1.2	8.1	13.1					
Trout II	Observed	1	1	9	0	5	13	29	>.90	.21	.10	.69
	Expected	1.2	1.2	8.3	.31	4.1	13.8					
Trout III	Observed	1	1	5	2	5	14	28	>.50	.14	.18	.68
	Expected	.57	1.4	5.4	.89	6.8	12.9					
Jackson	Observed	0	3	7	2	10	18	40	>.75	.12	.21	.66
	Expected	.6	2.1	6.6	1.8	11.3	17.6					
Lawrence	Observed	0	1	1	4	20	7	33	>.05	.03	.44	.53
	Expected	.03	.86	1.1	6.4	15.3	9.3					
Spaulding	Observed	1	2	10	2	9	11	35	>.75	.20	.21	.59
	Expected	1.4	3.0	8.2	1.6	8.8	12.0					
Sannes	Observed	2	3	7	2	4	13	31	>.30	.23	.18	.60
	Expected	1.6	2.5	8.4	.97	6.6	11.0					
Blake	Observed	0	2	7	5	16	8	38	>.25	.12	.37	.51
	Expected	.51	3.3	4.6	5.1	14.4	10.0					
Osceola Hatchery	Observed	0	0	0	24	20	7	51	>.44	0	.67	.33
	Expected	0	0	0	22.9	22.4	5.6					

Lactate dehydrogenase polymorphism

Six lactate dehydrogenase phenotypes were observed in eye homogenate (Figure 3). These are identical to those reported for eastern populations by Wright and Atherton (1970). The banding patterns observed are the expression of codominant alleles at the LDH-A, LDH-B and LDH-C loci which code for tetrameric LDH molecules (Morrison and Wright, 1966). Although the LDH-A and LDH-C loci were monomorphic in this study, allelic variants for these loci have been reported by Wright and Atherton (1970). The LDH-B locus was polymorphic for three alleles in Wisconsin brook trout. Wright and Atherton also found three alleles among eastern populations and determined genetic inheritance for the LDH-B locus. Their designation of the alleles as B, B', and B" has been adopted here.

Phenotypic frequencies for each population were tested for adherence to Hardy-Weinberg Equilibrium (Table 3). While most of the populations were similar to Hardy-Weinberg expectations, Basteen, Trout II and Blake were exceptions. Lack of fit in the Trout II sample is due to a statistical artifact caused by the presence of expected values less than one for rare phenotypes B"B", B'B", and B'B'. Both Basteen and Blake creeks contain an excess of heterozygotes BB' and B'B" respectively.

Comparison of hatchery and field collections

X^2 contingency tables based upon phenotypic frequencies at the Tf and LDH-B loci were used to compare the hatchery sample with each of the stream collections. At both loci, the Osceola population exhibited reduced genetic variability in comparison to wild populations (Tables 2 and 3).

Figure 3. LDH banding patterns, phenotypic designations, and tetrameric construction

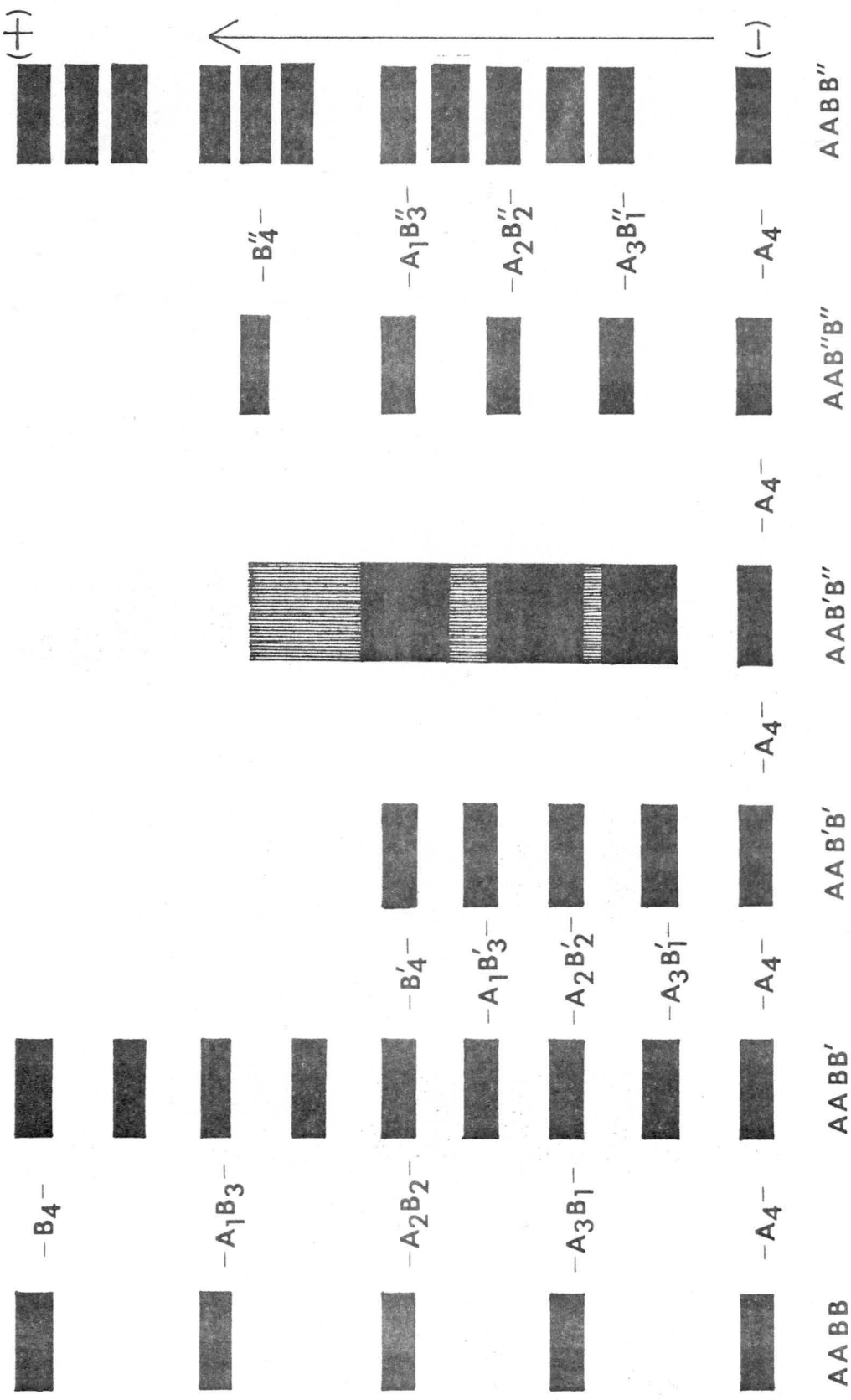

(+)
(−)
$-B_4-$
$-A_1B_3-$
$-A_2B_2-$
$-A_3B_1-$
$-A_4-$
AABB
AABB′
AAB′B′
AAB′B″
AAB″B″
AABB″
$-B'_4-$
$-A_1B'_3-$
$-A_2B'_2-$
$-A_3B'_1-$
$-B''_4-$
$-A_1B''_3-$
$-A_2B''_2-$
$-A_3B''_1-$

Table 3. LDH-B phenotypes and allelic frequencies with H-W equilibrium probability values

Population		B"B"	B'B"	B B"	B'B'	B B'	B B	To-tal	P of a larger X^2	Allelic frequency B"	B'	B
Allen	Observed	0	0	0	0	0	20	20	>.99	0	0	1.0
	Expected	0	0	0	0	0	20					
Stenson	Observed	2	0	14	0	1	19	36	>.75	.25	.01	.74
	Expected	2.25	.25	13.2	.007	.74	19.5					
Basteen	Observed	0	0	5	0	17	13	35	>.02	.07	.24	.69
	Expected	.18	1.2	3.4	2.1	11.7	16.6					
Trout I	Observed	0	0	1	2	8	18	29	>.75	.02	.21	.78
	Expected	.009	.21	.78	1.2	9.3	17.5					
Trout II	Observed	0	1	2	0	1	25	29	>.06	.05	.04	.91
	Expected	.08	.10	2.7	.03	1.8	24.2					
Trout III	Observed	0	0	4	0	3	21	28	>.45	.07	.05	.87
	Expected	.14	.21	3.5	.08	2.6	21.4					
Jackson	Observed	0	1	5	1	15	18	40	>.48	.07	.23	.70
	Expected	.22	1.35	4.2	2.0	12.6	19.6					
Lawrence	Observed	0	1	3	2	17	10	33	>.30	.06	.33	.61
	Expected	.12	1.3	2.4	3.7	13.3	12.1					
Spaulding	Observed	0	1	4	3	12	15	35	>.80	.07	.27	.66
	Expected	.18	1.4	3.3	2.6	12.5	15.1					
Sannes	Observed	0	0	0	2	6	24	32	>.10	0	.16	.84
	Expected	0	0	0	.78	8.4	22.8					
Blake	Observed	0	5	0	8	15	10	38	>.07	.07	.48	.46
	Expected	.16	2.4	2.3	8.5	16.6	8.1					
Osceola	Observed	0	0	0	4	20	27	51	>.99	0	.27	.73
	Expected	0	0	0	3.8	20.3	26.8					

At the transferrin locus, all three Tf alleles were usually present in moderate frequencies with the Tf^C most common (Table 2). In contrast, among the hatchery population Tf^B was common and Tf^A was absent. Comparisons of phenotypic frequencies of the Osceola hatchery sample with those of the field collections revealed statistically significant heterogeneity in every case (Table 4).

