Genetic Profiles of Selected Brook Trout *Salvelinus fontinalis* Populations from Minnesota Streams Tributary to Lake Superior

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Abstract

Brook trout inhabit many inland Minnesota rivers and streams as well as streams tributary to Lake Superior, some which may have populations of a migratory form of brook trout called the "coaster." Recent interest in rehabilitation of "coaster" brook trout in the wake of significant population declines has prompted management agencies to document the characteristics of existing brook trout populations, especially with respect to genetic diversity. The objectives of this study were to document levels of genetic diversity within and among wild brook trout populations in Minnesota streams tributary to Lake Superior. We compared genetic diversity between populations sampled above and below barrier dams, and we compared wild populations with several hatchery strains known to have been stocked in Minnesota waters. Overall, a substantial amount of genetic diversity is still present in wild Minnesota brook trout populations. Genetic diversity was greater above barrier dams than their below-barrier counterparts, but a greater number of different haplotypes were detected in below-barrier populations. There was no evidence of significant introgression between hatchery and wild populations, as the most common hatchery strain possessed a unique genetic type not found among the Minnesota populations sampled here. An analysis of molecular variance (AMOVA) showed significant partitioning of genetic variance between above and below-barrier populations in the same system, and among the group of below-barrier populations. In contrast, there were no significant differences in the distribution of genetic types among above-barrier populations or between pooled wild and hatchery populations. Levels of genetic diversity found among wild Minnesota brook trout populations suggest that individual populations should ideally be considered as independent entities for purposes of conservation and restoration.

Introduction

Brook trout, *Salvelinus fontinalis,* are indigenous to North America, and since about 1872 have had their range extended by man to include all major continents (MacCrimmon and Campbell 1969, Behnke 1972). In the Lake Superior drainage basin, brook trout are the only extant indigenous stream salmonid since the loss of several populations of river-run lake trout in the last century. Studies have shown that brook trout populations in some Lake Superior tributaries, including several in Minnesota waters, are self-sustaining (Newman and DuBois 1997). In some Lake Superior populations, significant genetic diversity was detected, suggesting that wild native fish are still present in the basin (Danzmann et al. 1991, Burnham-Curtis 1996).

The reliable identification of "native" brook trout is complicated by a diverse stocking history in many Minnesota rivers and streams. At least four different non-native strains may have been stocked at various times in the last century, and local lore holds accounts of numerous unofficial downstream – upstream stock transfers (Eddy and Underhill 1974, Waters 1977). In addition, some Lake Superior tributaries may have contained populations of "coaster" brook trout, a form of brook trout which migrates out into the open water of Lake Superior for part of its adult life and returns to shoreline reefs or some upstream sites to spawn. Coaster populations throughout the Lake Superior basin have suffered severe declines, and only a few streams are thought to still harbor remnant stocks. In Minnesota waters, an estimated 250 brook trout are

caught in lower reaches of tributary streams each spring, but it is unknown whether those fish are coasters or river-resident fish (Morse 1999).

Modern management of brook trout populations has begun to focus on the importance of native fish populations to the health of river and lake ecosystems. Interest in restoration of brook trout populations, particularly the "coaster" brook trout, has been identified by all agencies involved in Lake Superior fisheries management as a high priority for Lake Superior (Newman et al. 1999). Individual agencies have begun investing more resources into protecting and restoring wild brook trout populations. Identification of existing levels of genetic diversity and the relationship between wild and hatchery-derived brook trout is an important component of the management equation for this species. In the Lake Superior basin, characterization of different life history morphs adds complexity to both research and management activities. As consideration of the status of particular species as threatened or endangered becomes more common, managers increasingly need more accurate descriptions of population identities. Concerns about low genetic diversity, gene flow between hatchery and wild populations, and maintaining the genetic integrity of wild populations have prompted management agencies to reevaluate hatchery stocking programs for many native fish species in the U.S., including brook trout in the Great Lakes basin.

In the eastern U.S., genetic diversity among wild brook trout populations was undoubtedly reduced by bottlenecks from recent declines in population abundance. These populations were further impacted by interactions with hatchery-raised fish in almost every region where brook trout were native (Kreuger and Menzel 1979, Webster and Flick 1981, Danzmann et al. 1991, Perkins et al. 1993). Allozyme studies have demonstrated that considerable variation still exists among extant inland brook trout populations. Studies of mitochondrial DNA variation support the existence of barriers to gene flow among populations within and among regional river systems (Quattro et al. 1990, Danzmann et al. 1991, Bernatchez and Danzmann 1993, Burnham-Curtis 1996). Recent advances in molecular technology have made possible the development of genetic markers with the potential to provide population- and individual-specific profiles for use in parentage analysis (Angers et al. 1995, T. King and M. K. Burnham-Curtis, unpublished data).

In order to effectively manage fish populations, it is necessary to know the genetic characteristics of the existing populations, how the genetic variation is distributed, and whether there are discrete population-specific genetic markers available to identify populations of interest. Allozymes (Kreuger and Menzel 1979, Webster and Flick 1981) and mtDNA (Quattro et al. 1990, Perkins et al. 1993, McCracken et al. 1993) have been useful in distinguishing some brook trout populations which reside in discrete segments of inland streams. Recognition of hatchery versus wild brook trout has been limited to documenting lower levels of heterozygosity in hatchery populations as opposed to identifying population-specific markers (Quattro et al 1990, Perkins et al. 1993). Danzmann et al. (1998) demonstrated that significant levels of genetic diversity can be detected using characters derived from maternally-inherited mitochondrial DNA. In the Great Lakes drainage, two maternal clades predominate, having Mississippian and Atlantic refugial origins during the last glacial event (Danzmann et al. 1998). Numerous closely related mtDNA haplotypes derived from the post-glacial colonists could be

evidence of the survival of wild extant brook trout populations despite 19th and 20th century stocking efforts.

In this study, we surveyed the genetic diversity in the mitochondrial DNA of wild and hatchery-raised brook trout. Specific goals for this study were to: 1) determine if remnant brook trout stocks still exist in Minnesota tributaries below natural barriers; 2) compare genetic characteristics of brook trout populations found above and below natural barriers in tributary streams; and 3) determine if stocked brook trout have had any genetic impact on wild populations above and below natural barriers. This study is one component of a partnership between the Minnesota Department of Natural Resources (MNDNR), the Gitchee Gumee Chapter of Trout Unlimited (TU), and the USGS Great Lakes Science Center (GLSC) to investigate the genetic and biological characteristics of brook trout populations in Minnesota streams tributary to Lake Superior. Information needs identified in the MNDNR Lake Superior Management Plan (Schreiner 1995) included a basic understanding of the status of brook trout and genetic identification of strains or stocks present in Minnesota waters.

Methods

Brook trout were collected by the MNDNR and TU volunteers in 1995, 1997, and 1998 from 18 Minnesota streams tributary to Lake Superior (Figure 1). Additional populations were sampled by the Grand Portage Band and the Ontario Ministry of Natural Resources. Fish were collected by electrofishing with a Smith Root[a] model 11-A backpack electrofishing unit (400-600V, 60Hz). Some coaster brook trout are thought to migrate up tributary streams to spawn in the fall, so 1997 collections in areas below barrier dams were conducted during the fall spawning season (September through mid-November); collections in above-barrier streams were made in July and August 1998. Streams with above and below barrier dam collections include Cross River, Devil Track River, Kadunce Creek, Kimball Creek, Onion River, Spruce Creek, and Knife River. All other tributaries included in the analysis were sampled below-barriers. Only three individuals were sampled from below the barrier in the Knife River, so diversity analyses only included the above-barrier population. Samples were collected from hatchery populations to represent strains which were stocked above barriers in the last century. These include the St. Croix Falls strain (Spire Valley Fish Hatchery) , Owhi strain (Egan State Fish Hatchery; stocked 1986-1992), Phillips Maine strain (Phillips State Fish Hatchery, Maine; stocked 19??-1988), Rome strain (Rome New York Hatchery, stocked 19??-1983). The Minnesota Wild hatchery strain (currently stocked inland in southeastern Minnesota) was included for comparison to populations outside the Lake Superior watershed. Adipose fin clips were taken in the field and preserved in either a modified Queen's buffer (Seutin et al. 1981) or 95% ethanol and sent to the Great Lakes Science Center (GLSC) for genetic analysis. Biological data (length, weight, sex, maturity, scale samples for aging) were collected in the field for all samples and summarized by the MN DNR (Tillma et al. 1999).

