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CHAPTER I

INTRODUCTION

Overview

Advancements in the field of molecular genetics since the mid-1960's have

provided fisheries scientists with powerful investigative tools that can be used to answer

questions related to the genetics of fish. Fisheries biologists no longer have to rely upon

the uncertainty of phenotypic traits (length, weight, body condition, number of fin rays,

timings of maturity and spawning, etc.), that can be dramatically influenced by the

environment, to infer genetic relationships between and among fish populations. Genetic

data provides information on an organism's genotype, the precise information encoded by

its DNA that is transmitted from generation to generation. Innovative screening

technologies using molecular genetic markers, that allow researchers to investigate the

genetic composition and evolution of fish populations, are being applied to important

fisheries issues such as conservation, domestication, forensics, phylogeography,

reproductive success, stock identification, mixed-stock analysis, and taxonomy (Brown

and Epifanio 2003).

The conservation of native fish stocks has become an increasingly important issue

for fishery managers. The long-term survival of wild populations depends not only upon

preserving their natural environment, but also maintaining their capacity to evolve in that

environment. Maintaining a population's genetic (allelic) diversity is considered a key factor in this evolutionary process (Frankham et al. 2002). The ultimate source of genetic variation is heritable mutations, that is, changes in DNA sequence resulting in different alleles, which are passed to offspring. Natural processes, such as random genetic drift, bottlenecks, and inbreeding, can diminish the genetic diversity of a population (Frankham et al. 2002). Hybridization and introgression of nonnative genes can also result in a loss in allelic diversity, which can disrupt locally adapted genotypes and affect population fitness (Ferguson 1990). All of these processes can increase the risk that a population will become extinct. Information about the amount and distribution of genetic variability within and among populations is important in the development of rational conservation strategies for a species (Ryman 1981).

Salmonid fisheries (salmon, trout, and charr) have been a particular focal point for population genetics investigations because of their commercial and sporting value, and their relative ease of culture. The brook trout, *Salvelinus fontinalis*, is a charr native to coldwater streams and lakes in eastern North America (MacCrimmon and Campbell 1969; Scott and Crossman 1973) (Figure 1) and is highly valued for its aesthetic and sport fish qualities. This salmonid species has been the subject of numerous ecological studies (see studies cited by Scott and Crossman 1973; Raleigh 1982; and Schmitt et al. 1993). More recently Hudy et al. (2005) have documented the range-wide decline of brook trout in the eastern United States as a result of anthropogenic landscape changes, pollution, and competition from stocked salmonids.
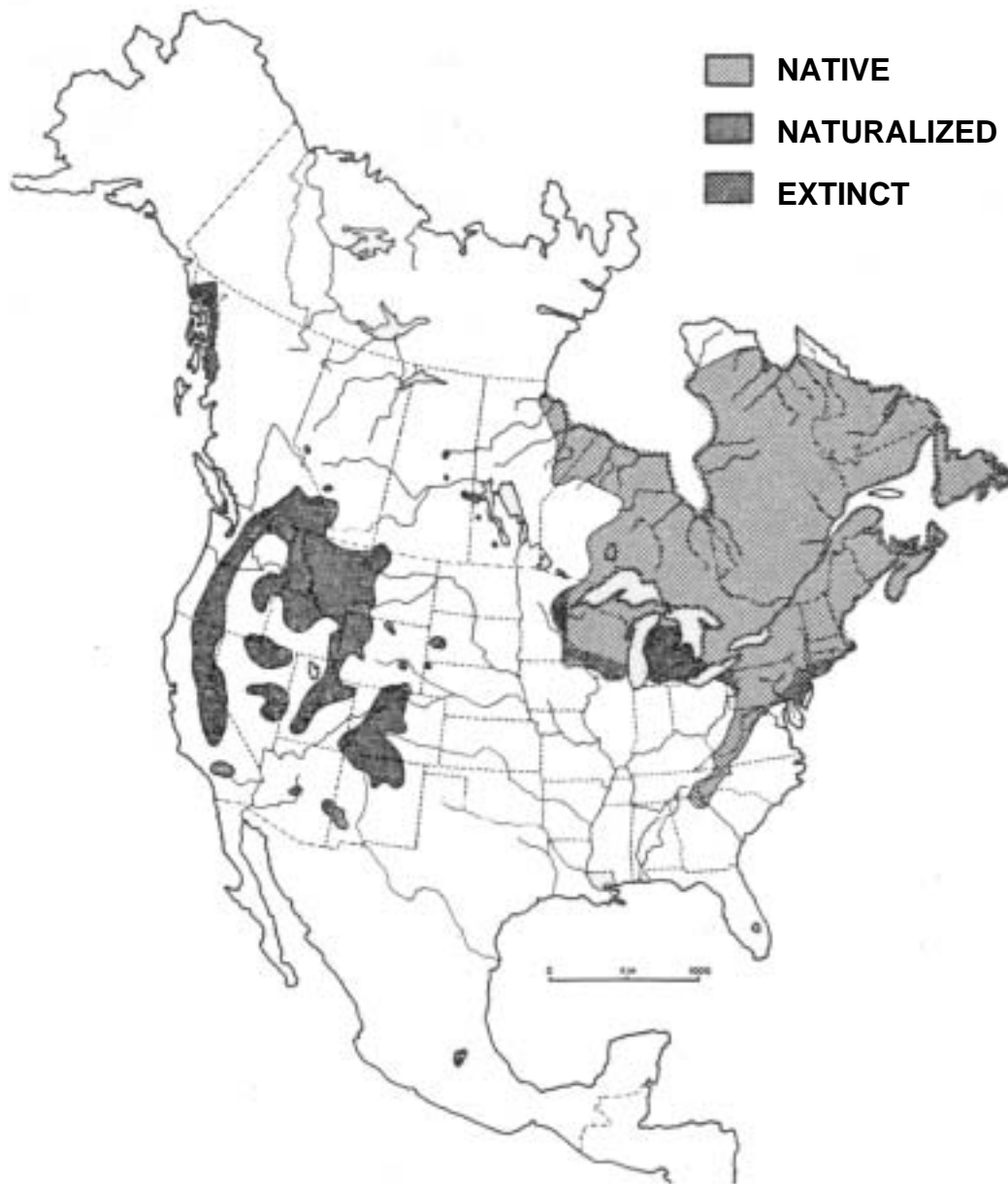
FIGURE 1.—Distribution of brook trout in North America (from MacCrimmon and Campbell 1969).

The distribution and population genetics of brook trout, and indeed many other freshwater fish faunas in North America, is deeply rooted in geological changes related to glaciation events. Repeated glacial advances and retreats during the Pleistocene Epoch, which commenced about 2.5 – 3.0 million years ago, profoundly affected the dispersal of northern temperate fishes and other freshwater organisms (Briggs 1986; Bernatchez and Wilson 1998). As glaciers advanced and receded, the distributional patterns of fishes were disrupted. Some populations were eliminated and those that were isolated lost genetic variation due to a reduced gene pool and genetic drift. Some fish populations occupying areas of refugia were able to re-invade glaciated areas where they could potentially differentiate (Briggs 1986). The last ice sheet retreated from the northern United States during the Wisconsinan glacial stage, 10,000 – 15,000 years ago.

The differentiation of evolutionary lineages and determination of native brook trout populations has been confounded by events far more recent than glaciers, and is directly related to the its popularity as a sport fish. In the United States, brook trout have been cultivated in hatcheries for more than a century, and both cultured and wild fish have been used to augment existing populations and establish new ones. The potential for introductions of nonnative brook trout strains to compromise the genetic integrity and fitness of wild populations through interbreeding is a major concern of fisheries managers (Perkins et al. 1993).

Prompted by questions regarding the phylogeography of brook trout populations across their native range, and the genetic hazards imposed by hatchery and transplantation programs, scientists began investigating the genetic structure and variation of wild brook trout populations in the 1970's. Over the last two decades,

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advances in laboratory techniques and computing technology have resulted in the

development of new classes of genetic markers and a rapid expansion in the power of

these markers to address a myriad of ecological questions (Selkoe and Toonen 2006;

DeYoung and Honeycutt 2005). Molecular markers used to assess genetic variation of

brook trout at the population level have been developed for proteins and also

mitochondrial and nuclear DNA. An overview of these molecular markers, the results of

investigations relevant to brook trout population genetics, the distribution and status of

brook trout in New Jersey, and the rationale and research objective for this study, are

presented in this chapter.

Molecular Genetics Approaches Used to Investigate Populations

One of the first molecular techniques developed for quantifying genetic variation

was protein electrophoresis, which can detect genetically different forms of proteins

encoded at the same locus (Avise 2004). However, the electrophoretic expressions of

proteins can be strongly affected by the length and conditions of sample storage (May

2003), and although proteins reflect differences at the DNA level, they are nonetheless

two steps removed from the gene itself and only a fraction of the genome codes for these

soluble enzymes (Avise 2004). Despite these shortcomings, protein electrophoresis

remains a viable tool for examining genetic diversity because the procedures are

relatively easy and inexpensive, large quantities of data can be produced quickly, and for

many species there are large baseline datasets (May 2003). However, more genetic

variation can be found at the DNA level, and in recent years molecular procedures have

been developed that can examine mitochondrial and nuclear DNA at the nucleotide level and provide a finer level of genetic resolution.

By the 1980's, technological advancements in molecular genetics gave scientists the ability to investigate the mitochondrial genomes of fish. Mitochondrial DNA (mtDNA) is a useful genetic marker, thanks to many of its unique attributes, such as the uniparental and nonrecombining mode of inheritance, simplicity of genomic organization, and relatively high point mutation rates compared to nuclear genomes (Moritz et al. 1987). The analysis of mtDNA sequence variation has proven most useful in defining major phylogenetic assemblages within species that were often undetected by allozymes and other genetic methods (Angers and Bernatchez 1998). Although many copies are present in each cell, early studies involving mtDNA often required the sacrifice of the fish so that purified mtDNA for whole-molecule analysis could be extracted from fresh or frozen tissue (liver or gonads).

The development of the polymerase chain reaction-based (PCR) method in 1986 allowed scientists to employ nonlethal sampling techniques to obtain minute amounts of mitochondrial and nuclear DNA from blood and fresh, frozen, alcohol-preserved, or dry tissue (fins, barbels, scales, muscle biopsy). Nuclear DNA (nDNA) contains most of the functional, protein-encoding DNA that provides instructions for making and, for the most part, maintaining an organism, as well as non-coding ("junk") DNA (Avise 2004).

Microsatellites, discovered in 1989, have become an increasingly popular and versatile means of assessing contemporary genetic variability. The term microsatellites refers to a class of co-dominant DNA markers that are inherited in a Mendelian fashion (DeWoody and Avise 2000). These markers are blocks of repetitive DNA, involving

tandem repeats of 1-6 nucleotides (such as $(AC)_n$ or $(GATA)_n$, where n lies between 5 and 50), that are scattered abundantly throughout the nuclear genome of most taxa. A pair of oliogonucleotide primers, designed to bind to the regions flanking the microsatellite, guide the amplification of the microsatellite locus during PCR.

Microsatellites typically far surpass allozyme loci in heterozygosity and number of alleles per locus (Avise 2004) and increase the probability that isolated populations diverge rapidly at these loci (Angers and Bernatchez 1998).   For genetic studies of processes acting on ecological time scales, high levels of allelic diversity are necessary and microsatellites are one of the few molecular markers that researchers can use to answer fine-scale ecological questions (Selkoe and Toonen 2006).

Studies Describing Genetic Variation in Populations of Brook Trout

Genetic studies of brook trout have employed a range of molecular markers, from allozymes and mtDNA to nuclear sequences and microsatellite DNA. Since the 1960's, researchers have used protein electrophoresis to analyze protein polymorphisms and compare the genetic diversity of brook trout populations. Building on earlier studies on protein polymorphisms in other fish species, Wright and Atherton (1970) surveyed allele frequencies at two protein loci, transferrin (*Tf*) and eye-specific lactate dehydrogenase (*LDH*), for seven northeast hatchery populations and eight wild brook trout populations. With only two loci, they were able to distinguish all hatchery strains, and some of the wild populations, and found the degree of variations of allele frequencies and the amount of heterozygosity was generally greater among hatchery fish than natural populations. Other early studies that examined protein polymorphisms in hatchery and wild trout also

found that some natural and hatchery populations brook trout were distinguishable from each other (Eckroat 1971; Eckroat 1973).

These early electrophoretic studies generally found that allele frequencies were often quite different among wild and hatchery populations of brook trout. They also provided limited biochemical evidence of possible genetic interchange between wild and hatchery brook trout stocks. However, study results were contradictory and the data interpretation was clouded by difficulties associated with the genetic interpretation of the isozyme banding patterns. In addition, the data could not be used to evaluate the genetic impact of stocking because stocking history information was lacking. Electrophoretic studies that included stocking histories soon followed and began to resolve lingering questions about the genetic relationships of wild brook trout populations over a broad geographical range, as well as the genetic effects of stocking.

Interest in brook trout population genetics was fueled by speculation that southern Appalachian brook trout (SABT) populations were taxonomically different from northern populations. This was based in part upon a limited amount of morphological data, such as smaller and more numerous red spots on the sides and different relative sizes of body parts (Lennon 1967). Researchers initially employed electrophoretic techniques to obtain genetic data that could be used to explore the taxonomic distinctness of SABT. Stoneking et al. (1981) compared allozyme variation among five wild northeastern populations and three wild southeastern populations with known stocking histories. The pattern of genetic variation observed suggested the existence of separate northern and southern phylogenetic lineages.

In a later study, stocked and unstocked populations of wild brook trout in the Great Smoky Mountains National Park (GSMNP), and brook trout from two northeastern U.S. hatcheries, were examined for variation in protein products encoded by 34 presumptive gene loci using starch-gel electrophoresis (McCracken et al. 1993). Putative native southeastern populations and northeast hatchery strains stocks were found to have substantial genetic divergence as a consequence of fixed genetic differences at one locus and allele frequency differences at nine loci. The CK-A2 locus, which codes for creatine kinase enzymes, was diagnostic for northern-derived and southern Appalachian strains of brook trout. Their data also showed relatively low average heterozygosity and polymorphism in all five native populations, relatively high variability in all three hatchery populations, and intermediate values of heterozygosity and polymorphism in all three of the populations comprised of mixed native and hatchery fish. These results were consistent with previous studies suggesting that native brook trout in the southeastern U.S. are taxonomically distinct from northeastern brook trout. Subsequent investigations involving allozyme analyses (Kriegler et al. 1995; Hayes et al. 1996; Guffey 1998, cited by Habera and Moore 2005; Galbreath et al. 2001) and molecular analyses that directly assayed DNA (discussed later in this chapter) support these earlier findings that northern-derived hatchery strains are genetically distinct from southeastern populations of brook trout. Protein electrophoresis has become the method of choice among fisheries management agencies to identify the genetic origin of brook trout populations in the southern Appalachians because of the existing large data set and relative ease of use.

As a result of these genetic and other ecological studies, fisheries managers in southeastern states began recognizing that brook trout populations in the southern

Appalachians had special management needs, which might include protecting and preserving their genetic integrity (Habera and Strange 1993). Kriegler et al. (1995) recommended that management programs that attempt to expand the current distribution of SABT should take into account the presence of hybrid and nonnative brook trout populations. They also cautioned that the genetic identity of brook trout populations can not be reliably inferred from stocking records, and genetic analyses are necessary to determine whether recorded or unrecorded stocking has affected the genetic composition of southern Appalachian brook trout populations. Continuing concern regarding distribution shrinkage and the long-term survival of SABT prompted the American Fisheries Society's Southern Division Trout Committee to release a position statement on managing SABT (Habera and Moore 2005). The authors indicated that the genetic identity of brook trout within this region is known for approximately 37% of the 3,000 km of stream length they inhabit, and of this, 47% supports SABT.

Investigators have also used protein electrophoresis to probe the genetic diversity of brook trout in other geographic regions. In Wisconsin, the long-term genetic impact of maintenance stocking upon wild brook trout populations was evaluated using blood and whole-eye proteins at several loci (Krueger and Menzel 1979). Hatchery stocks were genetically distinct from most wild populations at both loci, and reduced genetic variability was observed in the hatchery stock. Although significant correlation between allelic frequencies and stocking histories was found, the data did not provide compelling evidence of interbreeding between hatchery and wild stocks. The authors suggested that the study data indicated alteration of selective pressures induced by ecological interactions between the two stocks.