Table 4. X^2 homogeneity tests with the Osceola hatchery population using Tf phenotypic frequencies

Stream collection	X^2	d.f.	P of a larger value
Allen	45.4	2	<.005
Stenson	14.0	3	<.005
Basteen	28.3	4	>.005
Trout I	30.9	5	>.005
Trout II	43.0	5	<.005
Trout III	33.0	5	<.005
Jackson	36.0	4	>.005
Lawrence	7.3	2	>.025
Spaulding	34.9	4	>.005
Sannes	40.6	4	>.005
Blake	20.5	3	<.005

At the LDH-B locus the B, B' and B" alleles were present in all of the field populations with the exception of Allen and Sannes creeks (Table 3). In most collections LDH^B was most common and $LDH^{B"}$ was rare. In the

hatchery population LDH^{B} was also most common, however $LDH^{B''}$ was entirely absent. Phenotypic comparisons revealed the Osceola population to be most similar to the Trout I and Sannes collections (Table 5).

Table 5. X^2 homogeneity tests with the Osceola hatchery population using LDH-B phenotypic frequencies

Stream collection	X^2	d.f.	P of a larger value
Allen	14.2	2	<.005
Stenson	37.1	4	<.005
Basteen	11.6	3	>.005
Trout I	2.8	3	>.40
Trout II	19.7	4	<.005
Trout III	16.0	3	<.005
Jackson	9.1	3	>.05
Lawrence	9.3	4	>.05
Spaulding	7.9	3	>.05
Sannes	4.2	2	>.13
Blake	13.2	3	<.005

Within stream variation

Subsamples on Trout Creek were made to determine within stream breeding structure. The three collections were similar in Tf allelic frequencies (Table 2, Figure 4). A slightly higher Tf^{A} frequency occurred at site II than at either I or III. Statistically, however, evidence for similarity between the samples at the Tf locus was strong (Table 6).

Figure 4. Tf^A, Tf^B, Tf^C allelic frequencies for brook trout collections I, II, and III from Trout Creek

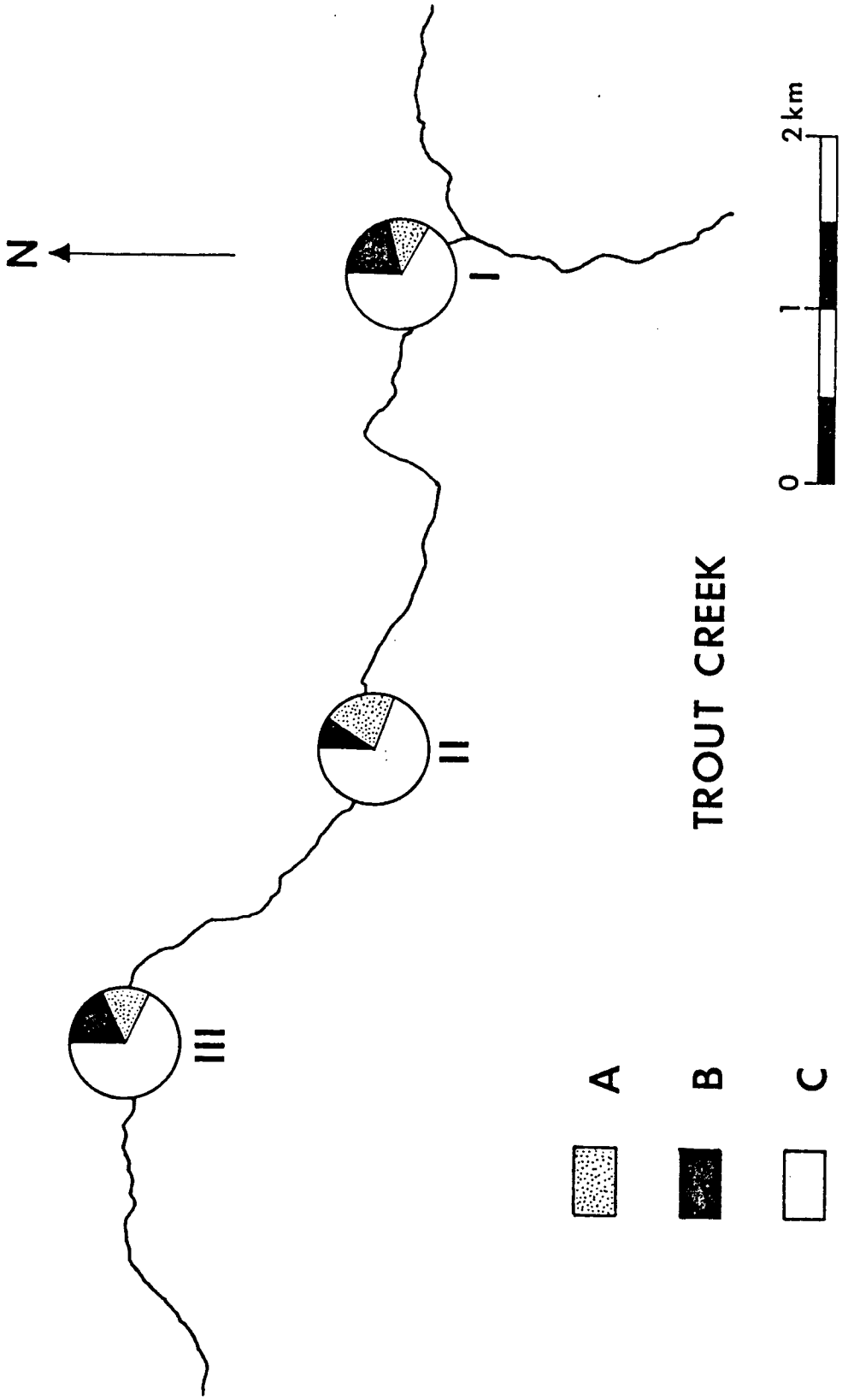

N
I
II
III
TROUT CREEK
0
1
2 km
A
B
C

Table 6. Tf and LDH-B loci homogeneity comparisons of Trout Creek collections using X^2

Comparison	Tf locus			LDH-B locus		
	X^2	d.f.	P of a larger value	X^2	d.f.	P of a larger value
Trout I Trout II Trout III	5.8	8	>.67	15.5	8	<.05
Trout I Trout II	5.1	4	>.25	9.9	4	<.05
Trout I Trout III	1.8	5	>.85	6.3	3	>.09
Trout II Trout III	3.2	5	>.65	3.0	3	>.40
Trout I Trout II & III	3.4	5	>.60	9.4	3	<.025

At the LDH-B locus allelic frequencies varied markedly between collections (Table 3, Figure 5). The site I collection had a much higher B' and a lower B allelic frequency than either II or III. Significant heterogeneity existed in a comparison of the three collections simultaneously ($p<.05$, Table 6). Partitioning of this X^2 value demonstrated that Trout I caused the heterogeneity, while II and III were a homogeneous subset ($p>.40$). The Trout II and III collections combined were considered representative of a distinct population from Trout I.

Between stream variation

The distinctiveness of each stream collection was tested by X^2 comparison of Tf and LDH-B phenotypic frequencies. The matrices in Tables 7 and 8 show paired comparisons for all possible combinations. In the 45

Figure 5. LDH-B, LDH-B', LDH-B" allelic frequencies brook trout collections I, II, and III from Trout Creek