Total genomic DNA was extracted from tissue subsamples using a commercial extraction kit (PureGene[1], Gentra Systems, Minneapolis, MN). Mitochondrial DNA was replicated and amplified using the polymerase chain reaction (PCR®, Applied Biosystems[1], Foster City , CA).

[1] Mention of tradenames does not imply U. S. Government endorsement of commercial products.

PCR amplifications were performed using Ampli-Taq DNA polymerase and PCR buffer II supplied by the manufacturer (PE-Applied Biosystems[1], Foster City , CA), 2.0-6.0 mM $MgCl_2$, 200μM dinucleotides (dATP, dTTP, dGTP, dCTP), and 0.1-0.3μM of each oligonucleotide primer flanking the mtDNA genes for the control region, NADH 5-6, and NADH 2 (Table 1). PCR products were electrophoresed in 1% agarose in 1 X TAE, post-stained in ethidium bromide, and visualized under long wave ultraviolet light to determine accuracy and quality of the PCR reaction.

PCR amplicons were digested with a set of Type II restriction endonucleases specific for the locus of interest. Restriction digestion products were electrophoresed in 2-4% agarose in 1 X TAE, post-stained with ethidium bromide and visualized under long wave UV light. MtDNA haplotypes were determined based on the composite pattern of presence or absence of restriction sites inferred from restriction fragment profiles. The combination of 3 gene loci and 6 restriction enzymes produced 12 distinct mtDNA haplotypes (Burnham-Curtis 1996).

Genetic diversity estimates were calculated from both the comparison of restriction site presence or absence, and from frequency of haplotype presence within and among populations and groups. These values (e.g. heterozygosity, nucleotide diversity, population pairwise distance) provide an estimate of the overall genetic variation among brook trout populations as well as means to evaluate the relationship between genetic distance and geographic distance among the sampled populations. Analysis of molecular variance (AMOVA, Arlequin Ver. 1.1, Schneider et al. 1997) was used to calculate population diversity estimates, and to estimate partitioning of genetic diversity within and among populations and groups in a hierarchichal manner. Groups tested included populations across all sampling sites, above versus below barrier dams, and wild versus hatchery. Population pairwise Fst estimates were input into the NEIGHBOR program of PHYLIP 3.5 (Felsenstein 1985) to generate a neighbor-joining network among populations. Gene frequency estimates were used to generate 5,000 replicate genetic distance matrices for a bootstrap analysis to generate confidence estimates in the neighbor-joining relationships using the PHYILP programs SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE. Comparisons were made between pairwise populations to estimate the significance of differences in haplotype composition with the X^2 Monte Carlo estimation method of Roff and Bentzen (1989) using 5,000 bootstrap replicates.

Results

A total of 1,056 fish were sampled for the genetic analysis; 801 were from wild populations and 255 were from 5 hatchery strains (Table 2). Among the wild populations, 338 were sampled from 7 populations above barrier dams, 363 were sampled from 19 populations below or absent barrier dams in tributaries to the Minnesota waters of Lake Superior. For comparison, 73 fish from Lake Nipigon, Nipigon River and Nipigon Bay, along with 27 fish from an inland stream (Spring Brook in southeastern Minnesota) were also included. Several below barrier populations had sample sizes of less than eight fish, and these were excluded from subsequent population diversity analyses.

Among the Minnesota populations, 10 of 14 possible brook trout haplotypes (as identified by a similar 3 locus/6 enzyme survey) were present (Table 3). One haplotype, designated BT1, dominated the sample. BT1 was present as the most common haplotype in all populations, and the only haplotype detected in 5 populations. The overall haplotype composition of the streams with above- and below-barrier counterparts appear to be significantly different (Figure 2). The Onion River was sampled both above and below the barrier dam in two separate years (1995 and 1998). In a separate comparison, the mtDNA haplotype distribution was compared across years to see if there were any temporal differences in the genetic profiles of these populations. There were no differences in haplotype composition across years for the above-barrier population ($P = 0.220$). In contrast, differences in haplotype composition between years for the population sampled below the barrier dam were highly significant ($P < 0.001$, Figure 3). The differences below the barrier were due to the presence of the BT5 haplotype in 1995 in 27% of the population, and of the BT3 haplotype in 1997, which made up 7% of the population, but was not found in other years either above or below the dam (Figure 3).

Although BT1 is most common in all populations, the above barrier populations have a significantly greater frequency of occurrence of the BT2 haplotype. One haplotype, BT10, was found in the Minnesota Wild hatchery strain, but only in one individual. This haplotype was also found in other Lake Superior brook trout populations (Burnham-Curtis 1996), but not from wild populations along the Minnesota shore. Of the 10 identified haplotypes, all but 1 (BT9) appear to fall within a single lineage of closely related haplotypes (Figure 4). It is interesting to note that with the exception of one individual from Kimball Creek above the dam, the BT9 haplotype appears only in below-barrier populations, and the wild Lake Nipigon sample.

Differential distribution of haplotypes is reflected among estimates of heterozygosity (the probability that two haplotypes in the sample are different) between above and below-barrier populations and between the wild and hatchery groups. Heterozygosity estimates were substantially higher for five of six above barrier populations than their below barrier counterparts. The Onion River below-barrier population was the only one that had slightly higher measures of heterozygosity than above-barrier. Levels of heterozygosity ranged from 0.319-0.725 for above-barrier populations and 0.000-0.540 for below-barrier populations. The range of diversity among hatchery strains (0.000-0.575) fell within the range of the wild populations, but varied widely among the hatchery groups (Table 3). The MN Wild strain had the highest estimates of genetic diversity of all hatchery strains.

The analysis of molecular variance (AMOVA) indicates that a significant proportion of genetic variation occurs among individual populations (11.5%, $P < 0.001$). However, a large proportion of the genetic variation was allocated within populations (88.5%, $\Phi_{ST} = 0.115$, $P < 0.001$; Table 4). Heterogeneity among above- and below-barrier populations was moderately significant and accounted for about 5% of the total variation ($\Phi_{CT} = 0.050$, $P = 0.015$). Differences among populations were generally due to frequency differences among closely related haplotypes. Comparisons between hatchery origin and wild populations showed no significant difference in partitioning of genetic variation ($\Phi_{CT} = -0.013$, $P = 0.786$) between groups; none of the observed variation was attributed to differences between populations of wild or hatchery origin.

We used a chi-square test to evaluate the differences in haplotype distribution between individual populations and among groups of populations. We used a bootstrap procedure to resample populations (with replacement) and estimate confidence limits for the χ^2 parameter. This test provides a measure of the statistical significance of differences among the haploytpe profiles of the sampled populations and a method by which the significance of geographic heterogeneity among populations can be evaluated. Chi-square tests of the distribution of the most common haplotypes, BT1, BT2, BT4, and BT5 showed significant differences in pooled above vs. below-barrier comparisons ($P < 0.001$), and in pooled wild vs. hatchery comparisons ($P < 0.001$). Differences between pooled above and below barrier dam populations were highly significant ($P < 0.001$). In individual rivers, above and below-barrier populations were also highly significantly different ($P < 0.001$) in Kadunce Creek, Spruce Creek, Devil Track River, and Cross River. Differences were moderately significant for the above/below comparison in Kimball Creek ($P = 0.050$) and Onion River ($P = 0.012$). There were significant differences among sample locations both above the barrier dams ($P < 0.001$), as well as below the barriers ($P = 0.002$).