In New York and Pennsylvania, the genetic variability of wild brook trout populations was found to be organized by river basin, suggesting colonization of river basins by genetically different groups of brook trout at different times (Perkins et al. 1993). A high level of genetic differentiation was found, even within the same minor river drainage, for wild populations. Other allozyme studies have also found that high levels of population differentiation exist among brook trout populations located close to one another (Eckroat 1971; Krueger and Menzel 1979; Jones et al. 1996). Perkins et al. (1993) suggest that management strategies for conserving the genetic variability of wild brook trout should focus on individual lake and stream populations within river basins as the primary management units.

In summary, allelic protein data sets obtained through electrophoresis have provided convincing evidence that (1) demonstrates substantial genetic differentiation between northeastern and southeastern brook trout, (2) shows native gene pools have been altered through interbreeding of wild and hatchery fish, and (3) high genetic variability is present among local populations. Although protein electrophoresis will continue to be a useful tool in fishery management, technical advances in molecular genetics over the last two decades has prompted many researchers to shift from this traditional approach to direct assays of DNA.

Mitochondrial DNA analysis of population structure has been a useful method to ascertain the postglacial dispersal routes and phylogeographical structuring in many freshwater fishes (Danzmann et al. 1998). In the 1990's, researchers began using mtDNA markers to probe the genetic variability and phylogeographic patterns of brook trout. Quattro et al. (1990), using RFLP analysis of mtDNA from ten brook trout

populations inhabiting two major drainages in western Maryland, found two distinct matriarchal lineages that fell on either side of a major geographical feature – the eastern continental divide. Mitochondrial DNA variability in 49 populations of brook trout from the Algonquin Park region suggested that fish from two different glacial refugia colonized the southern and northern regions of the park (Danzmann and Ihssen 1995). In eastern Canada, mitochondrial DNA variation of brook trout showed low divergence among mtDNA haplotypes, which suggested a single glacial refugium for the trout that recolonized that region (Jones et al. 1996).

In a large-scale phylogeographic survey, Danzmann et al. (1998) examined 155 brook trout populations from eastern North America using RFLP analysis of mtDNA and identified six major phylogenetic clades (evolutionarily divergent lineages) of brook trout. Large phylogenetic differences between northern and southern populations were found. Populations outside the zone of glaciation were the most genetically heterogeneous, while low mtDNA diversity was found in northern brook trout populations inhabiting recently deglaciated regions of Canada and northeastern United States. The phylogenetic patterning suggests that the extent of mtDNA variation found in brook trout is related to geological events. The least amount of divergence was found in northern populations and the greatest divergence occurred in populations from a southern, unglaciated region. The patterning also lends support to an earlier hypothesis that brook trout recolonizing deglaciated areas originated from different refugial zones. Danzmann et al. (1998) recommended that certain lineages/populations be recognized as evolutionary significant units and managed as such.

Subsequent studies have yielded similar phylogenetic results. A large-scale analysis using allozymes and mtDNA revealed that the majority of genetic variance in brook trout populations was partitioned along major drainages or regions associated with distinct glacial refugia (Hébert et al. 2000). The evolutionary genetic relationships among mid-Atlantic brook trout populations from Maryland drainages, augmented with data from previously studied populations in Virginia, West Virginia, and Tennessee, was examined using RFLP analysis of mtDNA (Hall et al. 2002). Genetic diversity among these populations was considered high, when compared with results from northern populations analyzed previously. The mosaic patterning of mtDNA variation observed in these mid-Atlantic brook trout populations suggests that the region may be a transitional zone between major historical lineages - the genetically diverse southern populations and the relatively homogenous northern groups.

Mitochondrial DNA studies also support the findings of earlier allozyme studies that indicated that Appalachian brook trout are distinct evolutionary entities. Comparisons of mtDNA have also been used to discriminate hatchery and wild stocks, by using mtDNA haplotype variation to determine the level of introgression of nonnative genes in wild brook trout populations. A high degree of genetic differentiation between two hatchery stocks and two wild brook populations in Ontario was detected through RFLP analysis using 51 restriction enzymes (Danzmann et al. 1991). This survey showed that by sampling a high number of restriction enzymes, unique clonal variants might be discovered that can unambiguously discriminated hatchery and wild fish. While the sharing of mtDNA haplotypes by both wild and hatchery brook trout does not indicate

that the wild fish are of hatchery origin, the presence of unique haplotypes in wild fish does preclude their being of hatchery origin.

A subsequent study showed no or very low frequencies of mtDNA 'hatchery' haplotypes in wild populations in Algonquin Park, Ontario despite extensive plantings of hatchery reared trout (Danzmann and Ihssen 1995). Comparisons of mtDNA haplotypic distributions in hatchery and wild fish also suggested that hatchery females had minimal spawning success and/or their progeny survived poorly in the wild. In the southern Appalachians a comparison of the genetic diversity of native, stocked, and hybrid brook trout populations showed that native fish were genetically distinct from hatchery-derived fish and could be distinguished using three restriction enzyme sites (Hayes et al. 1996).

Although protein electrophoresis and mtDNA analyses still have utility in the exploration of genetic variability in organisms, the development of newer screening technologies that allow direct assessment of nuclear DNA sequence variation are gaining in popularity. Researchers are increasing utilizing more recently developed PCR-based methods, particularly microsatellite analysis, which allows direct assessment of nuclear DNA variation.

The development of microsatellite primers for brook trout has lagged in comparison to other commercially important salmonid species, and much of the molecular work in this genus has relied upon cross-familial amplification of microsatellites from other salmonid species (Perry et al. 2005). Limited success in applying microsatellite primers developed for other salmonids to brook trout prompted efforts to isolate specific microsatellite loci from a partial genomic library brook trout. Angers et al. (1995) successfully isolated seven microsatellite loci and used them to

examine brook trout populations in five geographically proximal lakes in Quebec. Four of the microsatellites were moderately to highly polymorphic (5 – 18 alleles detected) and this contrasted with the low mtDNA variation generally observed in this species for the region surveyed. The results of this study suggested that microsatellite loci could be valuable in addressing fine scale population genetics structuring in brook trout.

In an expanded study, involving 26 brook trout populations in a National Park in Quebec, microsatellite and mtDNA variation was characterized and compared by Angers and Bernatchez (1998). Their analysis of microsatellite variation revealed extensive polymorphism, which resolved a finer population structuring than mtDNA. These results lent additional support to the authors' hypothesis that microsatellites may be more appropriate than mtDNA for inferring relationships among closely related populations.

Microsatellite studies have been used to analyze relationships between intrapopulational genetic diversity of brook trout and landscape features such as hydrogeography and habitat types. The relationship of hydrography and population genetic structure of brook trout from eastern Canada was explored using six microsatellites (Hébert et al. 2000). Each of the 24 populations examined represented distinct, nonrandomly mating populations, even when found in the same drainage over short distances (less than five kilometers). Riverine populations of brook trout have been shown to have consistently higher levels of allelic diversity than lacustrine populations (Hébert et al. 2000; Angers and Bernatchez 1998; Castric et al. 2001). No correlation was found between habitat size and intrapopulational genetic diversity (Hébert et al. 2000; Angers et al. 1999; Castric et al. 2001). However, altitude has been shown to strongly influence genetic variability among brook trout populations, with lower

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heterozygosity observed in higher elevation populations, presumably constrained by physical barriers that influence dispersal and gene flow processes (Angers et al. 1999; Castric et al. 2001).

A suite of 13 microsatellite markers for brook trout, developed by the U.S. Geological Survey (USGS) - Leetown Science Center, Kearneysville, West Virginia (T. King, personal communication), has been used to investigate the amount and patterns of genetic diversity of brook trout from 125 collection sites in Canada and the U.S. King (2006) found high levels of genetic diversity among brook trout and demonstrated genetic differences at scales ranging from local streams to river basins, including differences among regions, major drainages, watersheds, streams, and specific locations within streams. Much of the genetic diversity was found in the mid-Atlantic region, with differences associated with the geographical separation of major drainages (Atlantic slope and Ohio River), while very low levels of diversity were found in certain southern Appalachian populations. In some of the populations studied, the impacts of stocking were discernable. This, and previously mentioned research, has demonstrated the ability of microsatellite DNA analysis to reveal fine-scale population structure and patterns of genetic divergence that may prove useful in developing a conservation roadmap for this species.

A variety of molecular screening techniques have been used to obtain genetic data sets to investigate the genetic variability within and among brook trout populations in many geographic areas of their native range. These studies contribute to greater knowledge and understanding of wild brook trout resources and aid resource managers in the development of conservation strategies for indigenous populations. For example,

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existing populations of trout that have been determined to be remnants of fish that originally colonized an area after deglaciation have been termed "heritage" trout (Perkins et al. 1993). Efforts to identify and preserve the gene pools of genetically distinct southern Appalachian brook trout populations have been undertaken by state fish and wildlife agencies, most notably in North Carolina, Virginia, and Tennessee (Habera and Strange 1993). With interest in brook trout conservation growing, molecular genetics is poised to play an increasingly key role in management decisions that will affect the short and long-term survival of this fish species.

Distribution and Status of Brook Trout in New Jersey

Brook trout is the only salmonid species native to New Jersey, but unfortunately the distribution of this species in New Jersey prior to the late 1960's is poorly documented. Using available data dating back to 1862, Fowler (1920) published a list of the fishes of New Jersey, in which 16 (of 21) counties and a handful of localities therein were named where brook trout were known to occur. More than half the localities (21) were in central and southern counties, while only 10 were given for counties in north Jersey. In relation to his list for brook trout, Fowler stated "In many localities formerly, now largely introduced", but did not differentiate between wild or stocked trout for localities listed. Fowler's list does not appear to be particularly comprehensive, judging from the paucity of localities given for other, more ubiquitous native freshwater fishes, most notably cyprinids (minnows), catostomids (suckers), and ictalurids (catfishes).

Unpublished records kept by the NJDFW, including stream assessments conducted in the late 1800's, and surveys conducted from 1918 –1920 under the direction

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of four biologists (W.T. Foster, F.N. Miller, H.E. Schradieck, and H.M. Spandau), suggest brook trout were more widespread. However, the lack of detail (trout species not identified, no indication of wild vs. stocked trout, survey location not specified, etc.) limits the usefulness of these and other data in describing the distribution of brook trout in New Jersey prior to stocking activities. In a comprehensive range-wide review of the worldwide distribution of brook trout (MacCrimmon and Campbell 1969), a brief description of the brook trout's occurrence in New Jersey is given. Relying upon a personal communication with Charles Hayford, then the Director of the New Jersey Division of Fish, Game, and Shellfisheries, the authors stated that "in New Jersey, where the species was found in nearly all counties, native brook trout populations now exist only in headwater streams of the northwestern counties of Sussex, Warren, Morris, and Passaic." Their map depicting the North American distribution of brook trout (Figure 1) conveys the false impression that brook trout had been extirpated from New Jersey. The present day occurrence of brook trout in New Jersey is more widespread than previously reported in the literature. In addition to those counties cited by MacCrimmon and Campbell (1969), fish surveys conducted by the New Jersey Division of Fish and Wildlife (NJDFW) from 1968 to 2003 have documented wild populations in the counties of Hunterdon, Somerset, Bergen, and Camden (Hamilton and Barno 2005). During this period, wild brook trout populations were found in 120 streams scattered across forested hills and mountains in the northern tier of the state, and also in one south Jersey stream. These streams are located in the freshwaters of four major river systems (Delaware, Hudson, Passaic-Hackensack, and Raritan) within the Atlantic Slope drainage (Figure 2). No anadromous populations have been documented in rivers where access to marine
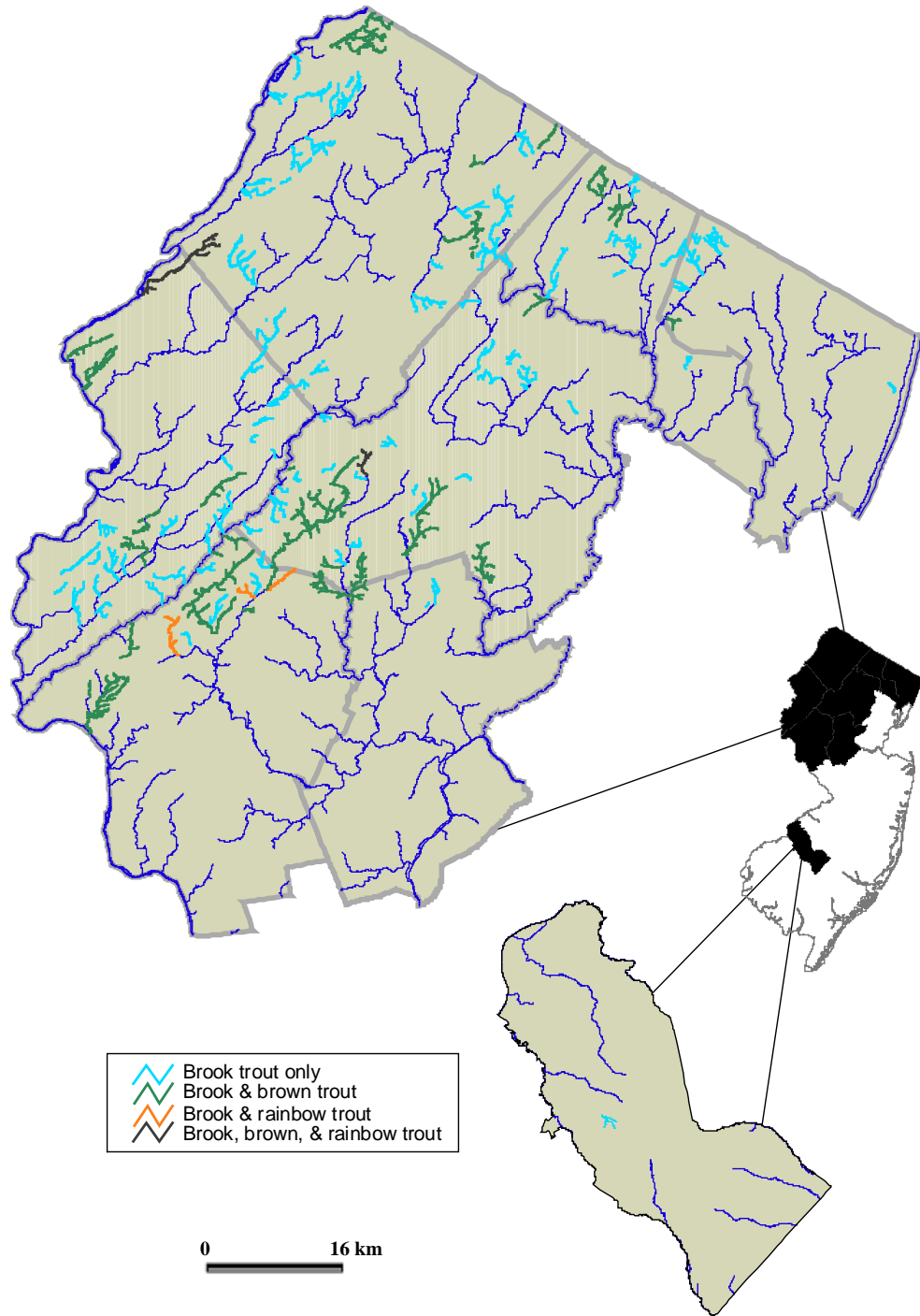
FIGURE 2.—Distribution of wild (spawning) brook trout populations in New Jersey as documented by stream surveys conducted by the New Jersey Division of Fish and Wildlife from 1968 through 2003 (from Hamilton and Barno 2005).

environments exists. Differences in coloration and markings on brook trout residing in different streams in New Jersey has also been observed (Figure 3).

The known distribution of brook trout in New Jersey, as documented by NJDFW over a 35-year period (1968 – 2003), appears to be strongly related to geomorphology. The majority of New Jersey's wild brook trout populations can be found in streams located within two physiographic provinces, the Valley and Ridge and the Highlands, and to a much lesser extent in the Piedmont province along its northern and western fringes (Figure 4). These three provinces are located within the Appalachian Rise and lie to the north and west of the Fall Line. The Fall Line separates the hard metamorphic rocks of these provinces from the older, unconsolidated sediments of the Coastal Plain provinces Dalton 2003).