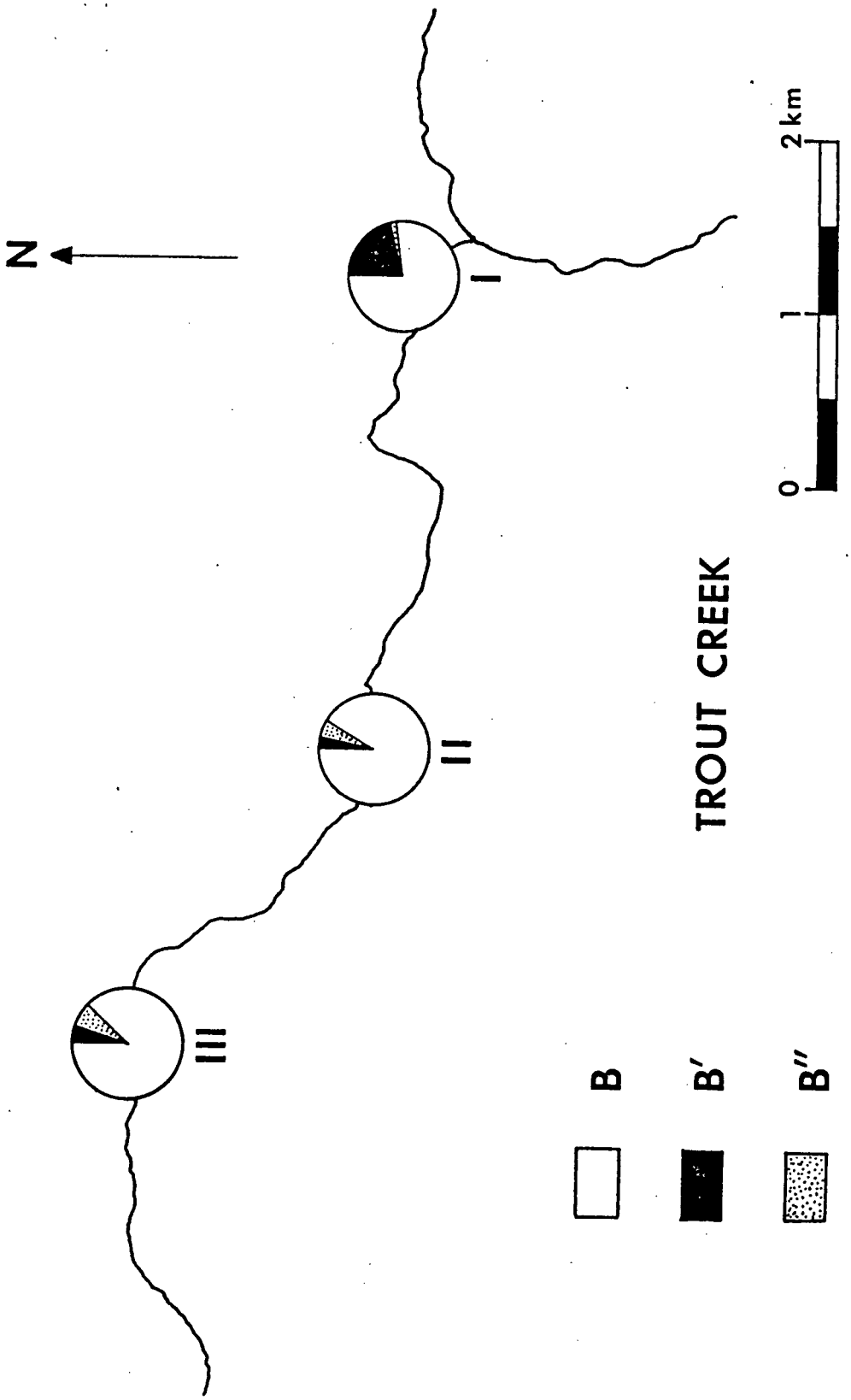

N
I
II
III
TROUT CREEK
0
1
2 km
B
B′
B″

Table 7. X^2 values (first row) and probability values (second row) for homogeneity hypothesis at the Tf locus in stream to stream comparisons

	Allen	Stenson	Basteen	Trout I	Trout II & III	Jackson	Lawrence	Spaulding	Sannes	Blake
Allen	-									
Stenson	56.0 <.005	-								
Basteen	34.7 <.005	15.9 <.005	-							
Trout I	14.9 <.025	32.2 <.005	14.8 <.01	-						
Trout II & III	17.2 <.005	50.3 <.005	27.3 <.005	3.4 >.60	-					
Jackson	17.4 <.005	34.6 <.005	14.5 <.01	2.2 >.75	3.5 >.50	-				
Lawrence	30.9 <.005	21.4 <.005	15.0 <.005	11.4 <.05	24.7 <.005	13.8 <.01	-			
Spaulding	24.3 <.005	31.9 <.005	12.1 <.025	4.0 >.50	2.7 >.70	1.9 >.70	14.4 <.025	-		
Sannes	17.9 <.005	34.8 <.005	11.1 <.025	3.8 >.50	2.5 >.75	2.8 >.50	20.6 <.005	2.9 >.50	-	
Blake	32.7 <.005	20.5 <.005	9.7 <.05	8.5 >.10	14.1 >.025	6.7 >.10	5.1 >.25	4.3 >.35	10.4 <.05	-

Table 8. X^2 values (first row) and probability values (second row) for homogeneity hypothesis at the LDH-B locus in stream to stream comparisons

	Allen	Stenson	Basteen	Trout I	Trout II & III	Jackson	Lawrence	Spaulding	Sannes	Blake
Allen	-									
Stenson	13.6 <.005	-								
Basteen	20.9 <.005	21.6 <.005	-							
Trout I	9.7 <.025	20.2 <.005	8.2 <.05	-						
Trout II & III	4.5 >.20	15.2 <.005	23.7 <.005	9.4 <.025	-					
Jackson	17.4 <.005	20.4 <.005	2.6 >.45	4.5 >.30	17.3 <.005	-				
Lawrence	24.6 <.005	29.1 <.005	3.8 >.40	7.3 >.10	29.9 <.005	2.6 >.50	-			
Spaulding	18.0 <.005	21.3 <.005	5.1 >.10	3.5 >.45	19.0 <.005	1.1 >.75	2.1 >.50	-		
Sannes	5.9 <.025	22.0 <.005	15.4 <.005	1.2 >.50	10.1 <.05	8.9 <.05	15.0 <.005	8.6 <.05	-	
Blake	28.5 <.005	44.0 <.005	18.4 <.005	13.0 <.025	44.1 <.005	15.4 <.005	9.1 >.05	10.2 <.05	17.8 <.005	-

comparisons, 14 were statistically homogeneous ($p>.05$) at the Tf locus. For the same comparisons at the LDH-B locus, 12 were homogeneous. Comparing Tables 7 and 8, only 6 comparisons in 45 were statistically similar with respect to both Tf and LDH. These comparisons were Trout I vs. Jackson, Trout I vs. Sannes, Trout I vs. Spaulding, Spaulding vs. Jackson and Blake vs. Lawrence.

Identification and genetic relationships of homogeneous groups

Two methods of analysis were used to identify similar populations with respect to either the LDH-B or Tf loci. First, cluster analysis was applied to each locus X^2 matrix in Tables 7 and 8. Second, to statistically confirm homogeneous relationships depicted by the cluster analysis, X^2 contingency tables were used. Statistically similar populations were then inspected for other common attributes.

Identification of similar populations Cluster analysis constructed a group consisting of Sannes, Spaulding, Jackson, Trout I, and Trout II & III which were statistically similar at the Tf locus ($p>.90$, Figure 6). The homogeneity of this core group was somewhat reduced by the addition of Blake Creek ($p>.25$). The addition of any other stream population substantially decreased homogeneity ($p<.05$). As indicated previously, Blake and Lawrence creeks are statistically similar at the Tf locus ($p>.25$, Table 7), however this relationship is not depicted by Figure 6.

At the LDH-B locus three population assemblages were identified (Figure 7). Jackson, Spaulding, Lawrence and Basteen Creeks formed the largest group ($p>.75$). The Osceola hatchery, Trout I, and Sannes collections formed another homogeneous subset ($p>.25$). The third group con-

Figure 6. Dendrogram based on cluster analysis of Tf X^2 values from population comparisons; X^2 homogeneity test probability values given for groups