Haplotype composition of individual hatchery populations were compared to individual wild populations (above or below barrier) in a bootstrap Monte Carlo simulation (Table 5). A majority of the comparisons were significantly different at $P < 0.001$. The St. Croix and Phillips strains had the greatest variability in significance comparisons. Only four of sixty comparisons showed no statistically significant differences at P=0.05, most likely due to frequency similarities for haplotypes BT1 and BT2. Of note, the Minnesota Wild hatchery strain was significantly different from all above- and below-barrier populations.

Genetic relationships among the brook trout populations were summarized in a neighbor-joining diagram (Saitou and Nei 1979) based on Cavalli-Sforza-Edwards chord distances derived from haplotype frequencies (Cavalli-Sforza and Edwards 1967). We compared 5,000 bootstrap replicates of the original frequency distributions, from which we estimated a consensus tree (Figure 5). While all populations are closely related, above-barrier populations cluster apart from below-barrier populations with two exceptions. One above-barrier population (Reservation River) clustered well within a group containing populations of Nipigon origin instead of among the other above barrier populations, and the Onion River below-barrier population clustered within the group of above-barrier populations. This arrangement is well-supported in bootstrap relpicates and indicates significant population structuring. The hatchery populations did not cluster together in the consensus tree; Phillips, Rome, and Dorion strains clustered with below-barrier populations, and the Owhi, St. Croix, and MN Wild strains were well-supported within the above-barrier cluster. Similarities among hatchery and wild populations are driven primarily by similarities in the frequency of the BT1 haplotype. Grand Portage Creek is one location that is known to have sustained substantial input of hatchery fish of Lake Nipigon origin; this population clustered on a well-supported branch with the Nipigon and Dorion strain (Nipigon origin) samples.

Genetic relationships among the brook trout haplotypes were compared to previous studies to infer evolutionary history of the Lake Superior brook trout populations. The majority of haplotypes detected appear to fall within one evolutionary clade (B clade, Figure 4) which is assumed to have its origin in an Atlantic refugium during the last glacial event (Danzmann et al.

1998). Of the 14 possible brook trout haplotypes detected to date in the Great Lakes drainage basin, 9 are present in the Minnesota populations sampled. All haplotypes except BT9 fall within the B clade; BT 9 falls within the A clade, assumed to be of southern Atlantic origin.

Discussion

The diversity of mitochondrial DNA haplotypes present among brook trout populations sampled from Minnesota streams indicates that a significant amount of genetic variation is still present among populations in the wild. One haplotype, BT1, dominated the sample and made up 40-100% of the haplotype distribution in a given population and 76% of the total sample. The haplotypes identified in this study fall into two evolutionary clades with origins in Atlantic glacial refugia. This corroborates results of Danzmann et al. (1998) showing the predominance of two brook trout lineages in the upper Great Lakes drainage basin. Differences in the distribution of mtDNA haplotypes among the streams sampled in this study suggest that gene flow among tributaries is minimal, but may not be completely absent. Temporal differences in haplotype frequencies in at least one river (Onion) suggest some heterogeneity in the composition of the migratory component of this population.

Above versus below barrier comparisons

Overall genetic diversity estimates were higher for all but one of the above-barrier dam populations than for their below-barrier counterparts. The AMOVA results suggest that there is partitioning between above- and below-barrier populations, and the amount of variation attributed to this comparison is much larger than the partitioning between wild and hatchery populations. The higher diversity estimates in above-barrier populations may be a reflection of higher frequencies of several different haplotypes in each of the six above-barrier populations, primarily BT2 and BT5. In contrast, the below-barrier populations had fewer individuals representing haplotypes other than BT1. With one exception, all above-barrier populations cluster together in neighbor-joining dendrograms, an arrangement that is supported in 70% of 5,000 bootstrap replications and is indicative of significant population substructuring. The clustering of the Reservation River population with Nipigon origin populations rather than other above-barrier populations may be due to the restricted haplotype distribution in the Reservation River sample. The Reservation River population was sampled near a headwater, and may have originated from a small number of fish from the resident Swamp Lake population (Newman and Johnson 1996).

Initial brook trout populations above the barrier dams are believed to have originated from below-barrier transfers that occurred during early settlement of the river systems, and in later years from widespread stocking events (Eddy and Underhill 1974, Waters 1977). Recent management records indicate that the Onion and Spruce Rivers support a lower level of fishing effort than the other rivers and streams, which may influence effective population size and allow the persistence of the slightly different suite of haplotypes in the above-barrier population than below in these two rivers. Despite the shared history of the above and below-barrier population pairs, it appears that enough differentiation has occurred between them that they maintain some level of genetic heterogeneity.

The presence of migratory brook trout in some Minnesota rivers with access to Lake Superior is supported by the significant temporal differences in haplotype composition of the below-barrier Onion River population. The BT5 haplotype detected in the 1995 sample made up over 25% of the sampled population, yet that haplotype only appeared in one individual the 1997 sample. In addition, individuals of haplotype BT3 appeared only in the 1997 population below the barrier, suggesting that there is some heterogeneity in the composition of the breeding population below the barrier that may be due to a migratory component of the population.

Hatchery versus wild comparisons

Genetic diversity of the hatchery populations varies from zero in the Phillips, ME strain to a relatively high 57.5% in the MN Wild strain. As in the wild populations, BT1 predominated in the hatchery samples, but six additional haplotypes were detected in significantly different distributions among the hatchery strains ($P < 0.001$) than among the wild populations. The MN Wild strain and the St. Croix strains were the most diverse of the hatchery strains tested, while the MN Wild strain was the only population sampled that contained the BT10 haplotype. No significant partitioning of genetic variance was detected between pooled hatchery populations and wild populations in the AMOVA, most likely because both groups shared a similar complement of mtDNA haplotypes, even if in different frequencies.

It is not likely that hatchery introgression is occurring at a substantial level among the above-barrier Minnesota brook trout populations. Only the MN Wild strain clustered with the above-barrier populations, most likely because of the similarity in the frequency distribution of the common haplotypes. However, the MN Wild population contained one individual with the BT10 mtDNA haplotype, a type not found in any other Minnesota brook trout population, and differences in genetic distance estimates were highly significant compared to all other above and below-barrier populations. There were also significant differences in mtDNA haplotype distributions among the other hatchery strains and the wild populations, suggesting that the stocked fish have not had a detectable genetic impact on the resident populations. Other studies of brook trout behavior and population genetics have also suggested that there is a generally low level of introgression between hatchery and wild genomes (Lachance and Mangan 1990, Danzmann et al. 1991).

Most of the below-barrier tributaries sampled in this study are suspected to contain migratory (coaster) brook trout. For example, in the Flute Reed and Cascade rivers, no brook trout were found in extensive surveys in the spring, however, spawning brook trout were encountered in fall surveys (Tillma et al. 1999). These fish were suspected to have migrated up the river from Lake Superior. The temporal difference in haplotype composition of at least one brook trout population lends credence to the hypothesis that some of these populations still have a migratory component. Lower levels of genetic diversity detected in the below-barrier populations may be a result of genetic bottlenecks from low effective population size coupled with a greater chance for gene flow if reproductively effective individuals are migrating among suitable tributaries. Substantial population declines of "coaster" brook trout have occurred throughout the Lake Superior drainage, and would likely be responsible for a decline in overall genetic diversity.