Phylogenetic studies of brook trout across its native range have demonstrated the importance of glacial events in shaping the distribution and genetic diversity of this species. New Jersey has undergone at least three glaciations during the last one and half million years of the Pleistocene Epoch (Witte 1998). The last ice sheet, which occurred during the late Wisconsinan advance, began to recede from its maximum extent roughly 17,000 – 18,000 years ago (Briggs 1986). In New Jersey, the furthest advance of the Wisconsinan ice mass is marked in most places by a terminal moraine known as the Ronkonkoma moraine (Figure 5). This moraine forms a nearly continuous low ridge, from Belvidere eastward through Perth Amboy to New York, and effectively delineates glaciated and unglaciated regions that resulted from this last glacial stage (Witte 1998).

FIGURE 3.—Examples of color variation in wild brook trout from New Jersey streams. (A) Burnt Meadow Brook (Passaic drainage), (B) Turkey Brook (Raritan drainage), (C) Cooley's Brook (Passaic drainage), and (D) Lake Stockholm Brook (Passaic drainage).
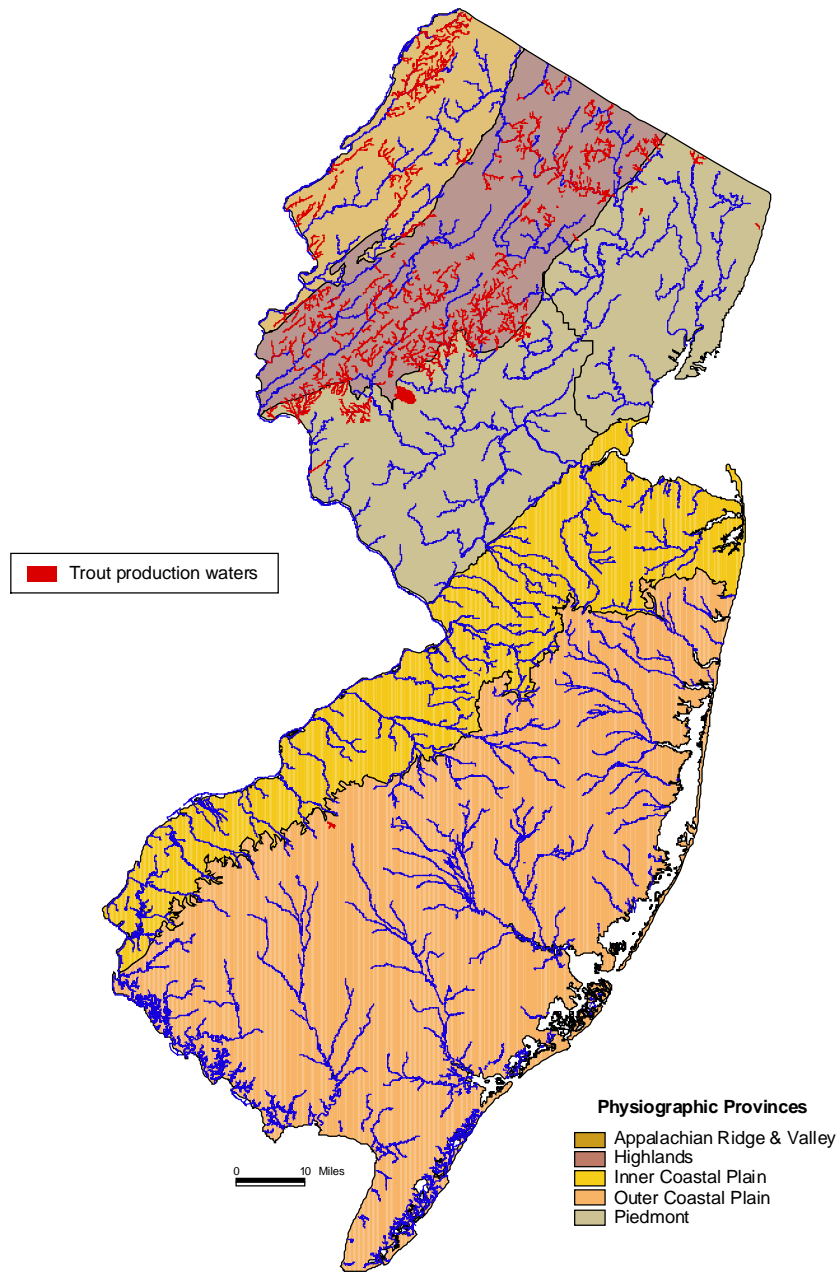
FIGURE 4.—New Jersey's physiographic provinces and freshwaters having self-sustaining salmonid populations (trout production waters), as documented through NJDFW surveys conducted from 1968 through 2003 (Hamilton and Barno 2005).
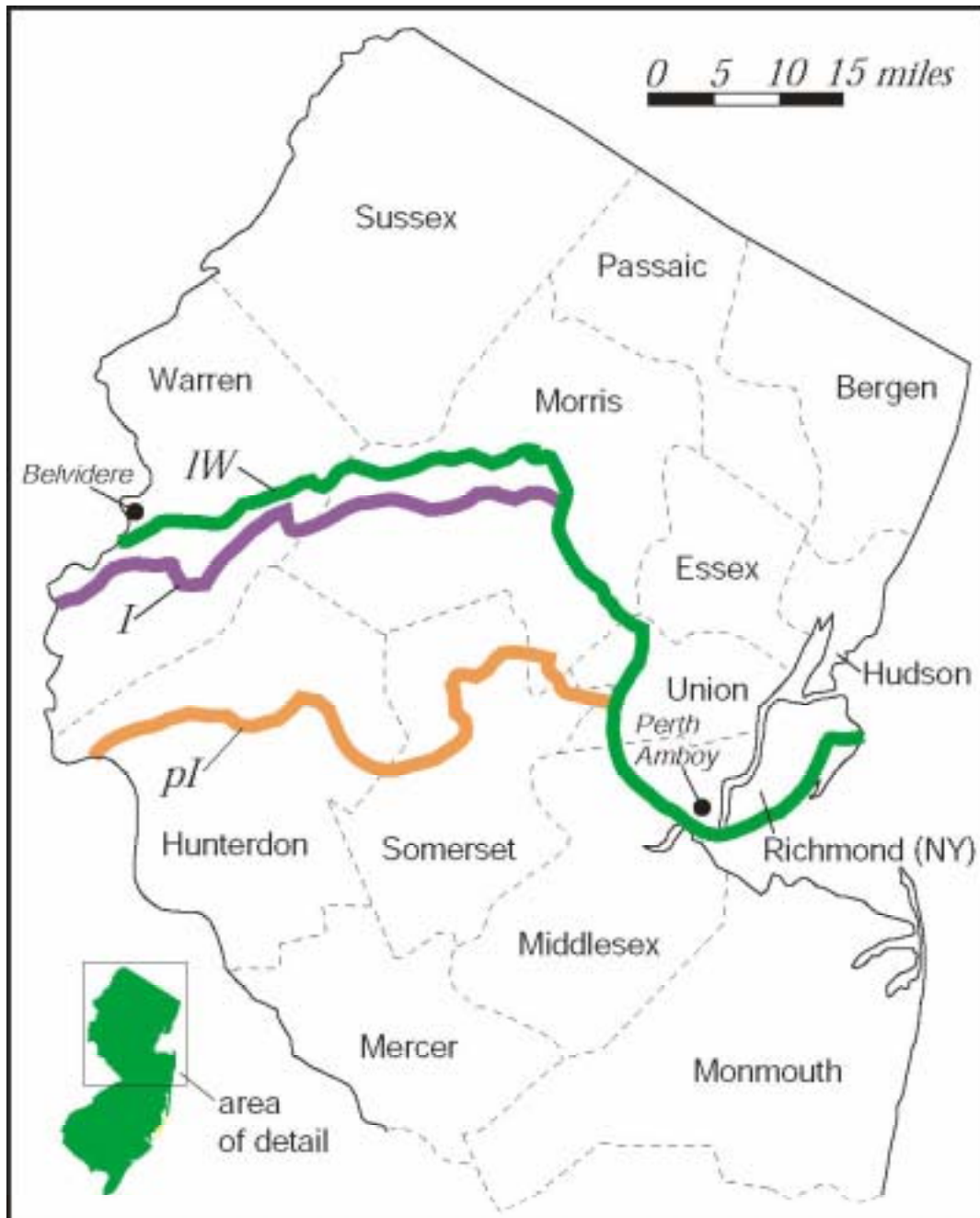
FIGURE 5.—Limits of glaciation in New Jersey and nearby New York. The trace of the *IW* limit generally marks the position of the Terminal (Ronkonkoma) Moraine. *IW* – late Wisconsinan, *I* – Illinoian, and *pI* – pre-Illinoian (modified from Witte 1998).

Although glacial events have likely shaped the distribution and genetic structure of brook trout populations in New Jersey, this relationship has not been confirmed. Events far more recent than glaciers, beginning with European colonization of North America, have likely impacted brook trout populations in New Jersey and throughout their native range. A recent range-wide assessment of brook trout in the eastern United States, based upon the professional opinion of experts from state and federal agencies, identified where wild brook trout populations remain strong, where they are struggling, and where they have vanished (Hudy et al. 2005; Figure 6a).

This assessment also categorized a variety of threats to brook trout and their habitats. In New Jersey, it was estimated that brook trout persist in less than half their original range (Figure 6b). The five most pervasive impacts considered to have affected New Jersey's native brook trout were sedimentation (roads), urbanization, dam inundation/fragmentation, high water temperature, stream fragmentation (roads), and one or more non-native fish species (trout). Man-made dams have not only contributed to the demise of many of New Jersey's brook trout populations, through elimination or degradation of habitat, but also fragmented their habitat, which has resulted in reproductive isolation of brook trout populations. Some wild brook trout populations may have benefited from habitat fragmentation, if artificial barriers successfully prevented interbreeding with cultured brook trout or intrusion and colonization by competing cultured trout species stocked in downstream waters.
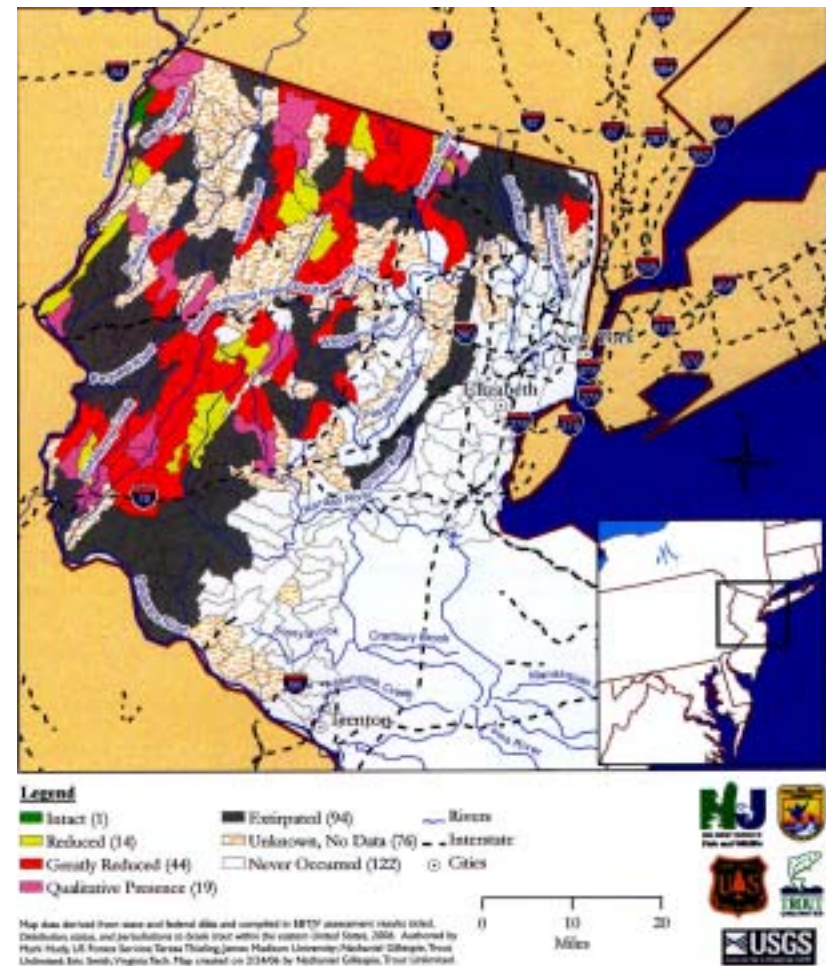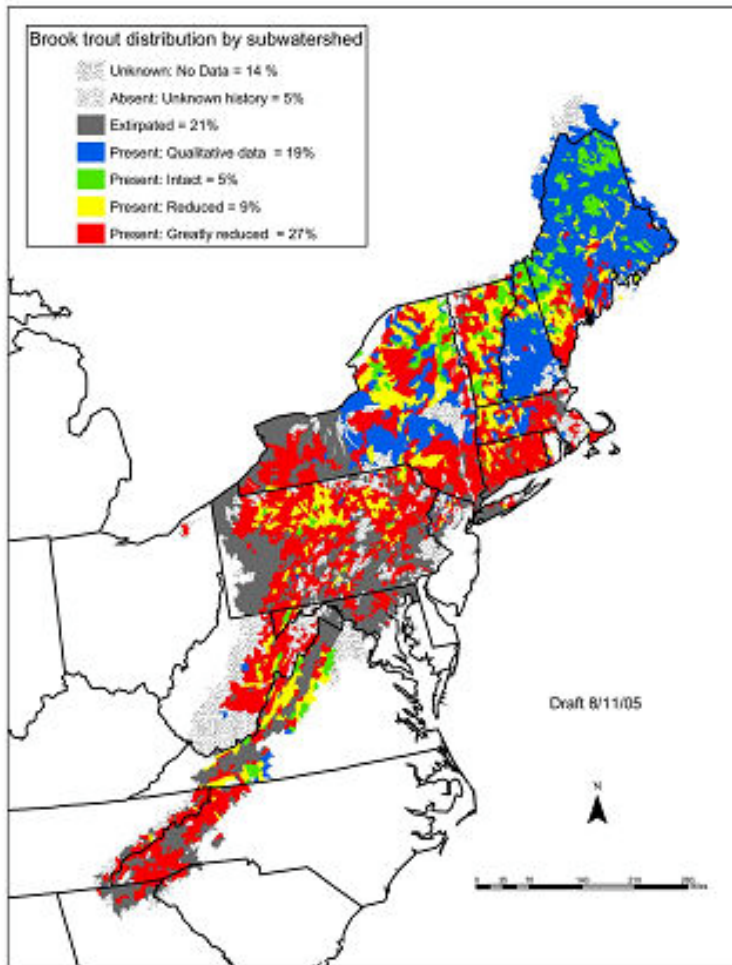
FIGURE 6.—Distribution and assessment of the status of wild brook trout in the eastern United States (left), with detail provided for New Jersey (right) (Hudy et al. 2005).

For many years, stocking hatchery-reared fish has been the most common way to meet the demand for recreational angling and to restore declining fish stocks, with little regard to the ecological and genetic consequences for native stocks (Nielson 1993). In New Jersey, a catastrophic drought in 1875 triggered the first stocking of hatchery-reared trout (fingerling brook trout) to re-establish trout populations in streams where they had been depleted. Soon after, in 1882, rainbow trout (*Oncorhynchus gairdneri*) were introduced and brown trout (*Salmo trutta*) followed in 1908 (Hamilton and Barno 2005). As rearing techniques were refined, and hatchery facilities expanded to meet angler demand for trout, the production and stocking of trout increased. The state's Hackettstown State Fish Hatchery, one of the oldest trout hatcheries in the U.S., discontinued production of approximately 500,000 brook, brown, and rainbow trout in 1985 after more than 70 years of operation (Hamilton and Barno 2005). The origin of the strain of brook trout cultured at this hatchery is not known.

In 1984, NJDFW began stocking trout reared at a newly constructed, disease-free facility, the Pequest Trout Hatchery. The brook trout at this facility originated from eggs obtained from North Attleboro National Fish Hatchery in Massachusetts (Nashua strain – Atlantic Slope origin). Currently, NJDFW produces and stocks more than 600,000 brook, brown, and rainbow trout in nearly 200 waters statewide to enhance recreational angling (Hamilton and Barno 2005). Of these trout, approximately 250,000 are catchable-sized brook trout that average 26 cm. Much smaller numbers of trout, purchased by local fishing clubs from privately owned fish hatcheries in New Jersey and surrounding states, are also stocked annually in New Jersey waters.