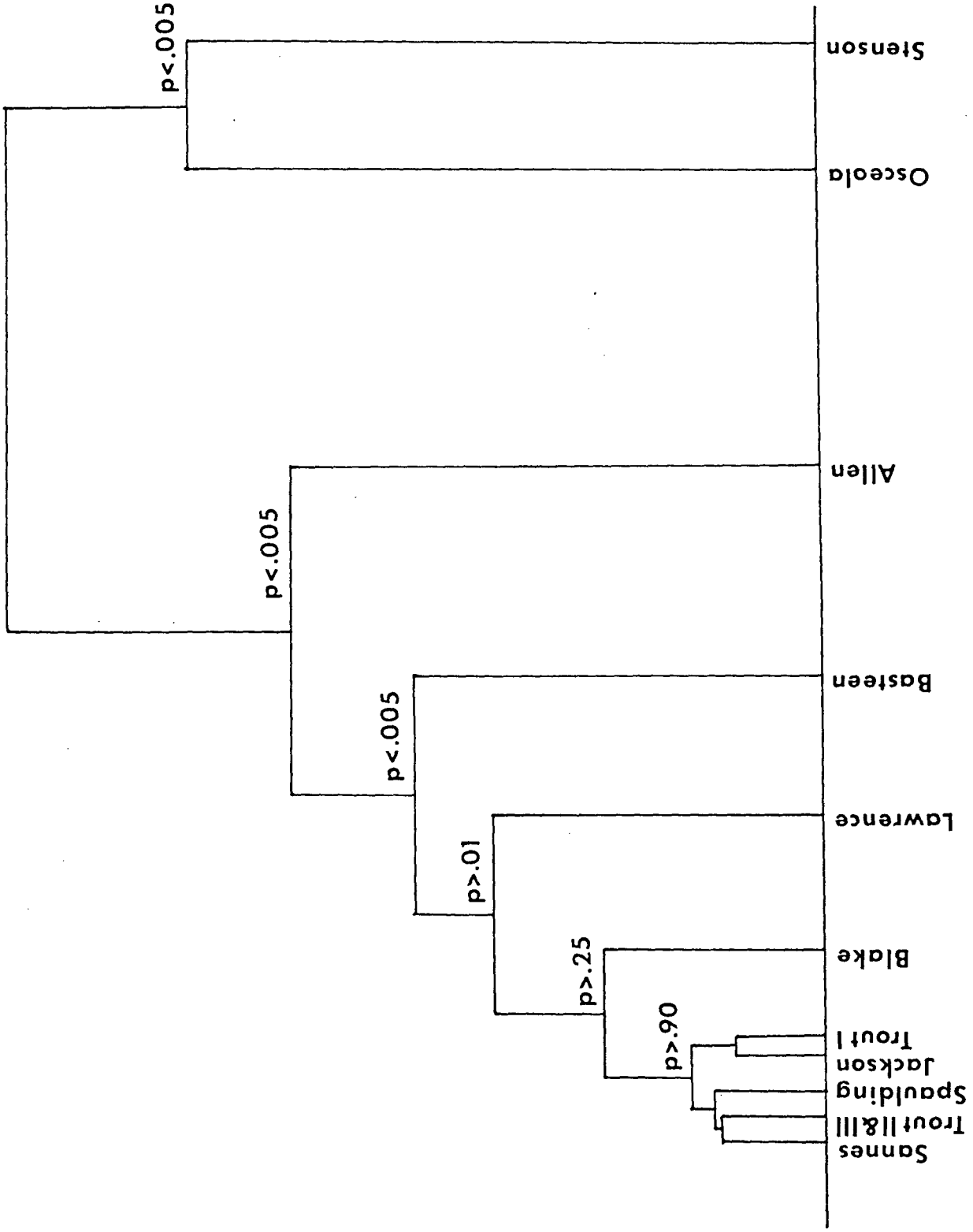
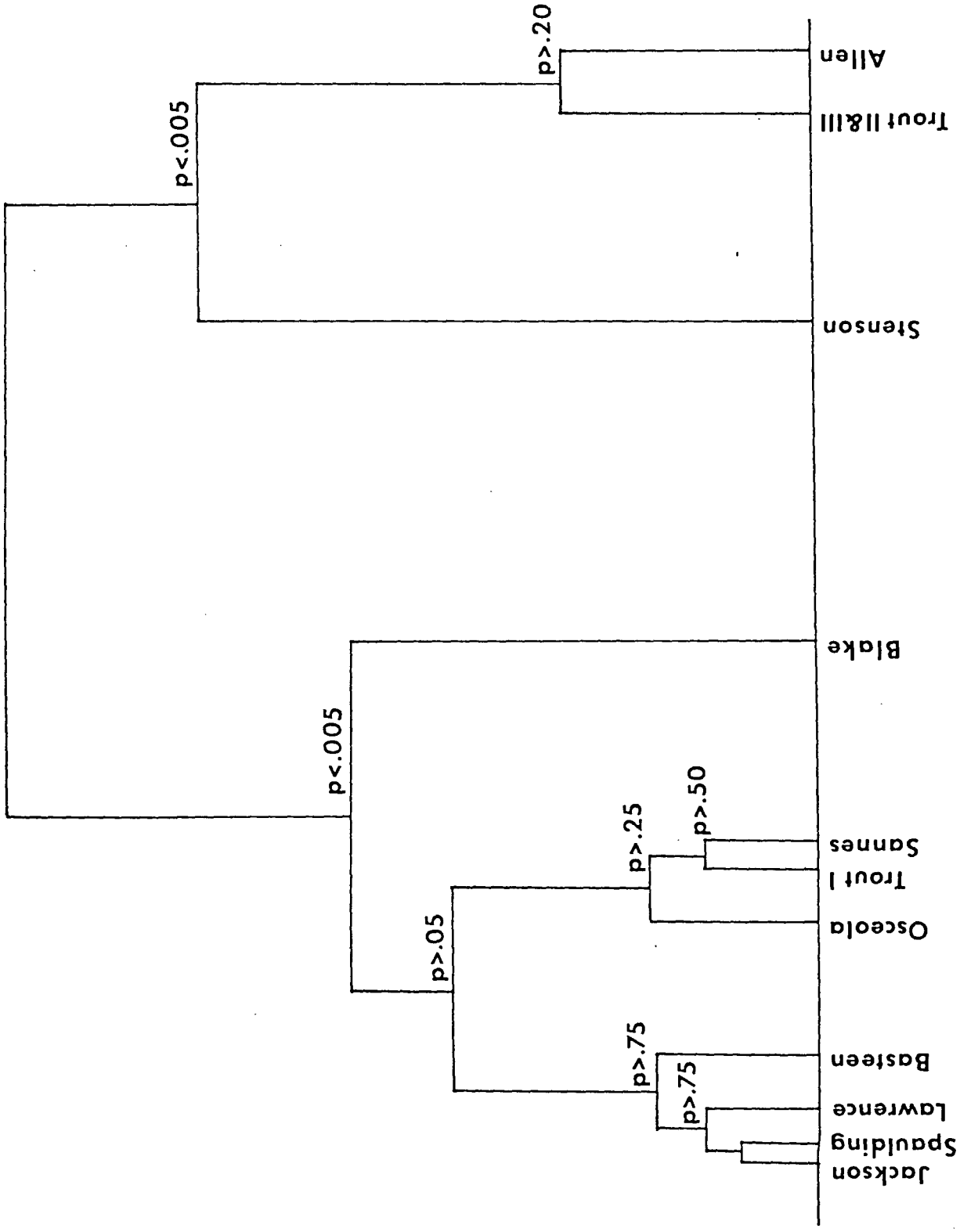

Stenson
Osceola
Allen
Bastеen
Lawrence
Blake
Trout I
Jackson
Spaulding
Trout II&III
Sannes
p<.005
p<.005
p<.005
p>.01
p>.25
p>.90

Figure 7. Dendrogram based on cluster analysis of LDH X^2 values from population comparisons; X^2 homogeneity test probability values given for groups


Allen
Trout II&III
Stenson
Blake
Sannes
Trout I
Osceola
Basteen
Lawrence
Spaulding
Jackson
p>.20
p<.005
p<.005
p>.50
p>.25
p>.05
p>.75
p>.75

consisted of Trout II & III, and Allen ($p>.20$). Weak evidence for statistical similarity between the first two groups at the LDH-B locus existed ($p>.05$). Additional X^2 tests were performed to determine the effects of the addition of the Osceola, Trout I, and Sannes data, singularly, to the first group of Jackson, Spaulding, Lawrence, and Basteen Creeks (Table 9). The addition of each of these populations to this group statistically retained homogeneity ($p>.05$), however Trout I and the Osceola hatchery fish were more similar to the first group than Sannes. Using the LDH-B locus information, the largest set of similar stream populations was Jackson, Spaulding, Lawrence, Basteen, Trout I and Sannes ($p>.10$, Table 9). The addition of any other stream population to this group reduced X^2 homogeneity significantly ($p<.005$).

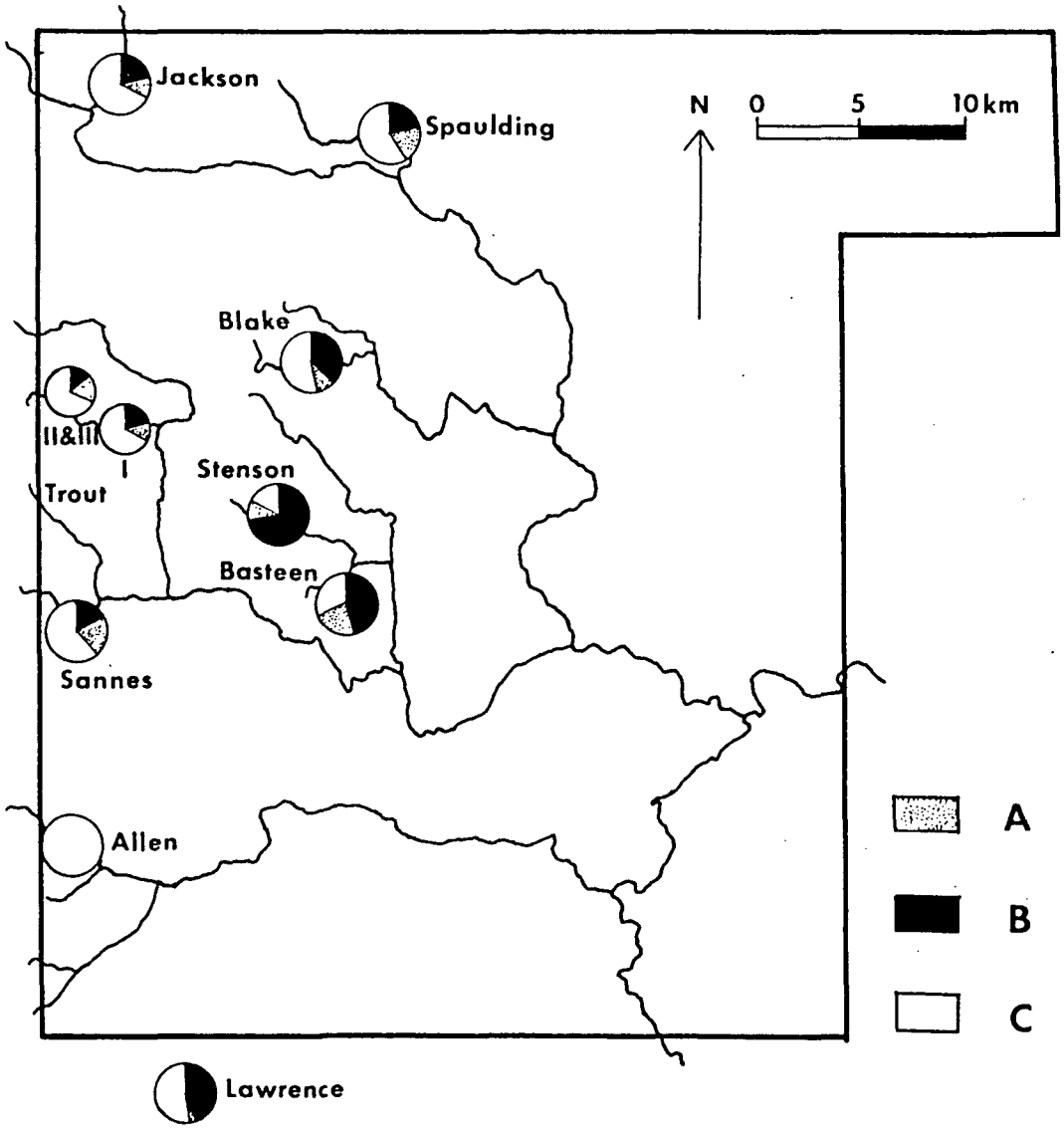
Relationships between similar populations Populations determined to be homogeneous at the Tf or LDH-B locus (Figure 6 or 7) were examined for similarities in environmental factors. Among factors such as stream length, or qualitative observations such as bank vegetation, trout abundance, trout species present or bottom substrate, none were found common between these populations.