Management implications

Substantial genetic diversity still exists within brook trout populations along Minnesota's shoreline. Resident brook trout populations suffered no significant genetic impact from stocked fish, however existing stock diversity should be preserved by protecting the wild populations. Genetic diversity is the substrate for natural selection and adaptation. Thus, the protection of this aspect of biodiversity is important to species conservation and restoration.

While a hatchery component may be the most economical method of conducting population supplementation, little is known about the actual potential for hatchery-raised fish to enter the effective breeding population. If stocking does occur, a long-term monitoring program should be established to evaluate the influence of stocking on the resident population. Such a program should include evaluating the reproductive success of stocked trout as well as checking for adverse genetic changes. Continuous review of stocking strategies should be conducted to determine their appropriateness, effectiveness, and impact in the wild.

If stocking is chosen as a potential restoration strategy, it should be limited and preferably use broodstock from Minnesota tributaries (local populations). While this and other studies (Danzmann et al. 1991, Burnham-Curtis 2000 unpublished data) of brook trout behavior and population genetics have suggested that there is a generally low level of introgression between hatchery and wild genomes, hatchery supplementation should proceed in the most prudent manner possible. In a compromised system (low abundance of resident fish, but still self-sustaining) there may be increased risk of physical swamping where the resident population will be negatively impacted through predation and competition by the planted fish.

Above and below barrier populations were slightly different but their genetic profiles reflected evidence of their common derivation. The fact that brook trout populations became established above barrier dams from upstream transfers, and that the above-barrier populations have retained a substantial amount of genetic diversity suggests that native stock transfers could be an effective means of population restoration. If this is a viable restoration option, it is essential that the habitat and genetic profiles of the resident brook trout in potential target stocking locations be evaluated for compatibility prior to transfer activity.

Fish stocked in some locations may be affecting the genetic composition of nearby resident populations. We understand very little about the migratory potential of brook trout stocked in locations with access to Lake Superior, and even less about their ability to navigate back to their stocked stream to spawn. The latter may introduce additional concerns for the integrity of resident brook trout populations in streams adjacent to stocking locations. For example, Grand Portage Creek was stocked for five years with eyed eggs and swim-up fry obtained from Nipigon strain fish from the Dorion Hatchery (Lake Nipigon origin). Returns of planted fish to Grand Portage Creek were documented (Newman and Johnson 1996), although most were yearling fish and there was no conclusive evidence that stocked fish returned to spawn. Increased numbers of brook trout that appeared in nearby rivers during the period in which Grand Portage Creek was stocked (Jones et al. 1995) suggests that the stocked fish may be straying to nearby streams. This could have a significant impact on the genetic composition of neighboring populations if survival to spawning is high for the planted fish. Continued

monitoring of the genetic profiles of Grand Portage Creek and adjacent populations would be prudent.

The importance of individual populations to the overall composition of Lake Superior brook trout supports the need for more finely detailed population genetic information. Newly developed microsatellite DNA markers are currently being evaluated for their ability to discriminate among individual populations of brook trout (T. L. King and M. K. Burnham-Curtis, unpublished data). Levels of genetic diversity among microsatellite DNA markers are exponentially higher than those found with mtDNA markers. The fine resolution that microsatellite DNA can provide will address such future questions as parentage assignment, effective breeding population size, extent of interbreeding among hatchery and wild fish, and extent of intermingling among geographically proximate populations. Distribution patterns of microsatellite DNA markers can also be used in landscape ecology applications to assess the correlation of genetic diversity with environmental variation and model changes in population structure over time. Populations sampled for this study will be included in the microsatellite DNA survey to provide a more detailed picture of population structure and the relationships among wild brook trout populations from the Lake Superior basin.

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Table 1. Specific gene loci targeted for analyses.

Gene	Location	Est. size (bp=base pairs)	Variable or Conserved	Primer source
Control region (D-loop)	mitochondria	1150 bp	variable	Bernatchez et al. 1992
NADH 2	mitochondria	1280 bp	variable	Park et al. 1993
NADH 5/6	mitochondria	1500 bp	variable	Park et al. 1993

Table 2. Genetic diversity estimates for Minnesota brook trout populations.

Population	N	Expected Heterozygosity (*H*)	Nucleotide Diversity	Mean No. Pairwise Differences
Above Barrier Dams				
Lake Nipigon	37	0.219 ± 0.087	0.011 ± 0.011	0.439 ± 0.403
Reservation River	15	0.000	0.000	0.000
Kimball Creek	43	0.653 ± 0.052	0.023 ± 0.017	0.943 ± 0.659
Kadunce Creek	50	0.529 ± 0.040	0.015 ± 0.013	0.608 ± 0.491
Devil Track River	50	0.671 ± 0.043	0.026 ± 0.019	1.072 ± 0.729
Spruce Creek	50	0.480 ± 0.035	0.012 ± 0.011	0.480 ± 0.423
Onion River 1998	49	0.319 ± 0.078	0.010 ± 0.010	0.399 ± 0.378
Cross River	68	0.725 ± 0.025	0.030 ± 0.021	1.250 ± 0.800
Knife River	28	0.701 ± 0.059	0.029 ± 0.021	1.169 ± 0.776
Spring Brook (MN inland)	27	0.000	0.000	0.000
Below Barrier Dams				
Nipigon River	13	0.000	0.000	0.000
Nipigon Bay	23	0.312 ± 0.114	0.014 ± 0.013	0.561 ± 0.475
Grand Portage Creek	27	0.000	0.000	0.000
Flute Reed River[a]	2	---	---	---
Kimball Creek	24	0.540 ± 0.109	0.021 ± 0.017	0.851 ± 0.624
Kadunce Creek	35	0.301 ± 0.091	0.009 ± 0.010	0.366 ± 0.361
Devil Track River	55	0.298 ± 0.079	0.010 ± 0.010	0.420 ± 0.389
Grand Marais Harbor	30	0.738 ± 0.052	0.031 ± 0.022	1.267 ± 0.820
Cascade River	8	0.250 ± 0.180	0.006 ± 0.008	0.250 ± 0.311
Spruce Creek	47	0.259 ± 0.073	0.006 ± 0.008	0.259 ± 0.292
Onion River 1997	57	0.483 ± 0.072	0.017 ± 0.014	0.703 ± 0.538
Cross River	35	0.516 ± 0.049	0.014 ± 0.013	0.595 ± 0.487
Knife River	3	---	---	---
Little Marais River[a]	5	---	---	---
Baptism River[a]	3	---	---	---
Split Rock River	11	0.327 ± 0.153	0.008 ± 0.009	0.327 ± 0.354
Encampment River[a]	1	---	---	---
Silver Creek[a]	2	---	---	---
Stewart River[a]	2	---	---	---
French River[a]	1	---	---	---
Hatchery Strains				
Owhi strain	52	0.317 ± 0.068	0.023 ± 0.018	0.950 ± 0.661
Phillips, ME strain	51	0.000	0.000	0.000
St. Croix strain	49	0.419 ± 0.084	0.021 ± 0.016	0.863 ± 0.619
Rome, NY strain	50	0.246 ± 0.071	0.006 ± 0.008	0.246 ± 0.283
MN Wild Strain	53	0.575 ± 0.058	0.021 ± 0.017	0.860 ± 0.617

[a] Populations with sample sizes of less than 8 were excluded from the analysis of population structure and genetic diversity.

Table 3. Frequency of occurrence of brook trout mitochondrial DNA composite haplotypes among Minnesota brook trout populations.