Repeated annual stockings of salmonids for nearly a century has resulted in the establishment of spawning populations of non-native salmonids in New Jersey. Stream surveys conducted by NJDFW from 1968 through 2003 documented 183 self-sustaining trout populations, and of these, barely half (94) were comprised solely of brook trout (Hamilton and Barno 2005). Of the remaining 89 streams, brook trout occurred in sympatry with naturalized populations of brown and/or rainbow trout in 27 streams (16% overall), and 62 streams (34% overall) had wild trout populations consisting exclusively of brown and/or rainbow trout. Hybridization between brook and brown trout has also been documented in two streams where wild populations of both species occur (Dunnfield Creek and the S/Br. Raritan River; NJDFW electrofishing surveys). These patterns suggest that hatchery supplementation with all three species, and perhaps translocations by well-intentioned managers and anglers, has caused displacement of native brook trout and facilitated potential interbreeding of non-native strains of brook trout with native brook trout populations.

Study Rationale and Research Objective

Brook trout are valued for their beauty, sport fish qualities, and as indicators of good water quality and a healthy ecosystem. Over much of their historic range in the eastern United States, wild populations of brook trout have declined due to a combination of land and water practices, and competition with non-native fishes (Hudy et al. 2005). Previous studies have described levels of genetic diversity in brook trout across their native range and demonstrated that geologic events, landscape features, and stocking of non-native salmonid species and brook trout strains have affected the occurrence and

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genetic structuring of brook trout populations. However, no genetic studies have evaluated brook trout from New Jersey waters.

The objective of this study was to characterize genetic variation within and among wild brook trout the populations in New Jersey, and evaluate patterns of fine-scale genetic variation to resolve questions regarding their genetic ancestry and integrity. Thirteen polymorphic microsatellite DNA markers were used to examine the genetic diversity of a subset of spawning brook trout populations in New Jersey. A hierarchy consisting of river drainages, subdrainages and individual populations was used to examine the distribution of gene diversity. The wild populations, some having a history of trout stocking and others suspected of being genetically "pure", were also compared with stock collected from a hatchery. In gathering this baseline information I hope to provide insight into the genetic variation of brook trout that will prove useful in shaping management strategies to ensure the long-term viability of wild brook trout populations in New Jersey and elsewhere in their native range.

CHAPTER II
MATERIALS AND METHODS

Study Design

  Twenty-two streams containing naturally reproducing populations of

brook trout were sampled during 2000 to provide data from all major New Jersey

drainages known to contain wild brook trout (Figure 7). Study streams were

generally small, first or second order streams that were primarily located in the

headwaters of larger river systems routinely stocked with catchable-size cultured

trout (Table 1). Nineteen of these streams, considered to have high potential for

harboring indigenous brook trout populations, were selected using the following

criteria: (1) no documented trout stocking history, and (2) absence of reproducing

populations of brown and/or rainbow trout (which indicate prior salmonid

stocking). Streams having natural barriers that could genetically isolate brook

trout populations and prevent interactions with cultured trout stocked downstream

were considered ideal candidates, but only one stream selected (Crooked Brook

tributary) was able to meet this additional criterion. Subsequent to sampling it

was learned that one of the 19 streams selected, Hacklebarney Brook, \was

stocked with trout in the past by NJDFW, and Cresskill Brook may have

Delaware Drainage

1. Forked Brook
2. Van Campens Brook
3. Independence Brook
4. Halfway House Brook
5. Kurtenbach's Brook
6. Mason's Run

Hudson Drainage

7. Mud Pond Outlet Stream

Passaic – Hackensack Drainage

8. Cresskill Brook
9. Preakness Brook
10. Havemeyer Brook
11. Cooley's Brook
12. Burnt Meadow Brook
13. Lake Stockholm Brook
14. Hibernia Brook
15. Crooked Brook tributary

Raritan Drainage

16. Flanders Brook
17. Krueger's Creek
18. Turkey Brook
19. S. of Hoffmans tributary
20. Rocky Run
21. Oakdale Creek
22. Hacklebarney Brook

Reproducing Trout Species

- Brook trout only
- Brook & brown trout
- Brook & rainbow trout
- Brook, brown, & rainbow trout

0   16 km

FIGURE 7.— Map indicating the location of 22 sites in New Jersey where brook trout, *Salvelinus fontinalis*, were collected in 2000.

TABLE 1.—Location and trout stocking history information for 23 brook trout collection sites in New Jersey. Trout stocking history information was obtained from NJ Division of Fish and Wildlife records, unless otherwise noted (M = mainstem stream; T = tributary to mainstem stream).

Site code	Drainage	Mainstem stream	Tributary	Latitude Longitude	Trout stocking history
FOR	Delaware	Big Flat Brook	Forked Brook	41°14'24.40"N 74°44'48.30"W	M - stocked annually 40+ yrs T - no record of stocking
VCB	Delaware	Delaware River	Van Campens Brook	41°04'36.00"N 74°57'30.59"W	M - generally not stocked along NJ/PA T - stocked extensively prior to 1979
IND	Delaware	Pequest River	Independence Creek	40°53'01.60"N 74°51'54.60"W	M - stocked annually 40+ yrs T - no record of stocking
HWH	Delaware	Pohatcong Creek	Halfway House Brook	40°44'44.67"N 75°02'46.34"W	M - stocked annually 40+ yrs T - no record of stocking
KUR	Delaware	Musconetcong River	Kurtenbach's Brook	40°54'33.24"N 74°45'17.61"W	M - stocked annually 40+ yrs T - no record of stocking
MAS	Delaware	Big Timber Creek	Masons Run	39°47'13.10"N 75°00'04.50"W	M - not stocked, but several off-stream impundments stocked regularly T - no record of stocking
MPO	Hudson	Wallkill River	Mud Pond Outlet Stream	41°08'00.00"N 74°33'18.90"W	M - stocked annually 40+ yrs T - no record of stocking
CRE	Newark Bay	Hackensack River	Cresskill Brook	40°56'43.20"N 73°56'30.40"W	M - stocked annually 40+ yrs T - no record of stocking
PRE	Passaic	Passaic River	Preakness Brook	40°58'10.81"N 74°13'52.90"W	M - not stocked extensively T - onstream impundment ? km downstream of sample site stocked (Barbours Pond)
HAV	Passaic	Ramapoo River	Havemeyer Brook	41°05'39.60"N 74°11'23.10"W	M - stocked annually 40+ yrs T - no record of stocking
COO	Passaic	Wanaque River	Cooleys Brook	41°09'18.37"N 74°21'25.13"W	M - stocked annually 40+ yrs T - stocked extensively prior to 1990

TABLE 1.—*Continued.*

Site code	Drainage	Mainstem stream	Tributary	Latitude Longitude	Trout stocking history
BMB	Passaic	Wanaque River	Burnt Meadow Brook	41°06'10.73"N 74°19'20.05"W	M - stocked annually 40+ yrs T - no record of stocking
LSB	Passaic	Pequannock River	Lake Stockholm Brook	41°04'48.25"N 74°31'39.17"W	M - stocked annually 40+ yrs T - no record of stocking
HIB	Passaic	Rockaway River	Hibernia Brook	40°58'04.12"N 74°29'26.59"W	M - stocked annually 40+ yrs T – stocked downstream of sample site, below on-stream impoundment (???)
CBT	Passaic	Rockaway River	Crooked Brook tributary	40°55'04.50"N 74°23'49.02"W	M - stocked annually since ????? T - no record of stocking
FLA	Raritan	S/Br. Raritan River	Flanders Brook	40°52'02.62"N 74°41'41.20"W	M - stocked annually 40+ yrs T - stocked annually prior to 1990
KRU	Raritan	S/Br. Raritan River	Krueger's Creek	40°50'29.89"N 74°42'07.97"W	M - stocked annually 40+ yrs T - no record of stocking
TUR	Raritan	S/Br. Raritan River	Turkey Brook	40°51'04.55"N 74°43'48.14"W	M - stocked annually 40+ yrs T - no record of stocking
SOH	Raritan	S/Br. Raritan River	S. of Hoffmans tributary	40°41'46.00"N 74°52'16.33"W	M - stocked annually 40+ yrs T - no record of stocking
ROC	Raritan	S/Br. Raritan River	Rocky Run	40°41'42.54"N 74°54'35.41"W	M - stocked annually 40+ yrs T - no record of stocking
OAC	Raritan	Lamington River	Oakdale Creek	40°47'48.13"N 74°41'51.57"W	M - stocked annually 40+ yrs T - no record of stocking
HAC	Raritan	Lamington River	Hacklebarney Brook	40°46'02.42"N 74°43'03.31"W	M - stocked annually 40+ yrs T - stocked annually prior to 19??
PTH	-	-	Pequest Trout Hatchery	-	Brook trout eggs obtained from the North Attleboro National Fish Hatchery in Massachusetts (Nashua strain) when hatchery production commenced in 1982.

been privately stocked with trout (anecdotal information provided by a caretaker of property bordering the brook when the stream was electrofished). Three additional streams, each from a different major drainage and having a long history of trout stocking (but not recently stocked), were also sampled. For comparison purposes, samples were taken from cultured brook trout reared at the NJDFW Pequest Trout Hatchery.

Sample Collection

Brook trout were collected from study streams using pulsed direct current backpack electrofishers (Smith-Root Model Type VII or 12-B) (Figure 8). A sample size of 10 – 15 fish (>10 cm) was targeted, though fewer were collected from streams with low population densities. The distance sampled therefore varied from stream to stream, and generally ranged from 100 – 300 m.

Figure 8.—Collection of brook trout using a backpack electrofisher.

Figure 9.—Cardiac puncture technique used to obtain blood samples.

Fish were anesthetized with tricaine methanesulfonate (Finquel) and approximately 100-μL of blood was taken by cardiac puncture using a 28-gauge insulin syringe (B&D) (Figure 9). Anesthetized fish were returned to the stream immediately following this procedure and monitored until they recovered sufficiently to swim away. Blood was initially stored in vacutainers containing ETDA and immediately placed on ice. Within 24 hours of

collection, samples were transferred to microcentrifuge tubes and frozen and stored at -55°C until DNA extraction was performed.

DNA Extraction

Genomic DNA was isolated from 247 blood samples using one of two protocols. Most extractions (193 samples) were performed at East Stroudsburg University using a commercially available DNA extraction kit (Biorad InstaGene™ Whole Blood Kit). The manufacturer's guidelines were followed, using 10-µL of blood. Extraction success was visually confirmed with electrophoresis on a 1% agarose gel stained with ethidium bromide, using 8-µL of the supernatant containing the extracted DNA, and 2-µL dye (Figure 10). Deer DNA was run in one lane for quality control purposes. Gels were photo-documented with Polaroid 667 film. The extraction process was repeated for failures until successful or the
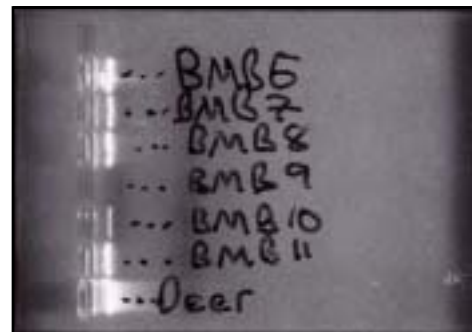
Figure 10.—DNA extraction success was confirmed electrophoretically and photo-documented

sample supply exhausted. Supernatants were placed in microcentrifuge tubes and stored at -55°C. For the remaining 53 samples, blood was placed on FTA® cards, air-dried, and sent to the USGS, Leetown Science Center, Kearneysville, WV for DNA extraction. For DNA extractions performed by USGS, the Puregene DNA extraction kit (Gentra Systems, Minneapolis, Minnesota; Buccal Cell Protocol used, p. 32 in Puregene instruction manual) was followed.

Microsatellite DNA Amplification

PCR was used to amplify 13 microsatellite loci using primer pairs designed specifically for brook trout (*SfoB52*, *SfoC24, SfoC28, SfoC38, SfoC79, SfoC86, SfoC88, SfoC113, SfoC115*, *SfoC129, SfoD75, SfoD91, SfoD100*; T. L. King, USGS, unpublished). The forward primers were fluorescently labeled with HEX, FAM, or NED dye (Applied Biosystems). Supernatants from DNA extractions were diluted 10:1 with deionized water, thoroughly mixed, and used for the DNA template. Reactions were generally successful using this dilution, therefore, DNA was not quantified prior to PCR. Reaction failures were repeated using undiluted supernatant for the template. Amplifications for each sample were carried out in three 15-µL reaction solutions, each containing a different set of four or five primer pairs. The components of each master mix solution are given in Table 2. The amplification cycle typically consisted of a 2-min initial denaturation at 94°C, followed by 35 cycles of 94°C denaturing for 45 s, 56°C annealing for 45 s, and a 72°C extension for 2-min. Cycling concluded with a 10- min extension at 72°C. PCR failures were repeated using single-locus reactions. Amplifications were carried out on either a PTC-200 or PTC-225 Thermal Cycler (MJ Research). All aspects of PCR were performed by the USGS.

Fragment Analysis

Fragment analysis (using fluorescently labeled DNA fragments obtained through PCR) was performed on an Applied Biosystems (Foster City, CA, USA) ABI 3100 Genetic Anaylzer, as described in King et al. (2001). Genescan™ 3.7 Analysis software and Genotyper™ 3.6 Fragment Analysis software (Applied Biosystems) was used to

TABLE 2.—Three master mixes used to amplify 13 microsatellite loci in 23 brook trout collections from New Jersey. Forward primers are labeled with fluorescent dye (*fam*, *hex*, or *ned*). Stock concentrations used: 10 mM trisHCl [pH 8.3] buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 5 mM, *Taq* DNA polymerase.

Master Mix A		Master Mix B		Master Mix C	
Quantity (µL)	Reagent concentration	Quantity (µL)	Reagent concentration	Quantity (µL)	Reagent concentration
3.96	dH20	3.39	dH20	2.34	dH20
2.625	0.875 1X bufer	2.625	0.875 1X buffer	2.625	0.875 1X buffer
2.25	3.75 mM $MgCl_2$	2.25	3.75 mM $MgCl_2$	2.25	3.75 mM $MgCl_2$
1.905	0.3175 mM dNTPs	1.905	0.3175 mM dNTPs	1.905	0.3175 mM dNTPs
0.225	0.075 uM *SfoC24 fam*	0.24	0.08 uM *SfoC86 hex*	0.42	0.14 uM *SfoC113 fam*
0.225	0.075 uM *SfoC24*	0.24	0.08 uM *SfoC86*	0.42	0.14 uM *SfoC113*
0.36	0.12 uM *SfoB52 fam*	0.27	0.09 uM *SfoC88 hex*	0.48	0.16 uM *SfoC115 fam*
0.36	0.12 uM *SfoB52*	0.27	0.09 uM *SfoC88*	0.48	0.16 uM *SfoC115*
0.15	0.05 uM *SfoD100 hex*	0.33	0.11 uM *SfoC129 hex*	0.42	0.14 uM *SfoC79 hex*
0.15	0.05 uM *SfoD100*	0.33	0.11 uM *SfoC129*	0.42	0.14 uM *SfoC79*
0.33	0.011 uM *SfoC38 ned*	0.69	0.23 uM *SfoC28 ned*	0.75	0.25 uM *SfoD91a hex*
0.33	0.011 *uM SfoC38*	0.69	0.23 uM *SfoC28*	0.75	0.25 uM *SfoD91a*
0.18	0.06 uM *SfoD75 ned*		-		-
0.18	0.06 uM *SfoD75*		-		-
0.27	0.09 units/uL *Taq*	0.27	0.09 units/uL *Taq*	0.24	0.09 units/uL *Taq*
1.5	DNA template	1.5	DNA template	1.5	DNA template
15	Total	15	Total	15	Total

score, bin, and output allelic (and genotypic) data. All aspects of the fragment analysis were performed by the USGS.