Examination of drainage relationships revealed that the populations homogeneous at the Tf locus were all located in the Little Wolf River drainage (Figure 8). Within this drainage these populations tended to occupy upstream positions with respect to the other populations. Blake Creek, located in the most downstream position relative to the other populations in the group, was the most statistically divergent. In general, populations in downstream locations in this drainage (Blake,

Table 9. LDH-B locus X^2 homogeneity tests

Group	X^2	d.f.	P of a larger X^2
Jackson Spaulding Lawrence Basteen Osceola	17.5	16	p>.25
Jackson Spaulding Lawrence Basteen Trout I	14.7	16	p>.50
Jackson Spaulding Lawrence Basteen Sannes	23.9	16	p>.05
Jackson Spaulding Lawrence Basteen Trout I Sannes	28.3	20	p>.10
Jackson Spaulding Lawrence Basteen Trout I Sannes Blake	58.6	24	p<.005

Figure 8. Waupaca County field collection locations and Tf^A, Tf^B, Tf^C allelic frequencies; Lawrence Creek included for comparison purposes


Jackson
Spaulding
N
0
5
10km
Blake
II&III
I
Trout
Stenson
Basteen
Sannes
Allen
A
B
C
Lawrence

Basteen, Stenson) had higher Tf^B allelic frequencies than upstream populations (Figure 8). Lawrence Creek, located in the Fox River drainage was statistically different from all populations except Blake Creek (Table 7). Allen Creek, located in the Waupaca River drainage was unique in being monomorphic for Tf^C. Examination of the stocking history of the various populations suggests that no relationship exists with the Tf locus.

Little association between LDH-B locus similarity and drainage position existed (Figure 9). Although some populations (Trout I and Sannes; Jackson and Spaulding) are similar at this locus and possess a similar drainage position, divergence between geographically proximate populations at the LDH-B locus was also apparent (Figure 9). The drainage position relationships at the Tf locus in the populations were not apparent at the LDH-B locus.

The stocking information in Table 1 was used to inspect for relationships with LDH population genetics. A survey of the indices revealed a possible relationship between total catchable trout stocked per stream kilometer (includes all species) and the LDH data. Basteen, Trout, Jackson, Lawrence and Spaulding creeks were similar in this stocking index (Figure 10). This same group (excepting Trout II & III) also had similar allelic frequencies for the LDH-B locus (Figure 11, 12, 13) and formed a statistically homogeneous set ($p>.50$, Table 9). The addition of Sannes Creek, which possesses a somewhat different stocking history (Figure 10) to this group reduced the homogeneity ($p>.10$, Table 9). Blake, Allen, and Stenson creeks were at the extreme range of the index (Figure 10) and differed significantly ($p<.005$) at the LDH-B locus from this group (Figure

Figure 9. Waupaca County field collection locations and LDH-B, LDH-B', LDH-B" allelic frequencies; Lawrence Creek included for comparison purposes

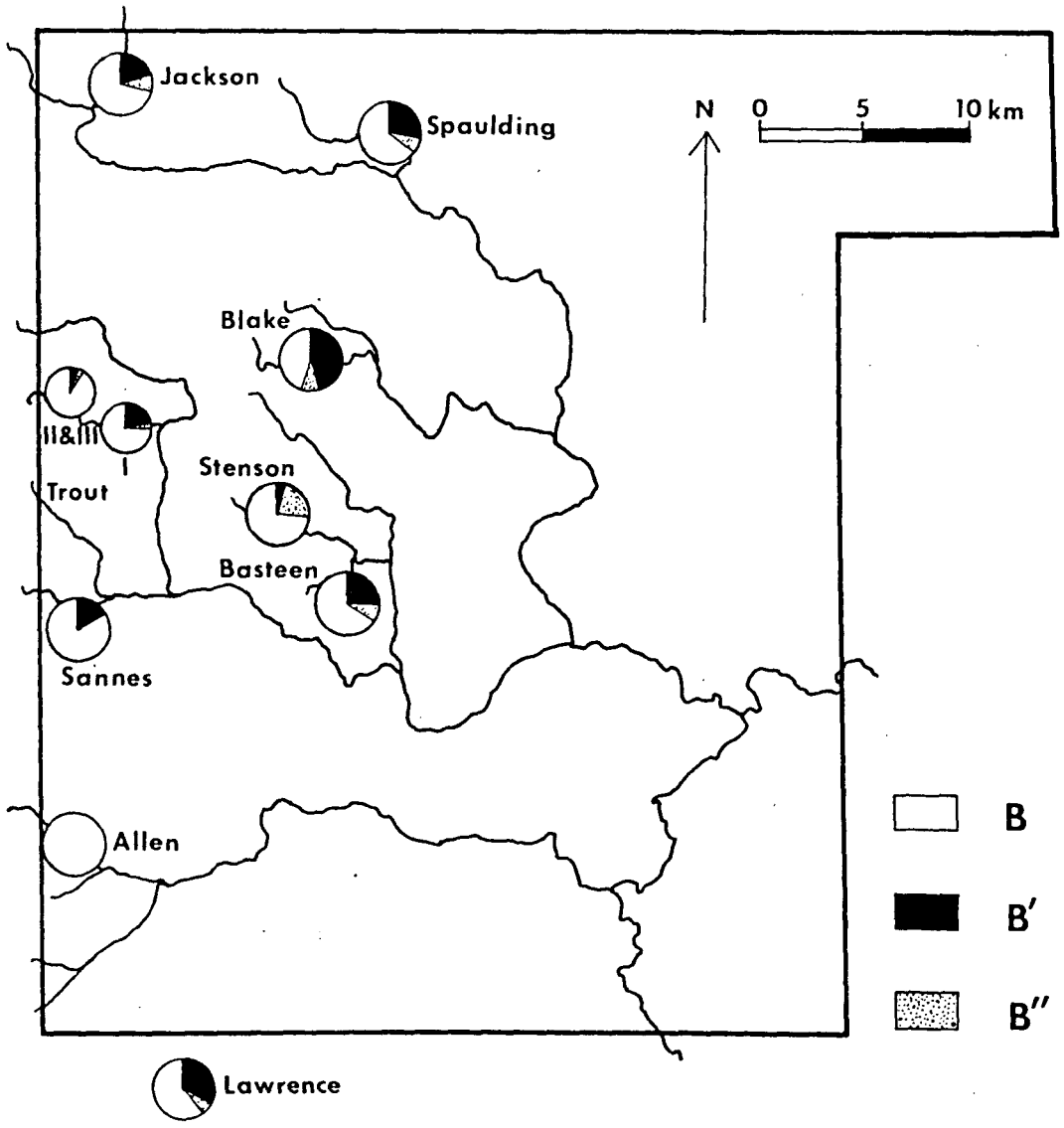

Jackson
Spaulding
N
0
5
10 km
Blake
II&III
I
Trout
Stenson
Basteen
Sannes
Allen
Lawrence
B
B′
B″

Figure 10. Stream stocking histories expressed as the number of catchable trout stocked per stream kilometer

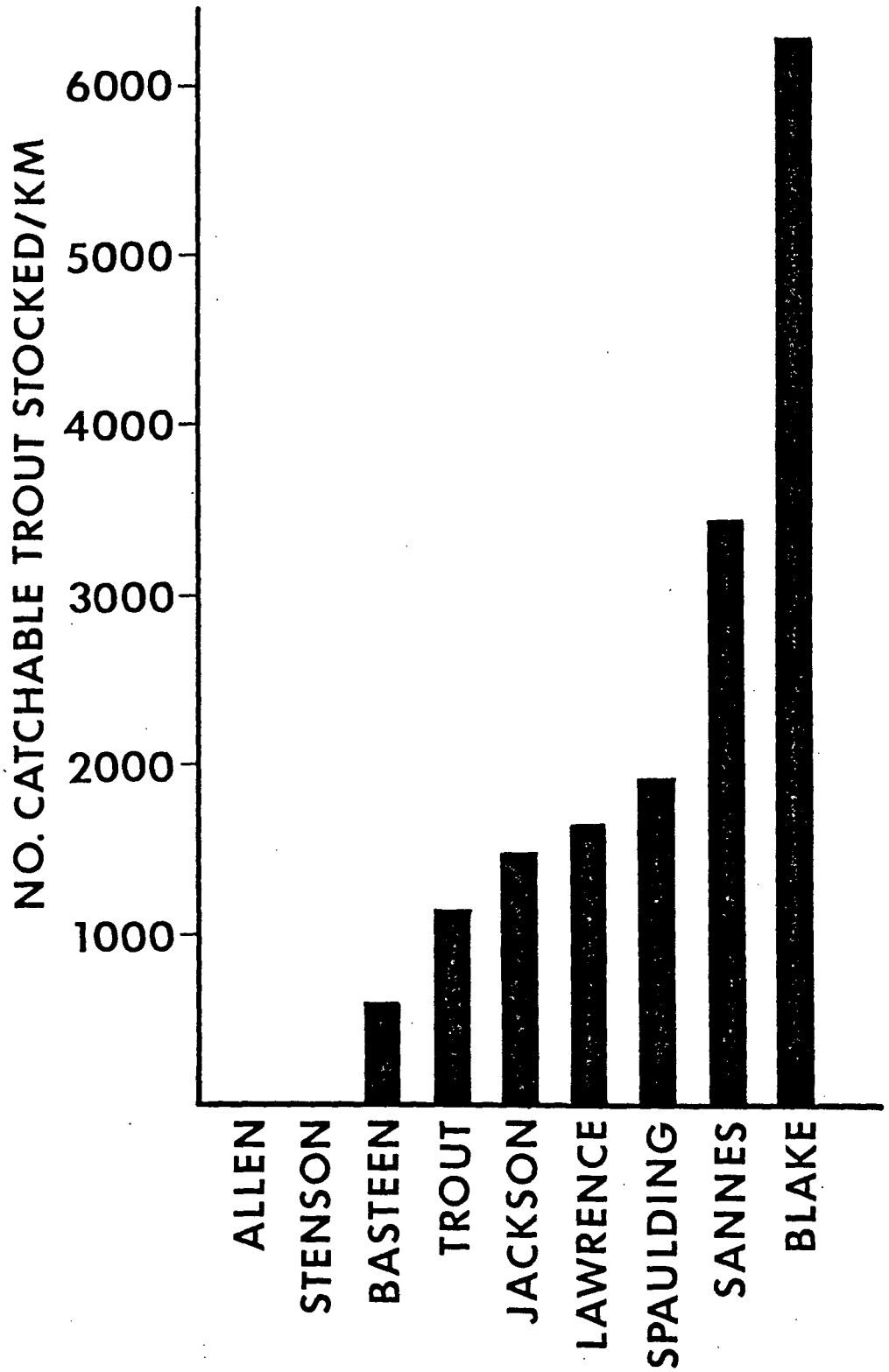
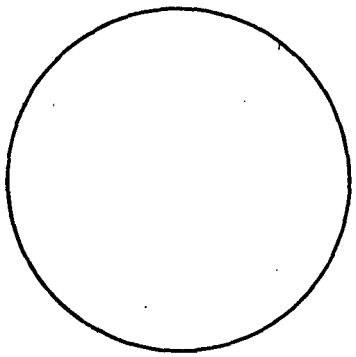
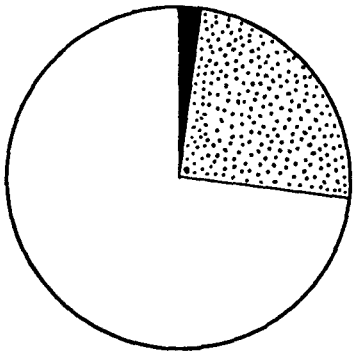