Population	N	BT1	BT2	BT3	BT4	BT5	BT6	BT7	BT8	BT9	BT10
Above Barrier Dams											
Lake Nipigon	37	0.88								0.12	
Reservation River	15	1.00									
Kimball Creek	43	0.51	0.28		0.14	0.05				0.02	
Kadunce Creek	50	0.58	0.38		0.02	0.02					
Devil Track River	50	0.46	0.34		0.06	0.10	0.02		0.02		
Spruce Creek	50	0.62	0.38								
Onion River	49	0.82	0.14		0.02	0.02					
Cross River	68	0.40	0.26		0.16	0.18					
Knife River	28	0.46	0.29	0.04	0.07	0.14					
Spring Brook (inland)	27	1.00									
Below Barrier Dams											
Nipigon River	13	1.00									
Nipigon Bay	23	0.83	0.04			0.08				0.13	
Grand Portage Creek	27	1.00									
Flute Reed River	2	1.00									
Kimball Creek	24	0.67	0.04		0.17				0.04	0.08	
Kadunce Creek	35	0.97								0.03	
Devil Track River	55	0.83	0.03	0.02	0.08				0.02	0.02	
Grand Marais Harbor	30	0.43	0.20		0.20	0.13	0.04				
Cascade River	8	0.87			0.13						
Spruce Creek	47	0.85			0.15						
Knife River	3	1.00									
Onion River	57	0.70	0.05	0.07	0.16	0.02					
Cross River	35	0.60			0.37					0.03	
Little Marais River	5	1.00									
Baptism River	3	1.00									
Split Rock River	11	1.00									
Encampment River	1			1.00							
Silver Creek	2	1.00									
Stewart River	2	1.00									
French River	1	1.00									
Hatchery Strains											
Owhi	52	0.81				0.19					
Phillips	51	1.00									
St. Croix	49	0.75	0.10			0.05	0.08		0.02		
Rome	50	0.86			0.14						
MN Wild	53	0.19	0.60		0.19						0.02

Table 4. Results of Analysis of Molecular Variance among Minnesota brook trout populations. Φ values are similar to fixation indices (Fst values) and P values are an estimate of the probability that random values would exceed observed values in 1023 permutations.

Source of Variation	df	Variance	% Variance	Φ	P
Pooled Wild Populations					
Among populations	22	0.044	11.5	$\Phi_{ST} = 0.115$	0.000
Within populations	818	0.334	88.5		
Grouped Above vs. Below vs. Hatchery					
Among groups	2	0.007	2.0	$\Phi_{CT} = 0.019$	0.106
Among populations within groups	27	0.043	11.5	$\Phi_{SC} = 0.117$	0.000
Within populations	1137	0.321	86.5	$\Phi_{ST} = 0.134$	0.000
Grouped Wild vs Hatchery					
Among groups	1	0.000	0	$\Phi_{CT} = -0.013$	0.786
Among populations within groups	28	0.050	13.6	$\Phi_{SC} = 0.134$	0.000
Within populations	1137	0.321	86.4	$\Phi_{ST} = 0.122$	0.000
Grouped Above vs. Below (no hatchery)					
Among groups	1	0.019	5.0	$\Phi_{CT} = 0.050$	0.015
Among populations within groups	21	0.033	8.6	$\Phi_{SC} = 0.091$	0.000
Within populations	818	0.334	86.4	$\Phi_{ST} = 0.136$	0.000

Table 5. Significance of differences in mtDNA haplotype frequency distribution (χ^2) in pairwise comparisons of hatchery populations and 6 wild populations in six Minnesota streams tributary to Lake Superior. Monte Carlo simulations were performed on 5,000 bootstrapped replicates (resampled with replacement) of pairwise population comparisons. Significance levels (n.s. = not significant; * = P<0.05; ** = P<0.005; *** = P<0.001) refer to the probability that the observed χ^2 estimate would be obtained by chance in 5,000 random samples.

		St. Croix	MN Wild	Owhi	Phillips	Rome
Above - Barrier	Kimball Creek	**	***	***	***	***
	Kadunce Creek	***	***	***	***	***
	Devil Track River	**	***	***	***	***
	Spruce Creek	***	***	***	***	***
	Onion River	n.s.	***	***	***	***
	Cross River	***	***	***	***	***
Below - Barrier	Kimball Creek	**	***	***	***	*
	Kadunce Creek	**	***	**	n.s.	***
	Devil Track River	*	***	***	*	n.s.
	Spruce Creek	***	***	***	**	n.s.
	Onion River	**	***	***	***	*
	Cross River	***	***	***	***	*

Figure Captions

Figure 1. Locations of brook trout samples taken for this study. Locations with asterisks were sampled above and below a barrier dam.

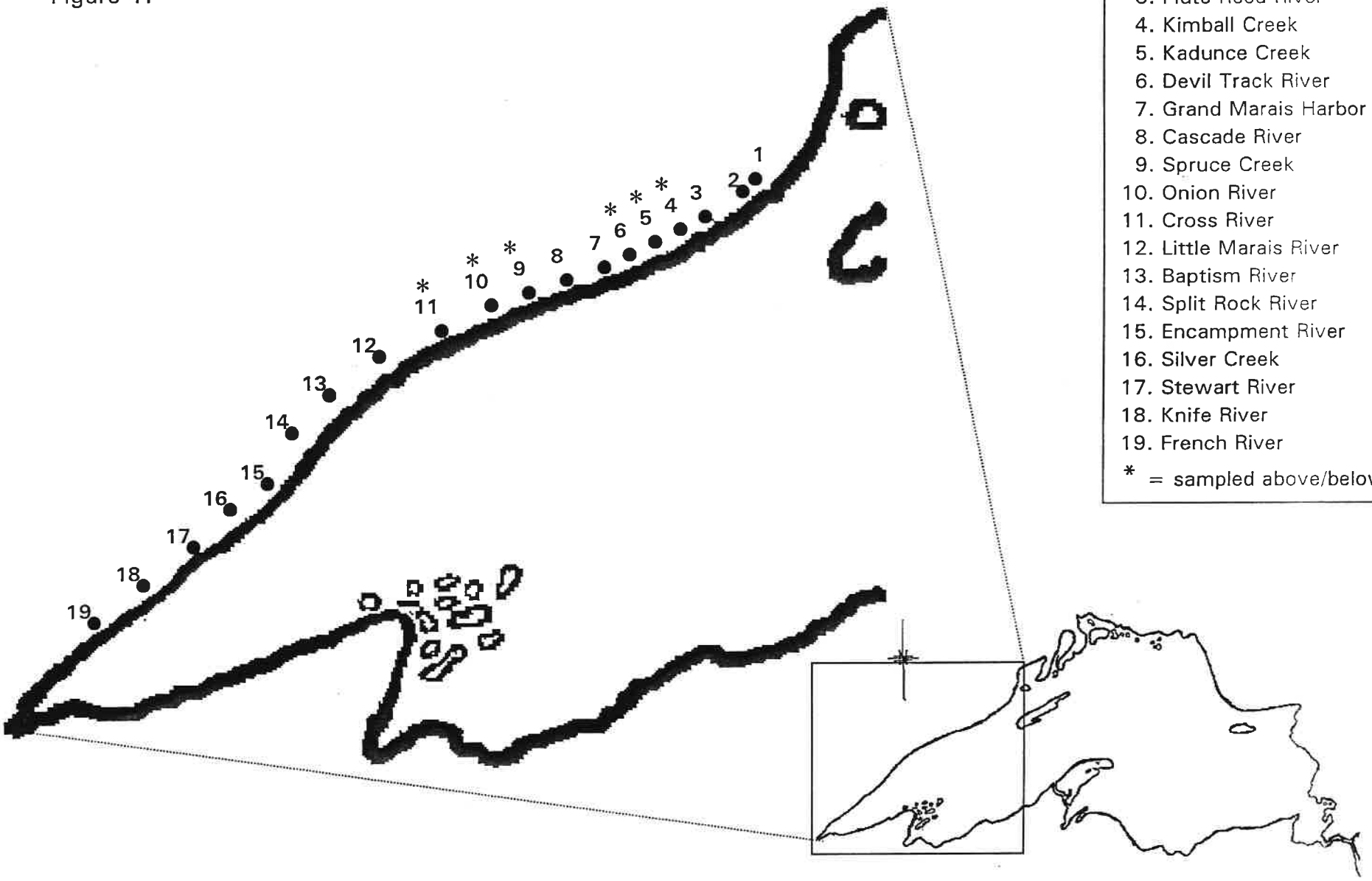
Figure 2. Cumulative frequency of mitochondrial DNA haplotypes compared among 6 tributary streams sampled above and below barrier dams. Although haplotype BT1 is most common across all sampling locations, there are significant differences between above- and below-barrier samples. 2 a) Locations arranged by river system; 2 b) locations arranged by above/below barrier source.