Data Analysis

The allelic data generated for 240 individuals were initially examined using Microsatellite Toolkit (Parks 2001), an add-in utility for Microsoft® Excel (Windows versions, Excel 97 or later) that contains tools for population geneticists working with microsatellites. Toolkit was used to identify data entry errors and detect genetically identical samples. Once the data set was finalized (Appendix, Table A1), Toolkit was used to bring the data into input file format for further analysis with other population genetics software. In this study, a null (nonamplifying) homozygote was detected at one locus (*SfoD91)* in one collection (Lake Stockholm Brook, LSB) (Table A1, Appendix). This locus was retained in subsequent analyses, unless otherwise noted, to maximize the number of independent alleles and reduce the coefficient of variation of estimates of genetic distance (Kalinowski 2002).

Genetic diversity within 23 collections was quantified using BIOSYS-1 (Swofford and Selander 1981) by calculating allelic frequencies, number of alleles per loci, loci polymorphism, observed heterozygosity (H_O), and expected heterozygosity (H_E). Corrected estimates of allelic diversity based upon the smallest sample size ($n = 7$ for collections and $n = 4$ for drainages) and Wright's (1969) inbreeding coefficient (F_{IS}) were estimated for each collection using FSTAT (Goudet 1995). Thirteen loci were used to derive all values for each collection except for the Lake Stockholm Brook collection

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(12 loci used; *SfoD91* excluded). The number of unique alleles, by collection and drainage, was determined using GenAlEx (Peakall and Smouse 2006).

The genotypes at each locus for each collection were tested for conformity to Hardy–Weinberg equilibrium (HWE) by comparing the observed genotype frequencies with the frequencies expected for an ideal population (large, randomly mating population of diploid organisms that reproduce sexually, have nonoverlapping generations, where the effects of mutation, migration, and selection are negligible). This test was performed in GenePop 3.1 (Raymond and Rousset 1995) using the Markov chain randomization test of Guo and Thompson (1992). Though not common, microsatellites can be clustered in the genome and therefore linkage disequilibrium should always be tested (Selkoe and Toonen 2006). To assess if loci assorted independently (i.e. not transmitted to offspring as a pair), linkage disequilibrium (LD) was tested for all pairs of loci using the randomization method of Raymond and Rousset (1995) in GenePop 3.1 with 10,000 dememorizations, 100 batches, and 5,000 iterations per batch. Significance levels for HWE and LD, and all other multiple comparison tests, were adjusted using sequential Bonferroni methods (Rice 1989) with an initial α level of $0.05/k$, k being the number of tests.

The statistical significance of allele frequency differences between each pair of samples was tested by means of the genetic differentiation randomization test in GenePop. Results were combined over loci using Fisher's method (Sokal and and Rohlf 1994) and adjusted for multiple tests with the sequential Bonferroni method. To test for genetic differentiation among the brook trout collections, pairwise F_{ST} values were obtained with GenePop 3.4. Pairwise R_{ST} values among collections were also calculated

using GenePop 3.4 and are provided for comparison purposes with F_{ST} values. F_{ST} assumes allelic diversity results from migration and gene drift, while R_{ST} also measures mutational differences between alleles (King et al. 2006).

Several techniques were used to describe the genetic relationships among collections and drainages. The population genetic structure was quantified at several levels using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992), performed in GenAlEx using pairwise R_{ST} values. To determine how much of the variation is due to differences among populations versus drainages, the total amount of genetic variation was partitioned into (1) the proportion due to genetic differences among collections, both within and between drainages and (2) the proportion due to genetic variation within and among drainages, with collections within drainages pooled.

To transform the allelic frequency data into a distance matrix, genetic distance estimates for all pairwise collection comparisons were determined using the chord distance measure of Cavalli-Sfzora and Edwards (1967), implemented by FSTAT (Goudet 1995). This metric measures the distance as though the collections were on a multidimensional sphere. It is based on the infinite allele model of mutation which assumes that most new mutations arise in a stepwise fashion by the gain or loss of repeated units (Shaklee and Currens 2003). This metric is generally considered more appropriate than the logarithmic-derived genetic distance metric developed by Nei (1972, 1978) when random genetic drift, rather than mutation, is the primary force of divergence (Shaklee and Currens 2003).

An unrooted phylogenetic tree was fitted using the distance matrix and the neighbor-joining algorithm implemented by PHYLIP (Felsenstein 1992), a package of

computer programs for inferring phylogenies. TreeView (Page 1996), a program for

displaying and printing phylogenies, was used to visualize the tree. Maximum likelihood

assignment tests (Paetkau et al. 1995) used to determine the likelihood of each

individual's multilocus genotype being found in the population and drainage from which

it was sampled, were conducted using GeneClass 1.0.02 (Cornuet et al. 1999) with the

Bayesian method ("leave one out" procedure). In the event of null frequencies, a

constant likelihood of 0.01 was assumed. The *SfoD91* locus was not included in the

AMOVA and assignment tests, due to the presence of null alleles in all animals from one

collection (Lake Stockholm Brook, LSB).

CHAPTER III
RESULTS

Genetic Diversity

Genotypes at 13 microsatellite DNA loci were determined for 238 brook trout sampled from 22 streams, representing 4 major river drainages in New Jersey, and 1 trout hatchery (see Table 1 for listing and abbreviations; Figure 1). The allele frequencies, individual locus heterozygosities, overall mean heterozygosities, and mean number of alleles per locus are provided in Table A1.2, Appendix. A total of 136 alleles was detected in 23 collections and the number of alleles per locus ranged from 2 (*SfoC79*) to 24 (*SfoC115*), with a mean of 10.5. When the hatchery collection excluded, the total number of alleles per locus was 133 (mean 10.2). Allelic richness was lowest in the Mason's Run collection from south Jersey (MAS, 1.7) and greatest in the hatchery collection (PTH, 4.7) (Table 3).

Observed heterozygosity (H_o) across the 23 collections averaged 0.541, and in a majority of collections (15 or 65%), ranged from 0.500 to 0.700 (Table 3). H_o was lowest in animals from Masons Run (MAS; 0.342), and highest in animals from Cooley's Brook (COO, 0.734), a stream having a history of trout stocking. The hatchery collection had

TABLE 3.—Summary of genetic diversity statistics for 23 collections of brook trout (*Salvelinus fontinalis*) from New Jersey surveyed at 13 microsatellite loci. Sample size (N), mean number of alleles per locus ($\hat{A}$), allelic richness ($\hat{A}_C$ corrected to $n=7$ for collections and $n=9$ for drainages), number of private alleles, polymorphism (frequency of most common allele did not exceed 0.95), observed and expected heterozygosity, and estimates of the inbreeding coefficient (F_{IS}).

Drainage (or hatchery) of origin / Stream of origin	Collection abbreviation	N	$\hat{A}$	$\hat{A}_C$	Private alleles[a]		Percent of loci polymorphic	Heterozygosity (H_O)	(H_E)	F_{IS}
Delaware drainage		**55**	**7.7**		**11**	**(12)**	**100.0**	**0.534**	**0.685**	**0.222**
Forked Brook	FOR	9	4.3	4.0	2	(3)	92.3	0.615	0.619	0.007
Van Campens Brook[b]	VCB	9	4.5	4.1	2	(2)	92.3	0.547	0.608	0.106
Independence Brook	IND	11	3.6	3.2	0	(0)	92.3	0.580	0.521	-0.121
Halfway House Brook	HWH	8	3.2	3.2	1	(1)	92.3	0.596	0.573	-0.043
Kurtenbach's Brook	KUR	9	3.0	2.9	3	(3)	92.3	0.521	0.522	0.002
Masons Run	MAS	9	1.7	1.7	0	(0)	61.5	0.342	0.309	-0.115
Hudson drainage		**10**	**3.5**		**0**	**(0)**	**92.3**	**0.575**	**0.546**	**-0.058**
Mud Pond Outlet Stream	MPO	10	3.5	3.3	0	(0)	92.3	0.575	0.546	-0.058
Passaic-Hackensack drainage		**80**	**7.7**		**10**	**(14)**	**100.0**	**0.501**	**0.668**	**0.251**
Cresskill Brook	CRE	11	2.5	2.5	1	(3)	84.6	0.508	0.452	-0.131
Preakness Brook	PRE	11	2.0	2.0	0	(1)	84.6	0.350	0.339	-0.320
Havemeyer Brook	HAV	7	2.8	2.8	1	(1)	76.9	0.549	0.469	-0.188
Cooleys Brook[b]	COO	11	4.5	4.1	0	(0)	100.0	0.734	0.679	-0.086
Burnt Meadow Brook	BMB	11	2.9	2.6	1	(1)	76.9	0.472	0.432	-0.097
Lake Stockholm Brook	LSB	10	2.9[c]	2.7[c]	0	(0)	91.7[c]	0.475[c]	0.430[c]	-0.110[c]
Hibernia Brook	HIB	10	3.4	3.1	2	(2)	92.3	0.554	0.534	-0.038
Crooked Brook tributary	CBT	9	2.2	2.2	1	(1)	84.6	0.353	0.346	-0.020
Raritan drainage		**73**	**7.9**		**5**	**(7)**	**100.0**	**0.549**	**0.694**	**0.211**
Flanders Brook[b]	FLA	13	4.2	3.7	2	(2)	92.3	0.613	0.619	0.010
Krueger's Creek	KRU	10	4.2	3.9	0	(0)	100.0	0.623	0.631	0.013
Turkey Brook	TUR	10	4.9	4.4	1	(1)	92.3	0.684	0.652	-0.052
S. of Hoffmans tributary	SOH	10	3.7	3.2	0	(0)	92.3	0.469	0.493	0.051
Rocky Run	ROC	10	3.0	2.9	1	(1)	92.3	0.507	0.523	0.032
Oakdale Creek	OAK	10	2.2	2.1	1	(1)	76.9	0.391	0.311	-0.275
Hacklebarney Brook[b]	HAC	10	4.5	4.1	0	(1)	100.0	0.541	0.625	0.141
Pequest Trout Hatchery	**PTH**	**20**	**6.1**	**4.7**	**3**	**–**	**100.0**	**0.677**	**0.695**	**0.026**
Total		**238**			**22**	**(24)**				

[a] ( ) indicates the number of private alleles when the hatchery collection is excluded; [b] Stream has a known history of stocking; [c] value based upon 12 loci (D91 not included).

the third highest observed heterozygosity (PTH, 0.677), which was substantially higher than those found when the collections were pooled by drainage (H_o ranged from 50.1 to 57.5 % in the 4 drainages). The percentage of polymorphic loci ranged from 61.5% in Mason's Run, to 100% in four collections (Cooley's Brook, Kruegers Creek, Hacklebarney Brook, and Pequest Trout Hatchery) (Table 3). Inbreeding ($F_{IS}>0$) was detected in eight collections from wild populations (0.002 – 0.141), and the hatchery collection (0.026).

Randomization tests showed that nearly all of the genotypic frequencies observed in the 23 collections conformed to Hardy–Weinberg (HW) expectations. Just by chance alone, 15 differences would be expected at the 0.05 level (0.05 x 23 x 13). A significant departure from HW proportions was detected in only 1 of 299 locus-by collection comparisons ($\alpha = 0.05$, $P < 0.002$). This departure was observed at the locus *SfoD75* in the collection from Hacklebarney Brook (*P*-value of 0.0002), and was the result of a heterozygote deficiency (homozygote excess) in 1 animal (HAC-08). A heterozygote deficit is the more common direction of HW equilibrium deviation, and can be due to the biological realities of violating the criteria of an ideal population, such as strong inbreeding or selection for or against a certain allele (Selkoe and Toonen 2006). Failure of this locus to meet Hardy–Weinberg expectations in one animal was not considered grounds for discarding the locus. Of the 1794 pairwise tests for linkage disequilibrium, no significant genetic linkage was observed between any paired loci across all collections ($\alpha = 0.05$, $P < 0.00017$), indicating the loci are segregating independently. Heterogeneous allele frequencies were observed through the study area. Among 3,267 single-locus pairwise tests (3,289 less 22 for no genotype at locus *SfoD91* in the LSB

collection) of allele frequency heterogeneity, 1283 (41.1%) exhibited departures from homogeneity after correction for multiple tests ($\alpha = 0.05$, $P < 0.00003$).

A total of 22 private alleles (16%) were found distributed in 14 of the 23 collections, at frequencies ranging from 0.025 to 0.333 (Table 4). The highest number per collection (3) was found in Kurtenbach's Brook (KUR) and the Pequest Trout Hatchery (PTH). Ten of the private alleles occurred at relatively high frequencies (at least 0.1) in 8 different streams (Kurtenbach's Brook (2), Flanders Brook (2), Halfway House Brook, Cresskill Brook, Burnt Meadow Brook, Hibernia Brook, Crooked Brook tributary, and Rocky Run). Five more private alleles were detected when the hatchery collection was excluded and one of those (in Cresskill Brook) occurred at a frequency in excess of 0.1. When the collections were pooled by drainage (with the hatchery collection considered a drainage), more private alleles (29, 21%) were found (Table 5). However, the highest frequency detected was 20.3%, and most (27, 93%) occurred at a frequency lower than 10%. When the hatchery collection was excluded from this analysis, 7 additional private alleles were detected at low frequencies (< 10%).

When the 22 collections representing spawning brook trout populations were grouped by drainage, quantitative estimates of hierarchical gene diversity indicated significant genetic diversity at every level. The greatest amount of variation occurred within populations (50.8%), followed by variation among populations within drainages (27.5%), and variation among drainages (21.7%) (Figure 11A). A comparison between the four drainages, with all collections pooled by drainage, determined that 73.4% of the genetic variation was due to differentiation within drainages and only 26.6% of the variation occurred between drainages (Figure 11B).

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TABLE 4.— Frequency of 21 unique alleles found in 238 brook trout collected from 22 streams and a hatchery in New Jersey, surveyed at 13 microsatellite loci. Additional unique alleles (those with corresponding frequencies shown in parentheses) were detected when the Pequest Trout Hatchery collection was excluded from analysis.

Drainage (or hatchery) of origin Stream of origin	Collection abbreviation	Locus	Allele size	Frequency
Delaware drainage				
Forked Brook	FOR	*Sfo-C28*	177	0.056
	FOR	*Sfo-D91*	256	(0.050)
	FOR	*Sfo-D100*	282	0.056
Van Campens Brook [a]	VCB	*Sfo-C113*	124	0.056
	VCB	*Sfo-C115*	329	0.056
Halfway House Brook	HWH	*Sfo-D100*	258	0.125
Kurtenbach's Brook	KUR	*Sfo-D75*	216	0.222
	KUR	*Sfo-D100*	242	0.333
	KUR	*Sfo-D100*	270	0.056
Hudson drainage		-	-	-
Passaic-Hackensack drainage				
Cresskill Brook	CRE	*Sfo-C86*	122	0.227
	CRE	*Sfo-C115*	333	(0.075)
	CRE	*Sfo-D75*	192	(0.125)
Preakness Brook	PRE	*Sfo-B52*	227	(0.050)
Havemeyer Brook	HAV	*Sfo-C115*	343	0.071
Burnt Meadow Brook	BMB	*Sfo-C113*	157	0.273
Hibernia Brook	HIB	*Sfo-C86*	128	0.200
	HIB	*Sfo-C115*	357	0.050
Crooked Brook tributary	CBT	*Sfo-C28*	203	0.333
Raritan drainage				
Flanders Brook [a]	FLA	*Sfo-C24*	125	0.154
	FLA	*Sfo-C115*	303	0.269
Turkey Brook	TUR	*Sfo-C115*	321	0.056
Rocky Run	ROC	*Sfo-C113*	136	0.111
Oakdale Creek	OAK	*Sfo-C28*	197	0.050
Hacklebarney Brook [a]	HAC	*Sfo-B52*	199	(0.025)
Pequest Trout Hatchery	PTH	*Sfo-B52*	207	0.050
	PTH	*Sfo-C28*	199	0.025
	PTH	*Sfo-D91*	280	0.075

[b] Stream has a known history of stocking.

TABLE 5.— Frequency of 29 unique alleles found in 238 brook trout collected from 4 drainages and a hatchery in New Jersey, surveyed at 13 microsatellite loci. Additional unique alleles (those with corresponding frequencies shown in parentheses) were detected when the Pequest Trout Hatchery collection was excluded from analysis.