NO. CATCHABLE TROUT STOCKED/KM
6000
5000
4000
3000
2000
1000
ALLEN
STENSON
BASTEEN
TROUT
JACKSON
LAWRENCE
SPAULDING
SANNES
BLAKE

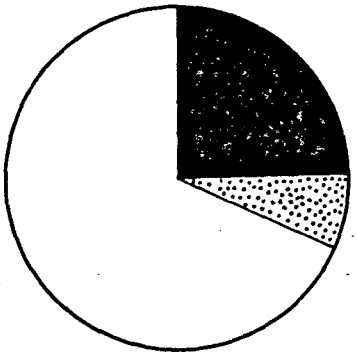
Figure 11. LDH allelic frequencies and stocking histories (adult trout stocked/km) in brook trout collections



0
ALLEN



0
STENSON



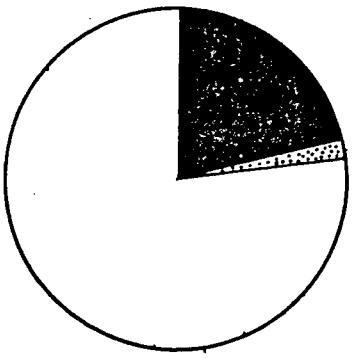
597
BASTEEN

B

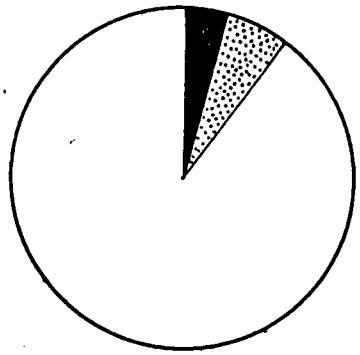
B′

B″

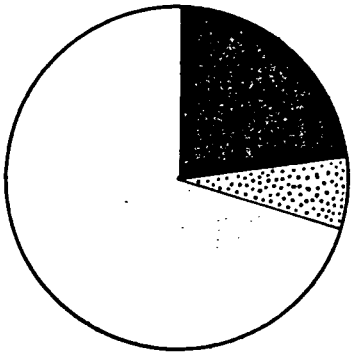
Figure 12. LDH allelic frequencies and stocking histories (adult trout stocked/km) in brook trout collections



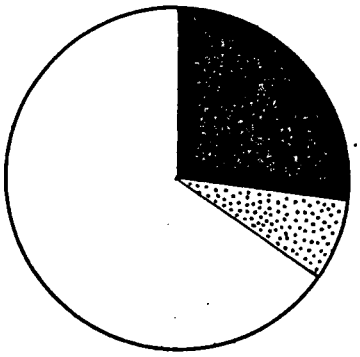
1147
TROUT I



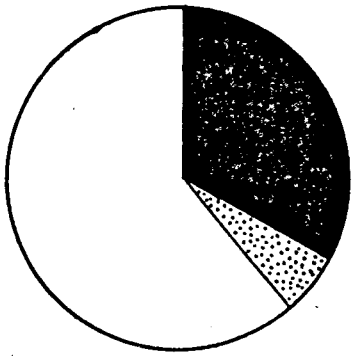
1147
TROUT II&III



1423
JACKSON



1928
SPAULDING



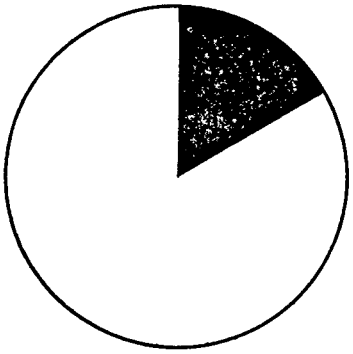
1642
LAWRENCE

B

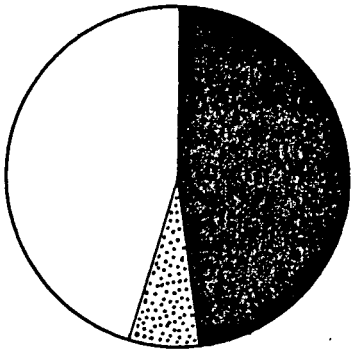
B′

B″

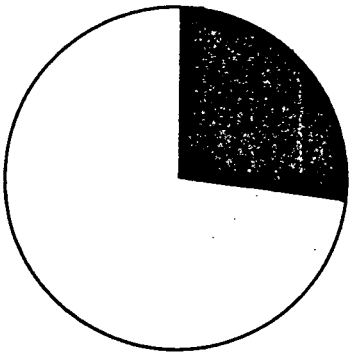
Figure 13. LDH allelic frequencies and stocking histories (adult trout stocked/km) in brook trout collections



3436
SANNES



6277
BLAKE



OSCEOLA

B

B′

B″

11, 13). The Trout II & III collection also was not similar with the homogeneous group above, yet it may have had similar stocking. This was a discrepancy in the stocking index and LDH-B locus relationship.

DISCUSSION

Deviations from Hardy-Weinberg Equilibrium

Among 11 population samples examined for Tf and LDH polymorphism there were four instances of statistical deviation from Hardy-Weinberg expectations. These were the Basteen, Trout II and Blake Creek samples with reference to LDH and the Lawrence Creek collection at the Tf locus (Tables 2 and 3). This number is in excess of the 2.2 deviations that would be expected by chance, if the .10 probability level is followed. The case of LDH in the Trout II sample has been previously explained as a statistical artifact. Among the Blake Creek sample, the deviation resulted from an excess of two heterozygote LDH B'B" individuals, an occurrence which may be attributable to chance sampling error. A general heterozygote overabundance accounted for the deviations at the LDH-B locus among Basteen Creek fish and at the Tf locus for the Lawrence Creek sample. Explanations for these latter deviations are not readily apparent although it may be speculated that extensive man-made habitat changes in Lawrence Creek (Hunt, 1969, 1971) have altered selective forces determining genetic balance at the Tf locus.

Comparisons with Other Studies

Wild populations

Transferrin genetic systems have been previously studied among wild brook trout populations in Connecticut and Pennsylvania (Wright and Atherton, 1970; Eckroat, 1973). In each case a Tf^B, Tf^C polymorphism

occurred with Tf^C typically predominating. Among Wisconsin stream populations a more complicated polymorphism existed involving different balances of Tf^B, Tf^C and a third allele which is presumed to be equivalent to Tf^A found among eastern hatchery stocks (Wright and Atherton, 1970). If the identification of Tf^A in Wisconsin populations is correct, this is the first record of wild occurrence. Moreover the existence of Tf^A in the homozygous combination among six apparently normal wild caught trout is surprising in view of Hoffman's (1966) conclusion that the genotype is lethal.

LDH variability in Wisconsin trout was characterized by three alleles (B, B', B") of intermediate frequency (Table 3). In contrast, among Pennsylvania, Connecticut, and Montana populations, LDH^B predominated and $LDH^{B'}$ or $LDH^{B"}$ were frequently absent (Wright and Atherton, 1970; Eckroat, 1973). Populations from Newfoundland have been reported variable, showing either predominance for one of the alleles or intermediate frequencies (Goldberg et al., 1971).

Although most wild populations of Wisconsin trout were polymorphic at both the Tf and LDH-B loci, the Allen Creek sample was unusual for its condition of allelic fixation at both loci. Allen Creek is a small stream that evidently supports only a limited trout population. Reduced genetic variability among this group, therefore, may be due to inbreeding. Similar degrees of reduced genetic variability have been reported among small Pensylvania populations isolated by acid mine drainage (Wright and Atherton, 1970).