Figure 3. Comparison of cumulative frequency distribution of mtDNA haplotypes from Onion River populations, above and below-barrier from two sampling years. There is no significant difference between years for the above-barrier population. In contrast, there is a significant difference in haplotype distribution between years for the below-barrier population. Below the barrier, significantly more BT5 individuals are found in 1995, and BT3 appears in only in 1997 collections.

Figure 4. Most parsimonious network of relationships among the brook trout mtDNA haplotypes detected in the Great Lakes drainage basin. Within the upper Great Lakes, only haplotypes BT1-BT10 are present. The A, B, and C clades represent major haplotype lineages according to Danzmann et al. (1998). The B clade origin is from a northern Atlantic glacial refugium.

Figure 5. Consensus neighbor-joining dendrogram based on Cavalli-Sforza-Edwards chord distances for Minnesota brook trout populations. Genetic distances were calculated among pairwise comparisons of all populations (wild and hatchery) and randomized in 5,000 bootstrap replicates to obtain a confidence estimate of the branching pattern. Numbers at branching points indicate the percent of trees in 5,000 bootstrap replicates that contained the pictured arrangement of populations.

Figure 1.



Legend:

1. Grand Portage Creek
2. Reservation River
3. Flute Reed River
4. Kimball Creek
5. Kadunce Creek
6. Devil Track River
7. Grand Marais Harbor
8. Cascade River
9. Spruce Creek
10. Onion River
11. Cross River
12. Little Marais River
13. Baptism River
14. Split Rock River
15. Encampment River
16. Silver Creek
17. Stewart River
18. Knife River
19. French River

* = sampled above/below

Figure 2 a.

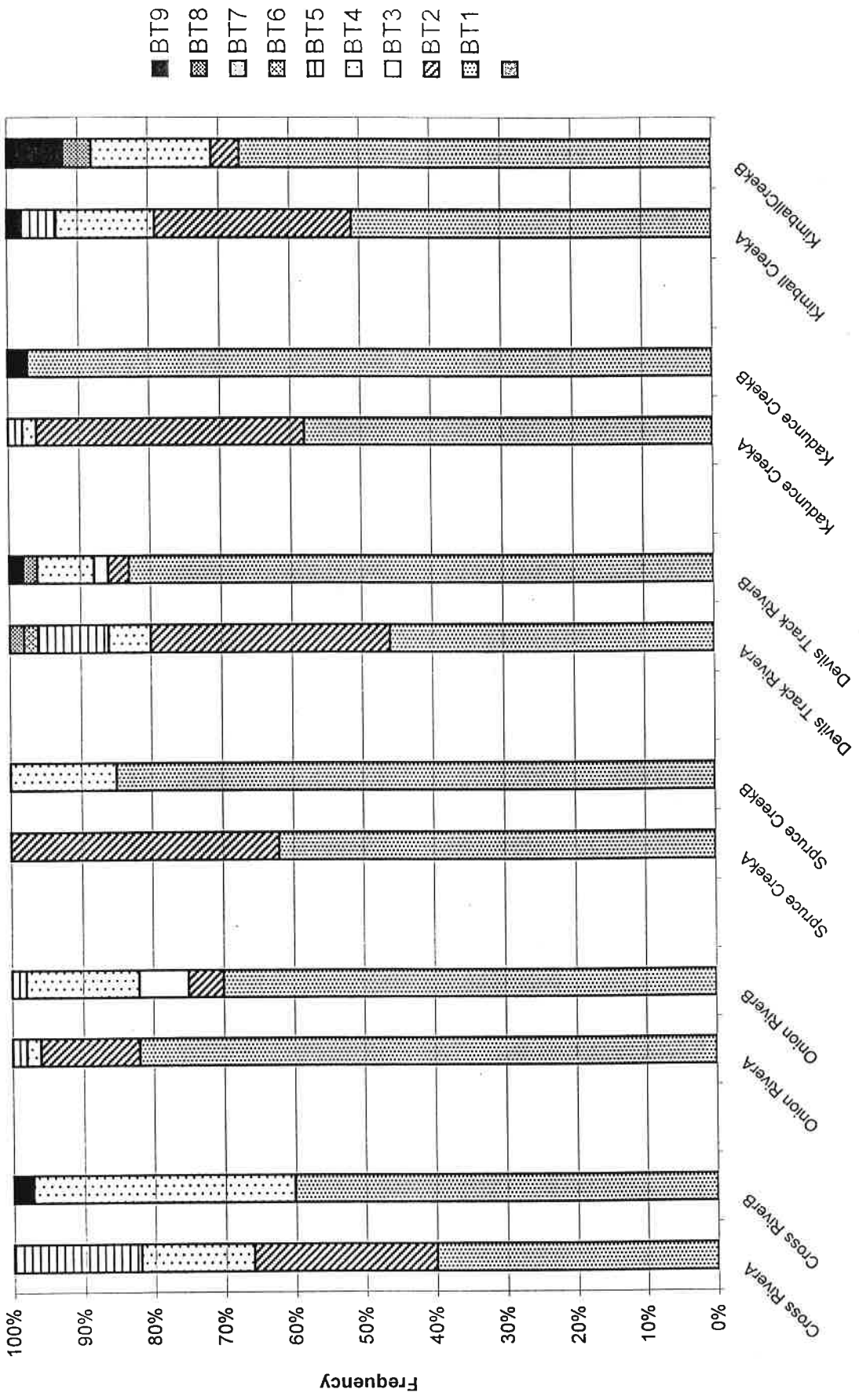

BT9
BT8
BT7
BT6
BT5
BT4
BT3
BT2
BT1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Frequency
Cross RiverA
Cross RiverB
Onion RiverA
Onion RiverB
Spruce CreekA
Spruce CreekB
Devils Track RiverA
Devils Track RiverB
Kaduncе CreekA
Kaduncе CreekB
Kimball CreekA
KimballCreekB

Figure 2 b.