Drainage or hatchery of origin	Locus	Allele size	Frequency
Delaware drainage	*Sfo-C28*	177	0.009
	Sfo-C113	124	0.009
	Sfo-C115	329	0.009
	Sfo-D75	216	0.036
	Sfo-D91	244	0.036
	Sfo-D91	252	0.018
	Sfo-D91	256	(0.050)
	Sfo-D100	242	0.055
	Sfo-D100	258	0.018
	Sfo-D100	270	0.009
	Sfo-D100	274	0.027
	Sfo-D100	282	0.009
Hudson drainage	-	-	-
Passaic-Hackensack drainage	*Sfo-B52*	227	(0.050)
	Sfo-C28	195	0.019
	Sfo-C28	203	0.038
	Sfo-C86	122	0.031
	Sfo-C86	128	0.025
	Sfo-C113	157	0.038
	Sfo-C115	249	(0.025)
	Sfo-C115	333	(0.075)
	Sfo-C115	343	0.006
	Sfo-C115	345	0.203
	Sfo-C115	349	0.057
	Sfo-C115	353	0.152
	Sfo-C115	357	0.006
	Sfo-D75	192	(0.125)
Raritan drainage	*Sfo-B52*	199	(0.025)
	Sfo-C24	125	0.028
	Sfo-C28	197	0.007
	Sfo-C113	136	0.014
	Sfo-C115	303	0.049
	Sfo-C115	321	0.007
	Sfo-C129	236	(0.050)
Pequest Trout Hatchery	*Sfo-B52*	207	0.050
	Sfo-C28	199	0.025
	Sfo-D91	280	0.075
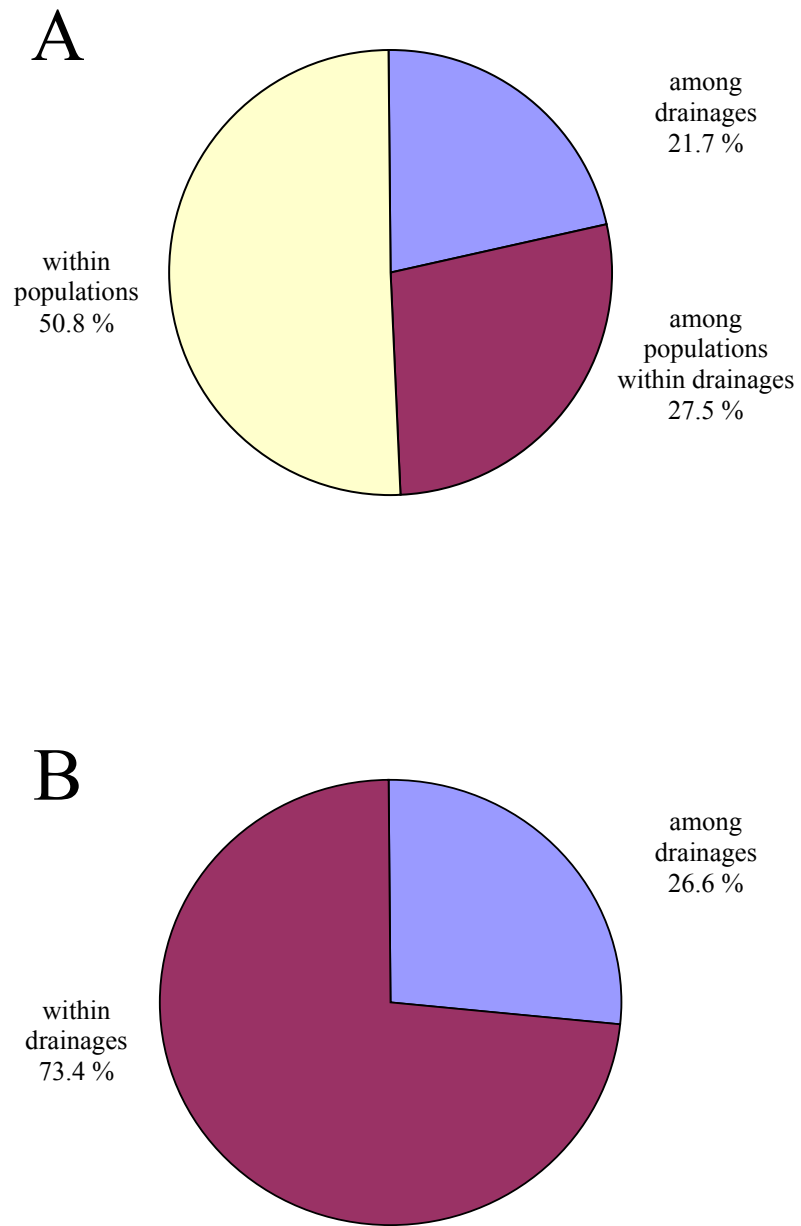
FIGURE 11.— Hierarchical gene diversity analysis (AMOVA) of 22 spawning populations of brook trout from New Jersey for 12 microsatellite DNA loci ($P < 0.010$). (A) Populations grouped by drainage but not pooled; (B) Populations pooled by drainage.

A considerable amount of genetic differentiation was also observed in comparisons of F_{ST} values. Pairwise F_{ST} estimates ranged from 0.07 between Cooley's Brook (COO), a stream having a history of trout stocking, and Pequest Trout Hatchery (PTH) collections, to 0.602 between collections from different drainages (Preakness Brook, PRE and Masons Run, MAS) (Table 6, below diagonal). Of the 253 comparisons, 250 (99%) were greater than 0.100, and 189 (75%) were greater than 0.200. R_{ST} values also indicated similar differences between pairs of collections, with values ranging from -0.001 to 0.935 (Table 6, above diagonal).

Population Structure

Pairwise genetic distance values (chord distance values; Cavelli-Sforza and Edwards 1967) were calculated between all collections to investigate evolutionary relationships among allele frequencies (Table 7). The greatest genetic distance occurred between two collections from different drainages (Preakness Brook, PRE and Rocky Run, ROC; 0.817), and 13 of 14 pairs having the greatest genetic distance involved the Preakness Brook collection. The lowest genetic distance was observed between the Pequest Trout Hatchery collection (PTH) and the collection from Cooleys Brook (COO), a stream having a history of trout stocking. The unrooted neighbor-joining tree depicting the underlying genetic structure of the distance matrix illustrates differentiation among collections by drainage (Figure 12). Two distinct groups were formed which were comprised of populations representing the Raritan and Passaic-Hackensack drainages. The Raritan drainage grouping contained 6 of 7 collections originating from the drainage,

TABLE 6.— Matrix of F_{ST} values (below the diagonal) and R_{ST} values (above the diagonal) for all pairwise comparisons among 23 brook trout collections from New Jersey. Measures were derived from data for 13 microsatellite loci. See Table 1 for collection abbreviations.

Collection	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB	LSO	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
FOR		0.251	0.065	0.387	0.145	0.805	0.380	0.870	0.930	0.525	0.244	0.756	0.619	0.516	0.880	0.522	0.387	0.225	0.258	0.250	0.577	0.242	0.321
VCB	0.091		0.079	0.017	0.278	0.339	0.079	0.577	0.760	0.160	0.084	0.460	0.180	0.178	0.655	0.111	0.050	0.040	0.031	0.256	0.147	-0.001	0.041
IND	0.120	0.203		0.194	0.194	0.561	0.168	0.705	0.833	0.328	0.153	0.622	0.377	0.382	0.758	0.271	0.164	0.093	0.074	0.079	0.363	0.112	0.160
HWH	0.189	0.244	0.266		0.372	0.328	0.129	0.575	0.754	0.158	0.085	0.428	0.206	0.126	0.632	0.171	0.096	0.080	0.076	0.379	0.174	0.043	0.063
KUR	0.200	0.279	0.315	0.251		0.795	0.404	0.881	0.932	0.533	0.151	0.745	0.682	0.498	0.885	0.568	0.466	0.193	0.318	0.409	0.603	0.267	0.328
MAS	0.283	0.358	0.382	0.364	0.464		0.355	0.743	0.901	0.216	0.445	0.298	0.468	0.083	0.781	0.327	0.415	0.429	0.570	0.822	0.385	0.422	0.161
MPO	0.178	0.270	0.287	0.167	0.310	0.337		0.610	0.790	0.207	0.119	0.497	0.257	0.235	0.690	0.082	0.090	0.189	0.219	0.417	0.217	0.099	-0.028
CRE	0.236	0.275	0.213	0.276	0.389	0.399	0.291		0.732	0.316	0.699	0.316	0.452	0.216	0.406	0.508	0.531	0.656	0.707	0.866	0.571	0.661	0.450
PRE	0.396	0.349	0.462	0.421	0.456	0.602	0.457	0.527		0.573	0.812	0.338	0.755	0.416	0.419	0.773	0.765	0.786	0.840	0.935	0.782	0.808	0.660
HAV	0.198	0.224	0.263	0.302	0.312	0.444	0.333	0.365	0.355		0.328	0.187	0.086	0.061	0.398	0.112	0.144	0.206	0.275	0.464	0.162	0.244	0.143
COO	0.122	0.156	0.192	0.145	0.264	0.298	0.138	0.259	0.373	0.260		0.574	0.421	0.291	0.738	0.309	0.220	0.098	0.137	0.345	0.323	0.061	0.110
BMB	0.234	0.272	0.346	0.340	0.323	0.452	0.331	0.385	0.378	0.250	0.277		0.357	0.143	0.306	0.441	0.482	0.513	0.592	0.747	0.427	0.533	0.394
LSB	0.153	0.190	0.207	0.262	0.368	0.451	0.292	0.331	0.426	0.214	0.197	0.300		0.144	0.582	0.097	0.088	0.329	0.322	0.595	0.139	0.268	0.169
HIB	0.175	0.245	0.216	0.199	0.319	0.368	0.226	0.234	0.439	0.305	0.188	0.336	0.281		0.289	0.172	0.178	0.249	0.307	0.510	0.096	0.226	0.177
CBT	0.270	0.350	0.355	0.337	0.452	0.483	0.333	0.414	0.524	0.380	0.281	0.330	0.315	0.286		0.653	0.633	0.698	0.748	0.879	0.658	0.718	0.572
FLA	0.132	0.171	0.196	0.237	0.251	0.341	0.245	0.270	0.400	0.215	0.200	0.277	0.194	0.213	0.299		0.040	0.253	0.290	0.499	0.122	0.173	0.024
KRU	0.170	0.170	0.232	0.172	0.266	0.397	0.220	0.287	0.318	0.214	0.170	0.240	0.195	0.196	0.307	0.184		0.110	0.141	0.370	0.101	0.083	0.052
TUR	0.122	0.176	0.215	0.185	0.195	0.322	0.209	0.283	0.330	0.163	0.185	0.256	0.237	0.169	0.306	0.132	0.177		0.041	0.161	0.289	0.069	0.149
SOH	0.188	0.232	0.227	0.293	0.320	0.469	0.322	0.352	0.464	0.256	0.218	0.344	0.173	0.297	0.394	0.188	0.206	0.189		0.200	0.343	0.094	0.173
ROC	0.214	0.270	0.276	0.242	0.298	0.398	0.276	0.308	0.520	0.261	0.233	0.380	0.292	0.276	0.417	0.228	0.284	0.212	0.279		0.604	0.311	0.337
OAK	0.276	0.366	0.262	0.414	0.428	0.543	0.415	0.439	0.580	0.416	0.336	0.481	0.312	0.374	0.484	0.326	0.351	0.336	0.335	0.435		0.149	0.100
HAC	0.127	0.184	0.172	0.226	0.252	0.345	0.258	0.291	0.420	0.242	0.165	0.327	0.184	0.244	0.343	0.142	0.189	0.137	0.140	0.236	0.205		0.053
PTH	0.110	0.154	0.188	0.110	0.225	0.259	0.092	0.217	0.324	0.238	0.072	0.239	0.191	0.173	0.271	0.177	0.142	0.137	0.209	0.189	0.313	0.145	

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TABLE 7.—Genetic distance (chord distance, Cavalli-Sforza and Edwards 1967) among 23 collections of brook trout from New Jersey using 13 microsatellite loci. See Table 1 for collection abbreviations.

Collection	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
FOR																							
VCB	0.455																						
IND	0.451	0.527																					
HWH	0.576	0.606	0.604																				
KUR	0.515	0.619	0.581	0.591																			
MAS	0.565	0.614	0.606	0.606	0.676																		
MPO	0.549	0.615	0.635	0.541	0.669	0.592																	
CRE	0.575	0.608	0.498	0.604	0.668	0.603	0.625																
PRE	0.708	0.651	0.738	0.687	0.707	0.751	0.715	0.762															
HAV	0.542	0.579	0.557	0.600	0.620	0.634	0.672	0.631	0.569														
COO	0.523	0.531	0.549	0.539	0.649	0.582	0.490	0.610	0.687	0.619													
BMB	0.590	0.613	0.634	0.663	0.601	0.637	0.637	0.655	0.571	0.531	0.583												
LSB	0.473	0.477	0.481	0.493	0.631	0.616	0.562	0.588	0.619	0.460	0.491	0.536											
HIB	0.533	0.568	0.540	0.571	0.633	0.615	0.613	0.552	0.679	0.590	0.561	0.654	0.534										
CBT	0.587	0.612	0.613	0.617	0.712	0.607	0.604	0.649	0.658	0.580	0.611	0.561	0.496	0.560									
FLA	0.507	0.543	0.543	0.604	0.598	0.610	0.614	0.617	0.724	0.568	0.609	0.633	0.516	0.576	0.619								
KRU	0.540	0.543	0.563	0.533	0.641	0.670	0.574	0.633	0.622	0.546	0.543	0.579	0.459	0.530	0.578	0.511							
TUR	0.487	0.540	0.525	0.550	0.535	0.600	0.572	0.628	0.624	0.496	0.588	0.593	0.513	0.528	0.593	0.501	0.448						
SOH	0.539	0.568	0.542	0.579	0.631	0.677	0.603	0.645	0.708	0.548	0.556	0.599	0.449	0.575	0.622	0.530	0.487	0.492					
ROC	0.551	0.629	0.543	0.573	0.645	0.634	0.616	0.586	0.817	0.548	0.597	0.666	0.566	0.601	0.644	0.580	0.612	0.556	0.552				
OAK	0.545	0.621	0.518	0.667	0.627	0.655	0.661	0.663	0.735	0.625	0.644	0.681	0.530	0.591	0.623	0.627	0.610	0.599	0.549	0.652			
HAC	0.467	0.524	0.487	0.569	0.600	0.587	0.609	0.635	0.721	0.593	0.529	0.656	0.488	0.575	0.615	0.500	0.528	0.487	0.472	0.569	0.467		
PTH	0.495	0.520	0.542	0.500	0.632	0.597	0.429	0.572	0.654	0.617	0.377	0.607	0.493	0.561	0.613	0.567	0.516	0.535	0.563	0.587	0.638	0.489	