Hatchery populations

The Osceola hatchery sample possessed two alleles in intermediate frequencies at both polymorphic loci (Tf^B, Tf^C, LDH^B, $LDH^{B'}$; Tables 2 and 3). Generally these allelic frequencies occurred within the range reported for eastern U.S. and Ontario hatchery stocks by Wright and Atherton (1970). Additionally the eastern stocks also occasionally possessed Tf^A and $LDH^{B''}$ in low frequencies. Lack of the uncommon alleles in the Osceola hatchery stock may be due to their absence originally or as a result of inbreeding and artificial selection. Breeding structures in the hatcheries reported by Wright and Atherton varied from semi-wild to a closed strain, randomly bred for many generations.

In summary both Goldberg et al. (1971) and Wright and Atherton (1970) noted greater heterozygosity in hatchery populations than in the wild, however, this was not apparent in Wisconsin trout. Among these, the Osceola hatchery stock exhibited reduced genetic variability and was distinct from all wild populations in terms of allelic balances (Tables 4 and 5).

Population Identification

Electrophoretic approaches to identification of stream fish populations have been employed in a number of investigations. Differing geographic distances, protein systems and fish species studied have no doubt contributed to the variable results produced. Koehn et al. (1971) found only minor variations in allelic frequencies of serum esterase for the sand shiner, Notropis stramineus, sampled over the 300 mile length of the

Kansas River, Kansas. Echelle et al. (1975) reported, on the basis of LDH, esterase and glutamate oxalacetic acid systems, evidence for genetic differentiation of darter (Etheostoma radiosum) populations between but not within tributaries of the Red River in Arkansas and Oklahoma. Koehn and Rassmussen (1967) and Koehn (1970) demonstrated correlations of serum esterase allelic frequencies with latitude among sucker (Catostomas clarki) populations from tributaries of the lower Colorado River. Huzyk and Tsuyuki (1974) found liver LDH allelic frequency variation between resident and anadromous rainbow trout from British Columbia streams. Genetic variation between Pacific and Atlantic salmon populations has also been demonstrated (Utter et al., 1970; Møller, 1970; Payne, 1974; Aspinwall, 1974). Aspinwall (1974) additionally reported variations in malate dehydrogenase and alpha-glycerophosphate dehydrogenase allelic frequencies between odd and even year spawners within streams, illustrating a form of temporal segregation. Northcote et al. (1970) found significant differences between LDH allelic frequencies of rainbow trout sampled above and below a waterfall on a British Columbia stream. Wright and Atherton (1970) and Eckroat (1971) reported significant LDH and lens protein loci differences between brook trout collections both within and between Pensylvania streams.

In the present study statistically significant differences ($p<.05$) at the Tf and LDH-B loci occurred between samples of various wild populations. Genetic distinctiveness occurred even between comparisons of proximate populations (e.g., Basteen and Stenson Creeks, Figures 8 and 9).

Wright and Atherton (1970) also found a high degree of genetic distinctiveness among Pennsylvania brook trout populations based on these protein systems.

Multiple samples were taken from Trout Creek to determine the extent of microgeographic genetic distinctions among wild trout. The electrophoretic evidence implied significant genetic differences at the LDH-B locus between samples located approximately 2 km apart (Table 6). The lack of known physical barriers to fish movement between collection locations suggests that semi-segregated breeding units result from limited longitudinal fish movement. Eckroat (1971) indicated that geographic distances of 300-800 yards might serve as an isolating barrier between brook trout populations. Actual mark and recapture evidence relating to this is inconclusive. Tagged brook trout recovered while spawning were found by Shetter (1937) to have typically traveled less than one mile (usually upstream) from tagging locations in a Michigan stream. Stefanich (1951) reported that tagged brook trout in a Montana stream, during an approximately one year period, were usually recovered within the same 600 foot section where they were marked. On the other hand, substantial numbers of young of year brook trout in Lawrence Creek, Wisconsin, have been found to disperse over the entire stream in both upstream and downstream directions (Hunt, 1965). Although adults exhibit less summer movement than young in Lawrence Creek, nothing is known about their movements during the spawning season (Hunt and Brynildson, 1964). If homing to natal areas occurs then population segregation within a stream is possible regardless of movement at other times of the year. The genetic

demonstrated for the Trout Creek samples suggests that admixture of the nature described by Hunt (1965) was not apparent at the time of collection (July) in this stream.

Relationships Within Genetically Defined Population Groups

Although stream population comparisons were usually significantly different, occasionally similarity at one or both loci was indicated. Statistically homogeneous groups were constructed separately for each protein, Tf and LDH (Figure 6 and 7). The membership differences between these groups suggest that the mechanisms causing homogeneity at each locus varied.

Transferrin locus groups

Samples which proved statistically homogeneous for Tf allelic frequencies were taken from widely separated localities in the Litte Wolf River drainage. Distance alone would seem to preclude the possibility that all of these samples were drawn from a single gene pool. The samples do, however, share the attribute of having been taken from upstream localities in the basin. Assuming that the fishes in these streams originated from a common stock, it is possible that the various groups have experienced similar selective pressures in these headwater areas. A number of literature reports suggest that transferrin systems respond to selective pressures. Fujino and Kang (1968) suggested that differential fitness among Tf genotypes in skipjack tuna may account for the observed relationship between heterozygote excesses and age. Balakhnin and Galagan (1972) noted that carp heterozygous for Tf were superior to homozygotes in

pond survival. Payne (1974) suggested that Tf heterozygote deficits in Atlantic salmon adults and allelic frequency variations between migratory and nonmigratory populations, were caused by differential fitness among genotypes.

Lactate dehydrogenase locus groups

Two groups of stream populations statistically homogeneous at the LDH locus were identified. Within the Allen and Trout II & III assemblage LDH^B predominated (>.87; see Table 3). No strong evidence in support of any particular genetic relationship exists for this group. The homogeneity observed may simply be a fortuitous occurrence. Members of the other group (Jackson, Spaulding, Lawrence, Basteen, Trout I and Sannes) displayed intermediate allelic frequencies and were similar in stocking history with respect to the number of catchable trout (all species) stocked per kilometer (Figures 11, 12, and 13). There were no obvious ecological, demographic, or geographic correlations between them. Some possible means by which stocking could affect LDH allelic frequencies in the wild populations are interbreeding between domestic and wild trout, angling pressure associated with stocking, and altered ecological interactions.

The possibility of interbreeding between wild and domestic trout has been theorized by Smith and Needham (1942) and Calaprice (1969). Among Wisconsin brook trout, however, such interbreeding should be limited in genetic impact because of the poor survival of stocked trout (Brynildson and Christenson, 1961; Mason et al., 1967). In the present study, the number of brook trout stocked was not correlated to LDH similarity which further implies that interbreeding is of little importance. Stocking of

all species, however, was related to the LDH homogeneity. Brown trout comprised the vast majority of the other trout stocked. Hybridization between brook and brown trout has been recorded in Wisconsin (Brasch et al., 1973) but is limited in occurrence.

Miller (1957) has suggested that intensive angling might result in altered genetic patterns of fish by selecting for characters such as growth or intelligence. Vaughan (1947) presented evidence that pink salmon (Oncorhynchus gorbuscha) populations, in response to heavy commercial fishing, may be developing strains which migrate to spawning streams after the close of the fishing season. Butler and Borgeson (1965) found that angling effort varied directly with trout stocking rates. In the present study if angling intensity was related to the rate of stocking and determined the degree of selection then similarly stocked populations could exhibit genetic homogeneity for selected characters.

Recent reports concerning standing crops of wild trout populations have indicated a negative impact of stocking both conspecifics and other species (Vincent, 1975; Thuemler, 1975). Butler (1975) proposed that behavioral interactions between hatchery and wild stocks may occur to affect space and spacing. Nyman (1970) reported that an ecological interaction between sympatric populations of brook and brown trout may have caused spatial segregation within pools in a Newfoundland stream. Large brown trout are also known predators on small trout including brook trout (Shetter and Alexander, 1970). Interactions between wild brook trout and hatchery brook and brown trout possibly could alter LDH genotype fitness.

Selection, operating on these genotypes, regulated by stocking rates could then account for the LDH homogeneity observed in Wisconsin populations.

Actual percentage species composition of catchable fish stocked varied widely from stream to stream. Brook trout comprised all of the catchable trout planted in Jackson and Spaulding Creeks whereas none were stocked in Basteen and Trout Creeks (Table 1). These streams, however, possessed similar catchable stocking rates and displayed LDH homogeneity which suggests an equal selection contribution by stocked trout independent of species. This may be surprising because stocking survival differences exist between brook trout and other species (Cooper, 1952; Brynildson and Christenson, 1961) and should affect the period and degree of the interaction.

The heterogeneity of the Trout II & III collections with other similarly stocked populations does not support the theory that stocking causes LDH homogeneity (Figure 12). No explanations for this discrepancy appear to exist.

Management Implications

Brook trout populations both within and between streams were readily identifiable using the Tf and LDH loci. Brook trout populations thus may be managed on at least a stream to stream basis where desirable and feasible. This management may possibly be extended to sections within a particular stream.

The evidence for genetic differences at the Tf and LDH locus between the Osceola trout and wild fish may be a reflection of a broad genetic

character variance between these fish. Such genetic differences could produce fish ill-adapted to survival in the wild and may explain the apparent inability of the Osceola fish to survive much beyond a year in Wisconsin streams (Brynildson and Christenson, 1961; Mason et al., 1967).