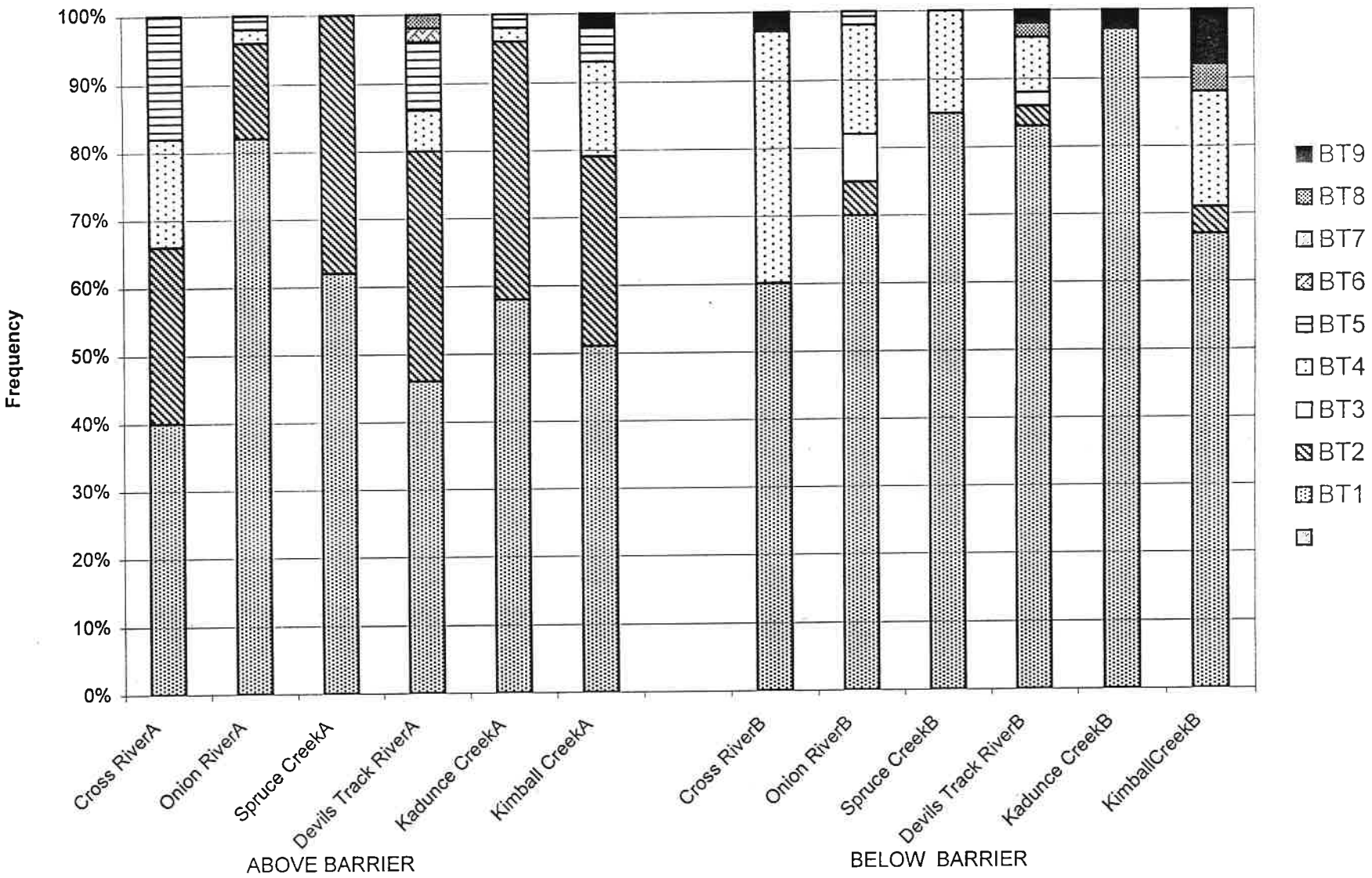

Frequency
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BT9
BT8
BT7
BT6
BT5
BT4
BT3
BT2
BT1
Cross RiverA
Onion RiverA
Spruce CreekA
Devils Track RiverA
Kadunce CreekA
Kimball CreekA
Cross RiverB
Onion RiverB
Spruce CreekB
Devils Track RiverB
Kadunce CreekB
KimballCreekB
ABOVE BARRIER
BELOW BARRIER

Figure 3.

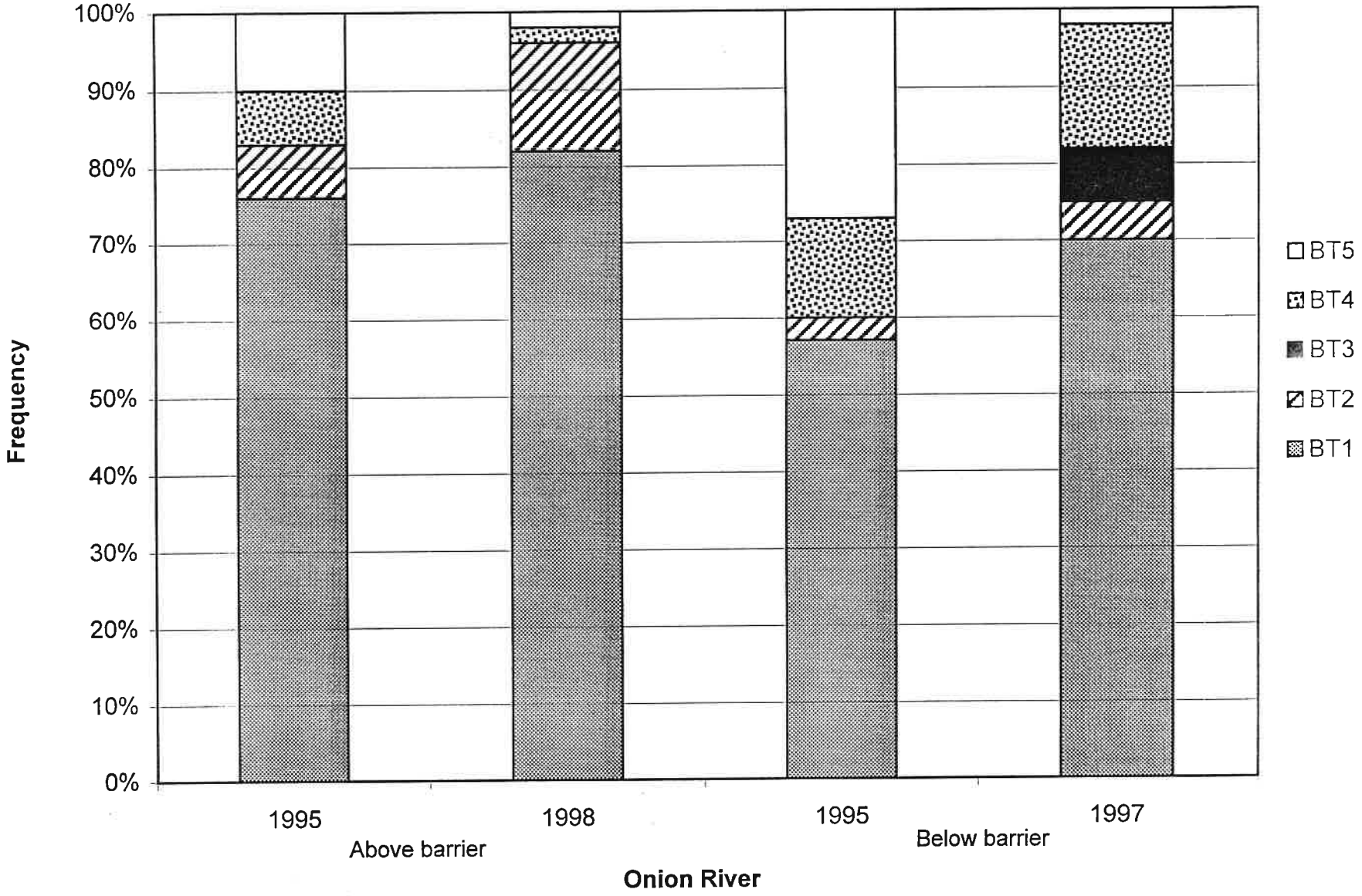

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Frequency
BT5
BT4
BT3
BT2
BT1
1995
1998
1995
1997
Above barrier
Below barrier
Onion River