including 2 streams having a history of trout stocking (Flanders Brook, FLA and Hacklebarney Brook, HAC). One collection from the Raritan drainage, Rocky Run (ROC), was left out of this genetically related group and one collection from the Passaic-Hackensack drainage, Lake Stockholm Brook (LSB), also clustered with this group. In the Passaic-Hackensack drainage, 5 of 8 collections also formed a group. One of the collections from the Passaic-Hackensack drainage not included in this subgroup was Cresskill Brook (CRE), the only collection from the Hackensack River watershed which drains into the Newark Bay complex and not directly into the Passaic River. The six collections from the Delaware drainage, which generally are more isolated from each other compared to collections within other drainages, separated into two smaller groups. The phenogram also illustrates a high level of divergence associated with the Pequest Trout Hatchery (PTH) collection, the only collection known to be comprised of brook trout not native to New Jersey. Animals from this collection were most closely related to animals from Cooley's Brook (COO), a stream having a long history of stocking whose collection did not group with others originating from the same drainage   (Passaic-Hackensack). The Hudson drainage, represented by only one collection (Mud Pond Outlet Stream, MPO), was highly differentiated from the two major groupings (Raritan and Passaic-Hackensack) and closely related to the collections from Cooley's Brook and the Pequest Trout Hatchery.
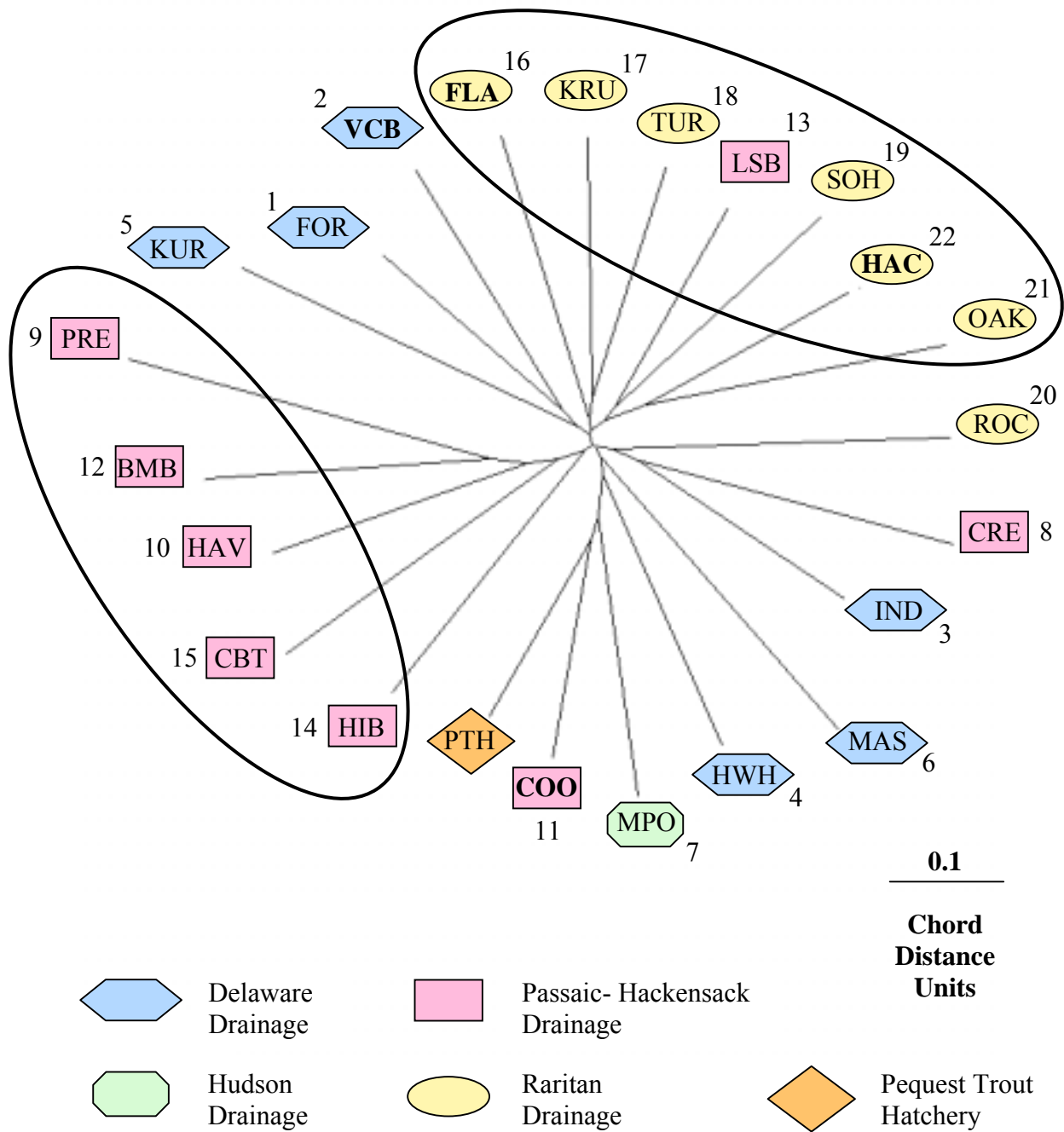
FIGURE 12.— Neighbor-joining phenogram constructed from the genetic distance matrix using the chord distance of Cavelli-Sforza and Edwards (1967), for brook trout collected from 22 streams and 1 hatchery in New Jersey. Collections in bold are from streams having a known history of brook trout stocking (see Table 1, Figure 7 for collection abbreviations and locations, and Table 7 for distance values).

Individual Assignment

Individual assignment tests using multilocus genotypes revealed that population differentiation was sufficient to identify the origin of individual fish with a high rate of success. Individuals were correctly assigned to their population of origin 94.5% of the time (on average) across all populations (Table 8). Fifteen populations had a 100% assignment success rate, and the remaining 8 populations had a total of 13 fish incorrectly assigned. There was no apparent pattern to the incorrect assignments, even within the 4 populations that had multiple fish (2 or 3) incorrectly assigned. When the populations were pooled by drainage, with the hatchery population considered a drainage, the assignment success dropped to 87.0% (207/238 fish assigned to the correct drainage) (Table 9).

TABLE 8.—Results of maximum likelihood assignment tests for 23 brook trout collections from New Jersey using multilocus genotypes derived from 12 microsatellite DNA markers. See Table 1 for collection abbreviations (collections in bold are from streams having a known history of brook trout stocking). Each row shows the sample size (N) and the assignment of individuals from the specified collection to all collections. The values along the diagonal (bold italics) indicate the number of correct assignments to each collection. Boxes highlight the assignment of individuals to collections within a drainage (n is the number of individuals collected from the drainage). The final column indicates the rate of correct assignment of individuals to their collection. Overall, of the 238 individuals collected, 94.5% (225) were assigned to their collection of origin.

Collection	N	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH	%
FOR	9	*8*		1																					88.9
VCB	9		*8*		1																				88.9
IND	11			*11*																					100.0
HWH	8				*8*																				100.0
KUR	9					*9*																			100.0
MAS	9						*9*																		100.0
MPO	10							*10*																	100.0
CRE	11								*11*																100.0
PRE	11									*11*															100.0
HAV	7										*7*														100.0
COO	11											*10*												1	90.9
BMB	11												*11*												100.0
LSB	10													*10*											100.0
HIB	10														*7*					1		1		1	70.0
CBT	9															*9*									100.0
FLA	13																*13*								100.0
KRU	10																	*8*	1	1					80.0
TUR	10			1											1				*8*						80.0
SOH	10														1					*9*					90.0
ROC	10																				*10*				100.0
OAK	10																					*10*			100.0
HAC	10																						*10*		100.0
PTH	20							1				1											*18*		90.0

Annotations within the table:
- Delaware drainage $n = 57$ (FOR, VCB, IND, HWH, KUR, MAS)
- Hudson drainage $n = 10$ (MPO)
- Passaic-Hackensack drainage $n = 80$ (CRE, PRE, HAV, COO, BMB, LSB, HIB, CBT)
- Raritan drainage $n = 73$ (FLA, KRU, TUR, SOH, ROC, OAK, HAC)
- Hatchery $n = 20$ (PTH)

TABLE 9.—Results of maximum likelihood assignment tests for 23 brook trout collections from four New Jersey drainages and a hatchery using multilocus genotypes derived from 12 microsatellite DNA markers. See Table 1 for collection abbreviations (collections in bold are from streams having a known history of brook trout stocking). Each row shows the sample size (*N*) and the assignment of individuals from the specified collection to each drainage. Boxes highlight the assignment of individuals to their drainage. The final column indicates the rate of correct assignment of individuals to their drainage. Overall, of the 238 individuals collected, 87.0% (207) were assigned to the correct drainage.

| Collection | N | Drainage | | | | Pequest Trout Hatchery | % |
		Delaware	Wallkill	Passaic - Hackensack	Raritan		
FOR	9	7			2		77.8
VCB	9	6		2		1	66.7
IND	11	11					100.0
HWH	8	6				2	75.0
KUR	9	9					100.0
MAS	9	9					100.0
MPO	10		10				100.0
CRE	11			11			100.0
PRE	11			11			100.0
HAV	7	1		6			85.7
COO	11	1		5		5	45.4
BMB	11	1		10			90.9
LSB	10	3		5	2		50.0
HIB	10	2		8			80.0
CBT	9			9			100.0
FLA	13				13		100.0
KRU	10			4	6		60.0
TUR	10	1			9		90.0
SOH	10			1	9		90.0
ROC	10	1			9		90.0
OAK	10				10		100.0
HAC	10				9	1	90.0
PTH	20		1			19	95.0

CHAPTER IV
DISCUSSION

This study represents the first evaluation of genetic diversity and population structure for spawning brook trout populations in New Jersey. The brook trout is the only salmonid species native to New Jersey, with spawning populations found primarily across the northern tier of the state, in the headwater and tributary streams within four Atlantic slope drainages. The 13 microsatellites used produced a data set that contained sufficient allelic diversity to reveal unique multilocus genotypes for all individuals sampled, identified moderately high levels of genetic diversity, and provided insight on the fine-scale genetic relationships within New Jersey's wild brook trout populations. The geographical distribution of genetic variability among the 22 wild brook trout populations in this study suggests remnants of ancestral brook trout exist in New Jersey and that the stocking of hatchery trout has minimally influenced the gene pools of most of these populations.

Genetic Diversity within Populations

Allelic diversity is often used to characterize the extent of genetic diversity within and across populations. Moderately high genetic diversity, 133 alleles at 13 loci (10.2 alleles per loci), with 2 to 24 alleles per locus, was observed among 218 individuals

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collected from 22 small streams in New Jersey. Genetic studies involving brook trout

populations from elsewhere in North America have revealed comparable levels of genetic

variation using microsatellite DNA markers. These other studies typically had larger

sample sizes, which generally yielded more alleles per locus. A study using 5

microsatellites to survey 496 individuals from 8 ponds within a watershed in

Newfoundland found the number of alleles per locus averaged 11 (67 total), and a range

of 2 to 25 alleles per locus (Adams and Hutchings 2003). Higher levels of polymorphism

(10 to 43 alleles per locus, average 18.8 per locus, 94 alleles total) were found in 779

individuals representing 26 populations in a national park in Quebec using 5

microsatellites (Angers and Bernatchez 1998). In Maryland, 100 alleles were observed

across 8 microsatellite DNA loci (12.5 average per locus) for 325 brook trout from 9

locations (King and Jullian 2000). A study of 30 populations (771 individuals)

representing 6 major river drainages in Maine found 10 to 57 alleles per locus (average

27.3) and 164 total, using 6 microsatellite loci (Castric et al. 2001). Samples from 12

sites (441 individuals total) in the Miramichi River drainage, New Brunswick, assessed

using 6 microsatellites detected 8 to 48 alleles per locus (Rogers and Curry 2004). King

(2006) has found very high genetic diversity (247 alleles) using the same 13

microsatellites used in this study, in more than 7,000 brook trout from 125 separate

collection sites across the eastern Unites States and Canada, with much of the diversity

represented in the mid-Atlantic region.

Heterozygosity is often used to characterize genetic diversity at the population level.

In the 22 wild populations included in this study, observed heterozygosities ranged from

0.342 to 0.734. Comparable levels of heterozygosity have also been found in other

studies of brook trout using microsatellites (0.594–0.766, King and Jullian 2000; 0.36–0.72, Castric et al. 2001; 0.17–0.79, Angers and Bernatchez 1998). Allelic richness is another important diversity measure because populations subjected to bottlenecks or to prolonged periods of low effective population size may retain high levels of heterozygosity while losing large numbers of alleles (Petit et al. 1998). Allelic richness for the wild populations in my study varied from 1.7 to 4.4; other microsatellite studies did not report allelic richness.

A relationship between levels of genetic diversity and population size was observed in this study. Low levels of heterozygosity and allelic richness generally coincided with field observations of low population abundance (i.e. inferred by difficulty obtaining individuals by electrofishing). Small, isolated populations in Mason's Run, Preakness Brook, Crooked Brook tributary, and Oakdale Creek had the lowest levels of heterozygosity (0.342, 0.350, 0.353, and 0.391 respectively) and allelic richness (1.7, 2.0, 2.2, and 2.1 respectively), whereas robust populations such as Cooley's Brook and Turkey Brook had the highest levels (0.734 and 0.684, and 4.5 and 4.9, respectively).

Smaller and more isolated populations are predicted to lose genetic diversity at a greater rate, and are more at risk of interbreeding, reduced fitness, and localized extinctions (Frankham et al. 2002). Since wild brook trout populations in New Jersey occur primarily in small streams, and often in small numbers (personal observation, based upon 28 years of electrofishing small New Jersey trout streams), they may be more vulnerable to extinction. Mason's Run, an isolated stream in south Jersey, had the lowest observed heterozygosity (0.342), allelic richness (1.7) and polymorphism (61.5%), and no unique alleles, though surprisingly inbreeding was not detected ($F_{IS} < 0$). The reason

for the low genetic diversity observed in this population is unclear, however, judging

from the difficulty obtaining just nine fish >10 cm over a considerable distance (in

comparison to other streams sampled) the population in Mason's Run is small. Processes

that diminish genetic diversity (genetic bottlenecks, random genetic drift, and inbreeding)

can be problematic in small populations. A founder event (the arrival of a few

individuals to a new area, either naturally or via stocking, that can result in a reduced

gene pool) is a plausible explanation, given the absence of other wild brook trout

populations in central and south Jersey. Whatever the explanation for the apparent low

genetic diversity, small populations such as Mason's Run, and others in New Jersey may

be at greater risk for extirpation.

Interestingly, the cultured trout from the Pequest Trout Hatchery collection had the

second highest heterozygosity (0.677) and the highest allelic richness (4.7). Surveys of

electrophoretic variation in brook trout have shown a similar pattern of relatively high

variability in hatchery populations and generally low variability in wild populations in the

southeastern United States (Wright and Atherton 1970; McGlade and MacCrimmon

1979; summarized in Stoneking et al. 1981). Similar results were found in a genetic

study of wild brook trout populations in the Great Smoky Mountains, some of which

were established through stocking (McCracken et al. 1993). The authors speculated that

the relatively high variability found in populations founded by hatchery strains could

reflect (1) the higher variability that is apparently carried in northeastern brook trout

populations, (2) the possible founding of the hatchery strains with fish from several

locations, and (3) possible interbreeding of hatchery fish with other hatchery stains or

wild populations. Stoneking et al. (1981) speculated that low variability in unstocked

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wild populations could result from isolation and small population size that promote genetic drift and inbreeding. The differences in heterozygosity observed between wild populations and the hatchery population in this study are likely due to differences in effective population size. The Pequest Trout Hatchery typically takes eggs from 400 to 450 females annually, and three times as many males as females are used to fertilize the eggs (W. Martka, NJDFW – Pequest Trout Hatchery, personal communication). Although the effective population size for wild brook trout in small New Jersey streams is not known, the degree of difficulty in obtaining fish greater than 10 cm in many of the streams sampled suggests that the effective population size is much smaller in small streams than in the hatchery.   It is also possible that the Nashua strain in the Pequest Trout Hatchery is more genetically diverse than wild trout populations.

Inbreeding ($F_{IS} > 0$) was detected in eight stream collections and the Pequest Trout Hatchery collection (Table 3). Sampling bias may explain why inbreeding was detected in some wild populations, where suitable specimens were abundant and typically collected over a shorter distance, thereby increasing the likelihood of relatedness. Small sample sizes may also contribute to the inbreeding detected.

Genetic Diversity among Populations and Genetic Structure

All of the statistical methods used in this study reject the null hypothesis of no genetic differentiation among brook trout from different streams and drainages in New Jersey. The microsatellite data in this study revealed a strong pattern of population subdivision among drainages, which suggests that geographic factors have played a major role in determining patterns of genetic structure among brook trout collections from New

Jersey. F_{ST} values measure population divergence and typically an F_{ST} above about 0.15 is considered to be an indication of significant differentiation among populations (Frankham et al. 2002). In my study, many pairwise F_{ST} estimates, and to a lesser extent R_{ST} estimates, for collections differed greatly from zero indicating divergence among populations. The AMOVA test also showed the highest variance at the population level (50.8%) rather than the drainage level. The general pattern of population uniqueness was further supported by the multilocus assignment tests, which correctly assigned individuals to their source population with a high level of accuracy (94.5%). The presence of 22 private alleles, 10 of which occurred at high frequencies, also indicates that populations have differentiated. Although the sample sizes and number of populations surveyed may limit the ability of the analyses to provide conclusive results, the results from my study agree with those from earlier genetic surveys involving microsatellite studies of brook trout, which have also shown a strong pattern of population sub-division (Castric et al. 2001, Angers et al. 1995, Adams and Hutchings 2003). The ability of the suite of 13 microsatellite loci to provide high resolution with small sample sizes was notable.