No evidence for interbreeding between wild and hatchery trout and the subsequent transmission of unusual genetic characters into the wild, was observed. The present study's results, however, do suggest that stocking may alter the genetic character of wild populations in a regular fashion independent of the species stocked. It is possible that this alteration may be linked to the previously reported reduction in wild trout standing crops in streams when stocking occurs.

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ACKNOWLEDGMENTS

This research was performed through a cooperative research agreement with the Wisconsin Department of Natural Resources which contributed personnel and equipment for the collecting of specimens. In particular the author is indebted to Robert Hunt, Harrison Sheldon, and Kent Niermeyer of the Coldwater Research Group near Waupaca and to Lee Haas of the Osceola State Fish Hatchery for their assistance in the sampling of trout populations.

Support was also provided financially through the Graduate College at Iowa State University and the I.S.U. Agriculture and Home Economics Experiment Station. Statistical assistance was provided by Dr. Paul Hinz of the Statistics Department at I.S.U.

Dr. Bruce W. Menzel, serving as major professor, generously gave time, technical skills, and consultation during the entire course of this study. In addition, he was responsible for initially stimulating the author's interest in population genetics.

Finally, I am grateful to my wife, Sharon, who cheerfully assisted in collecting trout, tissue preparation, figure preparation, and typing. In addition she has continually supported me through encouragement and prayers.

APPENDIX A: OMNITAB PHENOTYPE CHI SQ PROGRAM

```
FIXED WITH 4 DIGITS TO THE RIGHT OF THE DECIMAL POINT
READ INTO COLS 1 *** 7
$ INSERT PHENOTYPE DATA CARDS HERE
$ THE FIRST SIX COLUMNS ON THE DATA CARDS ARE THE OBSERVED PHENOTYPES IN A
$ SAMPLE (COL 1 = AA, COL 2 = AB, COL 3 = AC, COL 4 = BB, COL 5 = BC, COL
$ = CC).  IN A TWO ALLELE-ONE LOCUS SITUATION ENTER ZEROS IN COLUMNS 1, 2,
$ AND 3.  COLUMN 7 IS THE TOTAL NUMBER IN THE SAMPLE.  IF PRINTOUT CON-
$ TAINS EXPECTED PHENOTYPES THAT ARE LESS THAN 1.0 THEN THAT PHENOTYPE
$ SHOULD BE ERASED AND ADDED TO THE PHENOTYPES WITH THE NEXT SMALLEST
$ VALUES UNTIL THE EXPECTED VALUES ARE GREATER THAN 1.0.  THIS MAY BE
$ ACCOMPLISHED FOR EXAMPLE BY THE FOLLOWING COMMANDS INSERTED IMMEDIATELY
$ AFTER THE DATA CARDS:
$              ADD COL 1 TO COL 2 STORE COL 2
$              ERASE COL 1
SUM COL 7 STORE 15
1/ SUM COL 1 STORE 9
2/ MULT COL 7 BY 9 STORE 16
3/ DIV 16 BY 15 STORE 23
4/ SUB COL 1 FROM 23 STORE 30
5/ MULT 30 BY 30 STORE 30
6/ DIV 30 BY 23 STORE 30
7/ SUM 30 STORE 36
8/ ADD COL 36 TO 43 STORE 43
9/ INCREMENT INSTRUCTION 1 BY (1,1)
```

```
10/ INCREMENT INSTRUCTION 2 BY (0,1,1)
11/ INCREMENT INSTRUCTION 3 BY (1,0,1)
12/ INCREMENT INSTRUCTION 4 BY (1,1,1)
13/ INCREMENT INSTRUCTION 5 BY (1,1,1)
14/ INCREMENT INSTRUCTION 6 BY (1,1,1)
15/ INCREMENT INSTRUCTION 7 BY (1,1)
16/ INCREMENT INSTRUCTION 8 BY (1,0,0)
EXECUTE INSTS 1 THRU 16, 6 TIMES
HEAD COL 9/SUM AA
HEAD COL 10/SUM AB
HEAD COL 11/SUM AC
HEAD COL 12/SUM BB
HEAD COL 13/SUM BC
HEAD COL 14/SUM CC
HEAD COL 15/TOTAL FISH
HEAD COL 23/EXPECTED AA
HEAD COL 24/EXPECTED AB
HEAD COL 25/EXPECTED AC
HEAD COL 26/EXPECTED BB
HEAD COL 27/EXPECTED BC
HEAD COL 28/EXPECTED CC
HEAD COL 30/X2 CALC AA
HEAD COL 31/X2 CALC AB
HEAD COL 32/X2 CALC AC
HEAD COL 33/X2 CALC BB
```

```
HEAD COL 34/X2 CALC BC
HEAD COL 35/X2 CALC CC
HEAD COL 36/X2 SUM AA
HEAD COL 37/X2 SUM AB
HEAD COL 38/X2 SUM AC
HEAD COL 39/X2 SUM BB
HEAD COL 40/X2 SUM BC
HEAD COL 41/X2 SUM CC
HEAD COL 43/TOTAL CHI SQ
PRINT COL 9 *** 15
PRINT COLS 23 *** 28
PRINT COLS 30 *** 35
PRINT COLS 36 37 38 39 40 41 43
STOP
```

APPENDIX B: OMNITAB HARDY-WEINBERG PROGRAM

```
FIXED WITH 4 DIGITS TO THE RIGHT OF THE DECIMAL POINT
READ INTO COLS 1 *** 7
$ INSERT PHENOTYPE DATA CARDS HERE
$ THE FIRST SIX COLUMNS ON THE DATA CARDS ARE THE OBSERVED PHENOTYPES IN A
$ SAMPLE (COL 1 = AA, COL 2 = AB, COL 3 = AC, COL 4 = BB, COL 5 = BC, COL
$ 6 = CC).  IN A TWO ALLELE-ONE LOCUS SITUATION ENTER ZEROS IN COLUMNS 1,
$ 2, AND 3.  COLUMN 7 IS THE TOTAL NUMBER IN THE SAMPLE.  IF PRINTOUT CON-
$ TAINS EXPECTED PHENOTYPES THAT ARE LESS THAN 1.0 THEN BOTH OBSERVED
$ PHENOTYPES AND GENERATED EXPECTED VALUES SHOULD BE ERASED AND ADDED TO
$ THE NEXT SMALLEST VALUES UNTIL THE EXPECTED VALUES SUM GREATER THAN 1.0.
$ THIS MAY BE ACCOMPLISHED FOR EXAMPLE BY THE FOLLOWING COMMANDS INSERTED
$ IMMEDIATELY BEFORE STATEMENT 5/
$               ADD COL 1 TO COL 2 STORE COL 2
$               ERASE COL 1
$               ADD COL 15 TO COL 16 STORE COL 16
$               ERASE 15
MULT 1 BY 2. STORE 8
ADD 8 TO 2 STORE 8
ADD 8 TO 3 STORE 8
MULT 4 BY 2. STORE 9
ADD 2 to 9 STORE 9
ADD 5 TO 9 STORE 9
MULT 6 BY 2. STORE 10
ADD 3 TO 10 STORE 10
```

```
ADD 5 TO 10 STORE 10
MULT 7 BY 2. STORE 11
1/ DIVIDE 8 BY 11 STORE 12
2/ INCREMENT INSTS 1 BY 1,0,1
EXECUTE INSTS 1 THRU 2, 3 TIMES
MULT 12 BY 12 STORE 15
MULT 13 BY 13 STORE 18
MULT 14 BY 14 STORE 20
MULT 2.0 BY 12 STORE 16
MULT 16 BY 13 STORE 16  $ 2AB
MULT 210 BY 12 STORE 17
MULT 17 BY 14 STORE 17  $ 2AC
MULT 2.0 BY 13 STORE 19
MULT 19 BY 14 STORE 19  $ 2BC
5/ MULT 15 BY 7 STORE 21
6/ SUB 1 FROM 21 STORE 27
7/ MULT 27 BY 27 STORE 27
8/ DIV 27 BY 21 STORE 27
9/ INCREMENT INST 5 BY 1,0,1
10/ INCREMENT INST 6 BY 1,1,1
11/ INCREMENT INST 7 BY 1,1,1
12/ INCREMENT INST 8 BY 1,1,1
EXECUTE INSTS 5 THRU 12, 6 TIMES
ADD COL 27 TO 28 STORE 33
13/ ADD COL 33 TO 29 STORE 33
14/ INCREMENT INST 13 BY 0,1,0
```

```
EXECUTE INSTS 13 THRU 14, 4 TIMES
HEAD COL 8 / A ALLELES
HEAD COL 9 / B ALLELES
HEAD COL 10 / C ALLELES
HEAD COL 11 / TOTAL ALLELE
HEAD COL 12 / FREQ OF A
HEAD COL 13 / FREQ OF B
HEAD COL 14 / FREQ OF C
HEAD COL @L / EXPECTED AA
HEAD COL 22 / EXPECTED AB
HEAD COL 23 / EXPECTED AC
HEAD COL 24 / EXPECTED BB
HEAD COL 25 / EXPECTED BC
HEAD COL 26 / EXPECTED CC
HEAD COL 27 / AA X2
HEAD COL 28 / AB X2
HEAD COL 29 / AC X2
HEAD COL 30 / BB X2
HEAD COL 31 / BC X2
HEAD COL 32 / CC X2
HEAD COL 33 / CHI SQ H-W
PRINT COL 8 *** 14
PRINT COL 21 *** 26
PRINT COL 27 *** 33
STOP
```