Fig. 4

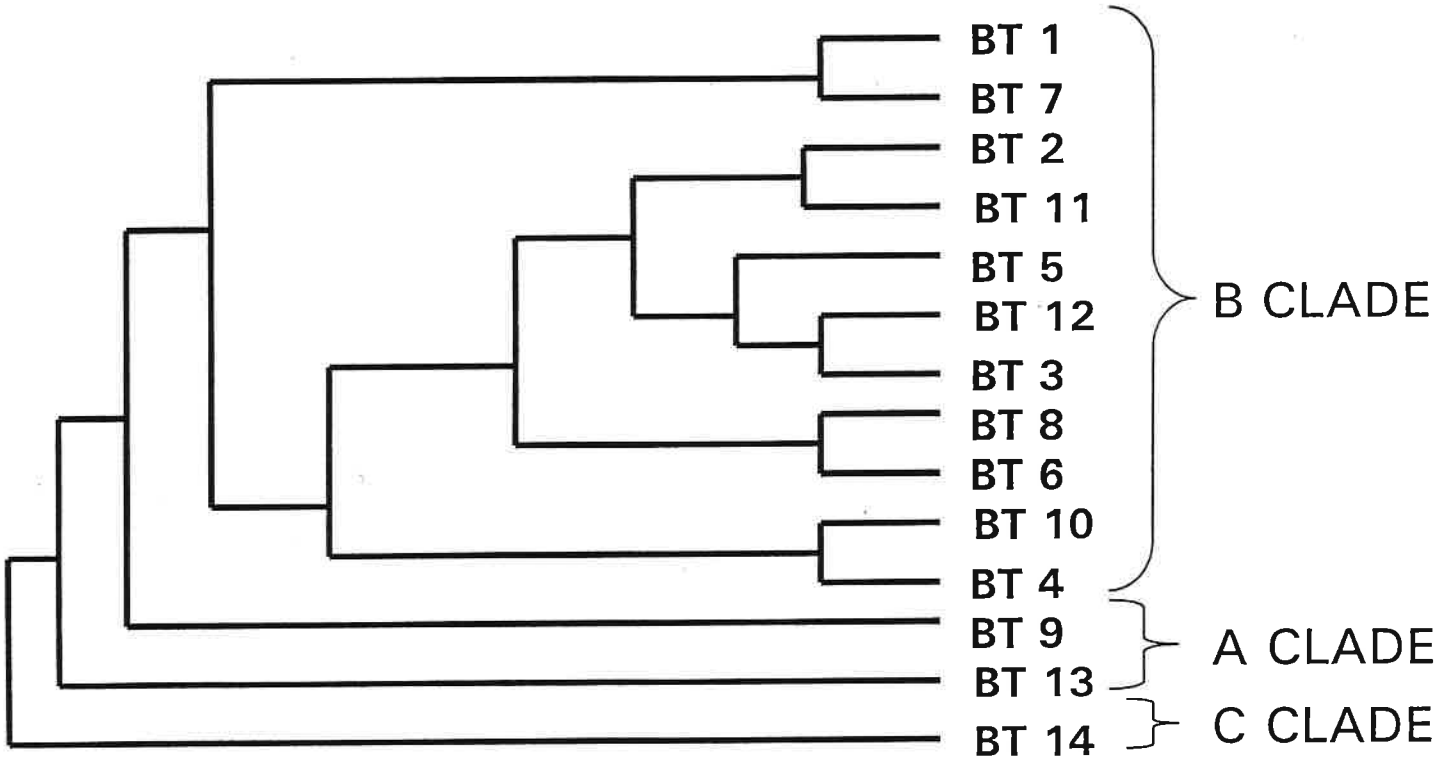
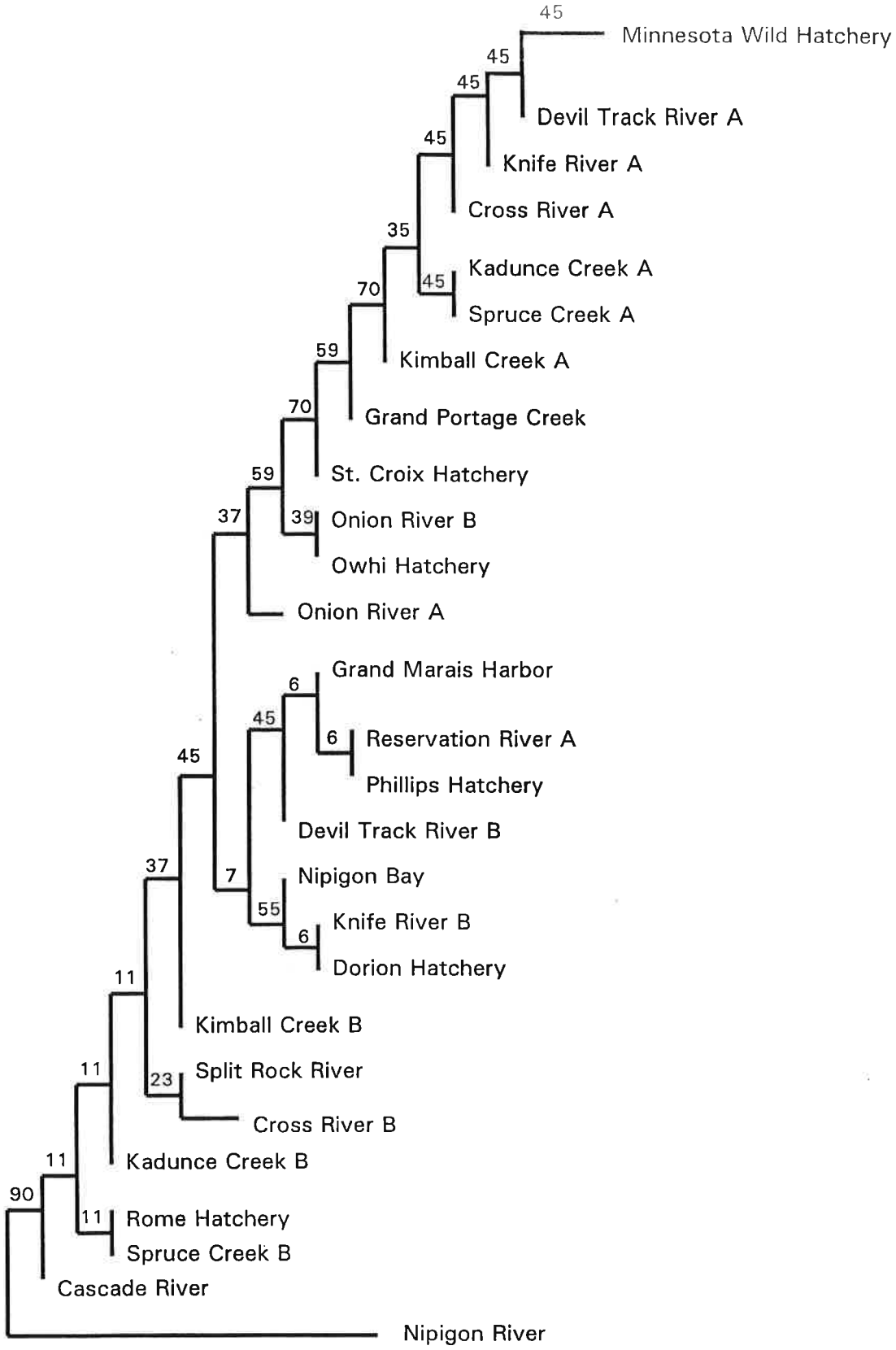

BT 1
BT 7
BT 2
BT 11
BT 5
BT 12
BT 3
BT 8
BT 6
BT 10
BT 4
BT 9
BT 13
BT 14
B CLADE
A CLADE
C CLADE

Fig. 5


45
Minnesota Wild Hatchery
45
45
Devil Track River A
45
Knife River A
Cross River A
35
Kadunce Creek A
70
45
Spruce Creek A
59
Kimball Creek A
70
Grand Portage Creek
59
St. Croix Hatchery
37
39
Onion River B
Owhi Hatchery
Onion River A
Grand Marais Harbor
6
45
6
Reservation River A
45
Phillips Hatchery
Devil Track River B
37
7
Nipigon Bay
55
Knife River B
6
Dorion Hatchery
11
Kimball Creek B
11
23
Split Rock River
Cross River B
11
Kadunce Creek B
90
11
Rome Hatchery
Spruce Creek B
Cascade River
Nipigon River