The pattern of genetic variation revealed by the 13 microsatellites indicates that population differentiation has occurred on a hydrogeographic scale in New Jersey. Populations within the Passaic and Raritan drainages showed the strongest genetic groupings, perhaps because these drainages are considerably smaller than the Delaware drainage and confined, for the most part to New Jersey. Furthermore, within these two drainages the streams that were geographically closest (i.e. connected by the shortest fluvial distance) were consistently shown in the tree topology to be most closely related.

For example, in the Raritan drainage, Oakdale Creek (OAK) and Hacklebarney Brook (HAC) in the Lamington River sub-drainage formed a subgroup, and Flanders Brook (FLA), Kruegers Creek (KRU), and Turkey Brook (TUR), which are located in the headwaters of the S/Br. Raritan River sub-drainage, also grouped together. Similarly in the Passaic drainage, Crooked Brook tributary (CBT) and Hibernia Brook (HIB) in the Rockaway River sub-drainage paired, as did Burnt Meadow Brook (BMB) and Havemeyer Brook (HAV) in the Wanaque-Ramapo subdrainage. Another striking feature of the NJ tree is that none of the populations from the Delaware and Hudson drainages, and the Hackensack subdrainage, grouped with populations from the Raritan or Passaic drainages. The tree topology suggests that the Hackensack River system, where Cresskill Brook (CRE) is found, should be considered a separate drainage from the Passaic, or alternatively, perhaps stocking has influenced the gene pool of this stream. Collectively, these patterns of gene diversity appear to reflect colonization of different drainages by genetically distinct fish and populations within drainages subsequently became further differentiated due to geographic isolation.

The grouping of populations by drainage or major basin has also been found in New York (Perkins et al. 1993), Tennessee (Kriegler et al. 1995), eastern Canada (Jones et al. 1996), and Maryland (Quattro et al. 1990; Hall et al. 2002). In contrast, a genetic study of brook trout populations inhabiting an open water system (Miramichi River drainage, New Brunswick) found that geographical factors play only a minor role in determining the patterns of genetic structure among drainages within a large river system (Rogers and Curry 2004). In open-river systems the potential for brook trout to disperse is much greater than in more closed systems having natural or manmade barriers. In New Jersey,

natural conditions and manmade barriers result in relatively closed river systems that separate populations of brook trout inhabiting small streams and inhibit their dispersal within the same drainage. This separation can effectively restrict or limit gene flow among these populations. Over time, this reproductive isolation, in concert with genetic drift and local mutations, has apparently resulted in sufficiently different allelic frequencies among populations, such that individuals can be correctly assigned to their population of origin with remarkable accuracy. Yet despite this divergence, many populations within drainages have retained sufficient genetic similarity, which allows them to form distinct groupings by drainage. The pattern of genetic structuring observed in this study suggests that a single panmictic population may have initially colonized each drainage. If true, then the presumed historical genetic relationships of populations within several New Jersey drainages may be relatively intact.

The Delaware drainage populations did not form a strong group compared to those from within the Passaic and Raritan drainages. Only three of the six Delaware drainage populations grouped together, and of these, two (Van Campens Brook, VCB, and Forked Brook, FOR), were proximate hydrogeographically while the third population (Kurtenbach's Brook, KUR) was much more distant. Of the three remaining populations from the Delaware drainage, a close genetic relationship was observed between two populations from Halfway House Brook (HWH) and Mason's Run (MAS), the isolated south Jersey stream, while Independence Brook (IND) grouped with two populations from two separate drainages. The failure of the Delaware drainage populations to group as a unit may be more a reflection of the sheer size of the Delaware drainage, and its more linear nature, when compared to the Passaic and Raritan drainages. Perhaps the

stocking of nonnative brook trout strains has impacted these Delaware drainage populations.

Finally, several populations failed to exhibit any affinity to their drainage of origin. Given the strong grouping of five Passaic drainage populations, the failure of Lake Stockholm Brook (LSO) and Cooley's Brook (COO) to group in this drainage suggests other forces have affected these populations. In the case of Lake Stockholm Brook, the presence of a null allele at one locus may have caused this aberration. With others, it is possible that the legacy of widespread stocking of cultured brook trout in New Jersey over the last century has left a lasting footprint on the native gene pools of some wild populations. This may be particularly true in Cooley's Brook, which was routinely stocked with trout prior to 1990. Rocky Run (ROC) in the Raritan drainage may also have been affected, as trout have been stocked downstream in Spruce Run Creek. Several wild brown trout were encountered when sampling for brook trout in this stream, indicating trout have been stocked in the stream or that stocked fish have migrated from downstream areas. Yet, other streams having a history of trout stocking (Flanders Brook and Van Campens Brook) do not show evidence of having been affected by stocking. Therefore, a history of stocking does not necessarily indicate that the genetic integrity of a wild population has been compromised by introgression of non-native genes.

Land use practices and widespread stocking of cultured salmonids over the last century have likely influenced the current distribution of this species and may have affected some native gene pools. This study provides evidence of genetic structure among wild brook trout populations in New Jersey and suggests that the stocking of hatchery-reared brook trout or transference of brook trout between drainages has likely

affected the genetic integrity of some native gene pools. I emphasize that small sample

sizes for each collection were used in this study and single populations were used to

represent entire drainages or large sub-drainages, which may limit the ability of the

analyses to provide conclusive results. However, the data suggests that the detection of

population structure is possible with a small sample size (10 individuals per population)

when 12-13 microsatellite loci are used. Additional studies using a larger sample size

and more collection sites are recommended to reinforce the inference gained in this study.

Management and Conservation Implications

As concerns increase for brook trout across its native range, the distribution and

pattern of genetic variation in brook trout populations have emerged as important

considerations in conservation of the species. Several pieces of evidence in this study

suggest that brook trout in New Jersey drainages should be considered a conservation

priority. The study revealed (1) distinct genetic structuring of brook trout in two

drainages, (2) genetically distinguishable populations in all four drainages, and (3) the

influence of hatchery stock on the Cooley's Brook population and possibly others in this

study. These results have important implications for managing and conserving New

Jersey's wild brook trout populations and the natural ecosystems they depend upon.

First, the pattern of fine-scale genetic variation, as indicated by the genetic distance

tree structure, the distribution of genetic variation, as measured by pairwise F_{ST} values,

and hierarchical gene diversity analysis, suggests that local populations of wild brook

trout in small streams should be treated as separate management units in order to preserve

their genetic integrity. However, separate management of every stream in New Jersey

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suspected of containing ancestral brook might not be feasible due to economic, legal, and sociocultural limitations. As suggested by Perkins et al. (1993), conservation efforts may therefore have to focus on a subset of populations that at a minimum maintains the genetic differentiation observed at two fundamental levels – among populations within drainages and among drainages.

Second, the low level of genetic diversity observed in small populations emphasizes the importance of restoring habitat connectivity and quality. Habitat loss and fragmentation are among the biggest threats to the long-term survival of brook trout populations in New Jersey (Hudy et al. 2005). Unfortunately, the restoration of historically connected streams may be impossible in much of New Jersey, given the realities of water development (streams and wetlands dammed by property owners to create permanent impoundments). Fisheries managers may have more success restoring physical habitat rather than re-connecting stream fragments.

Third, the genetic integrity of many of the wild populations in this study appears to be relatively unaffected by past stocking practices, allowing for potentially successful restoration efforts using locally adapted wild stock. The pattern of population structuring by drainage indicates that drainage and geographic proximity appear to be effective surrogate indicators of genetic relationships between populations. A restoration program should, therefore, rely upon transfers of wild stock from adjacent areas within the same drainage, preferably ones with no history of stocking. Translocation of fish among major drainages and stocking with cultured trout is not recommended because of large genetic differences observed among drainages and between wild fish and fish from the Pequest Trout Hatchery.

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Fourth, since stocking appears to have affected the genetic integrity of at least one of three brook trout populations that has been stocked in the past, resource managers should consider strategies to avoid or minimize further genetic interactions between cultured and wild brook trout. Hybridization between native and hatchery-produced salmonids is considered a serious threat to the long-term persistence and genetic integrity of native stocks (Allendorf and Leary 1988).   If a stocking program is widespread and interbreeding frequent, locally adapted native stocks will be replaced by larger more homogeneous populations (Krueger and May 1991). Therefore, genetic diversity should be an important consideration when stocking hatchery-reared trout in drainages where wild brook trout occur.

In New Jersey, the annual stocking of catchable-size hatchery-reared brook, brown, and rainbow trout has led to strong public support and high demand for trout. In recent years, NJDFW has instituted changes that address the ecological and genetic impacts of stocking while minimally impacting harvest-oriented anglers. Since the mid-1980's the stocking of cultured brook trout in small streams having wild brook trout has been, for the most part, discontinued, though non-native cultured salmonids were often substituted. When the *Wild Trout Stream* fishing regulation was adopted in 1990, stocking was discontinued on 29 designated streams, and some of those had spawning brook trout populations. Since then, nearly all small streams having wild brook trout have been removed from the stocking program, and a policy implemented in 2005 prevents stocking in most streams having reproducing trout populations (Hamilton and Barno 2005). An increase in the statewide minimum harvestable size for trout, from seven to nine inches,

has been proposed for 2008 which would further curtail the harvest of larger, potentially sexually mature trout, by anglers.

To protect the genetic integrity of New Jersey's native brook trout populations it may be prudent to consider additional strategies. For example, developing a sterile (triploid) trout program would allow for the continued stocking of brook trout at existing stocking locations while preventing introgression of non-native genes. Sterile trout, when stocked as catchables in streams, may provide recreational fisheries that are equal or superior to normal diploid fish (Kozfkay et al. 2006). The use of sterile trout would have the added benefit of further limiting the establishment of non-native salmonids in existing or potential brook trout habitat. Technical and economic considerations (equipment and manpower costs) associated with the production of sterile trout may limit the ability of an agency to undertake such a program.

Another strategy to preserve the genetic integrity would be to restrict the use of hatchery-produced (nonnative) brook trout in drainages where spawning brook trout populations occur or stock exclusively non-native trout species at existing stocking locations wirhin these drainages. Because brook trout declines in New Jersey have been attributed in part to the intrusion of non-native brown trout (Hudy et al. 2005), rainbow trout may be the preferred non-native species to stock in this situation. Although ecological hazards are still associated with sterile and non-native trout, these strategies would allow the stocking of hatchery-reared trout for harvest-oriented anglers to continue, with quantities of trout that anglers are accustomed to receiving. Perhaps combination of these and other options that capitalize on the flexibility of the existing

stocking program, while striking a better balance between ecological, economic, and social needs, have the best chance of succeeding.

Clearly, the recreational and intrinsic value of brook trout, coupled with an alarming decline in its distribution in parts of the eastern U.S., has triggered a concerted effort to manage and conserve the species. To protect the long-term viability of wild brook trout, management decisions regarding stewardship of this valuable resource must be based upon the best biological information available. Ancestral brook trout populations represent an irreplaceable part of the natural resources in New Jersey, and indeed elsewhere in its native range. Management agencies should make a concerted effort to identify native populations and safeguard their gene pools to preserve their genetic variability and potential to evolve in response to environmental change.

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Appendix 1: Genetic Variation in Brook Trout Collections from New Jersey

TABLE A1.1.—Allele sizes at 13 microsatellite DNA markers for 238 brook trout from 23 collections from New Jersey. See Table 1 for collection abbreviations that correspond to sample prefixes.

							Locus						
Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
FOR-14	221 221	116 116	175 183	143 143	120 123	101 101	184 187	130 151	243 243	221 230	176 188	228 256	226 226
FOR-15	219 219	110 116	175 179	143 143	120 120	101 101	184 184	130 151	243 243	221 230	200 208	252 260	274 282
FOR-16	219 219	110 116	179 179	143 143	120 120	110 110	181 190	142 145	241 243	224 224	176 200	236 240	218 218
FOR-17	221 221	116 119	175 177	143 143	120 120	101 110	184 184	130 151	239 241	221 221	176 208	228 244	218 238
FOR-18	221 223	110 116	175 183	143 143	120 123	101 101	181 184	151 151	241 243	230 230	188 208	236 256	218 218
FOR-19	219 223	116 119	175 179	143 143	120 120	119 119	184 184	148 148	241 243	221 230	176 176	232 236	218 234
FOR-20	221 221	113 116	167 175	143 143	120 120	101 116	184 187	130 145	239 239	221 230	180 200	216 220	218 226
FOR-21	221 223	116 119	179 183	143 143	120 123	101 110	184 184	130 151	239 243	230 230	188 208	236 256	226 238
FOR-22	221 223	116 119	175 183	143 143	120 120	101 101	181 184	142 151	241 243	221 230	180 208	232 248	218 226
VCB-01	219 221	119 119	167 175	143 146	120 120	101 101	184 184	145 151	243 305	224 230	180 208	216 252	226 274
VCB-02	211 223	119 119	167 175	143 146	120 120	101 119	184 193	133 133	239 305	221 221	208 208	212 224	218 234
VCB-04	219 223	119 119	175 187	143 143	120 120	113 113	184 184	142 145	237 239	221 224	212 212	232 232	218 234
VCB-08	221 223	119 119	167 175	143 143	120 120	101 101	187 187	142 142	243 305	221 221	176 212	216 216	214 218
VCB-09	221 221	119 119	175 187	143 143	120 120	101 101	187 193	142 145	309 325	221 224	176 208	216 224	218 218
VCB-10	219 221	119 119	167 175	143 143	120 120	101 101	184 190	142 145	243 305	221 221	176 208	224 236	210 274
VCB-11	211 221	119 119	187 187	143 143	120 120	101 113	187 187	133 133	243 329	230 230	176 204	216 224	214 218
VCB-12	219 221	119 119	175 175	143 146	120 120	101 101	184 184	145 154	243 305	224 230	176 184	216 216	222 226
VCB-13	215 221	119 119	175 179	146 146	120 123	101 119	187 190	124 130	241 243	230 230	176 176	232 236	214 214
IND-01	223 223	119 119	179 183	143 143	120 120	101 119	184 190	133 151	239 243	230 230	176 184	232 240	210 246
IND-02	223 223	113 116	179 179	143 143	120 120	101 110	184 184	133 151	243 341	230 230	180 224	212 240	210 246
IND-03	219 223	116 122	175 179	143 143	120 120	101 101	184 190	133 151	235 243	221 230	176 188	236 240	218 218
IND-04	223 223	116 122	175 179	143 143	120 120	101 119	190 190	133 151	235 243	230 230	176 180	212 240	210 218
IND-05	223 223	116 116	175 179	140 149	120 120	101 119	184 184	148 148	239 243	221 230	180 180	212 236	210 218
IND-06	219 223	116 116	179 179	143 143	120 120	101 113	184 184	133 133	239 241	221 221	180 224	236 240	210 234
IND-07	223 223	116 119	179 179	143 143	120 120	101 119	184 190	133 151	243 341	230 230	180 184	240 240	210 246
IND-08	221 223	116 119	175 179	143 146	120 120	101 119	190 196	133 148	235 341	230 230	176 180	228 240	210 218

						Locus							
Sample	*SfoB52*	*SfoC24*	*SfoC28*	*SfoC38*	*SfoC79*	*SfoC86*	*SfoC88*	*SfoC113*	*SfoC115*	*SfoC129*	*SfoD75*	*SfoD91*	*SfoD100*
IND-09	223 223	116 116	179 179	143 143	120 120	101 119	184 184	133 148	241 243	221 233	180 180	232 236	210 234
IND-10	223 223	116 119	179 183	143 143	120 120	119 119	184 184	133 151	239 243	230 230	176 224	212 240	210 234
IND-11	223 223	116 122	179 179	143 143	120 120	101 119	184 190	133 133	235 239	221 230	176 180	236 240	218 218
HWH-01	215 215	116 116	179 183	143 143	120 120	110 110	187 190	130 130	241 305	224 230	180 220	208 208	206 230
HWH-02	215 221	116 116	175 183	146 146	120 120	101 110	187 190	130 139	243 317	230 233	176 220	212 224	218 234
HWH-03	219 221	116 116	175 183	146 146	120 120	101 110	187 193	130 139	243 317	230 230	180 220	208 224	218 258
HWH-04	217 221	116 116	179 183	143 146	120 120	101 116	190 193	133 139	241 317	230 233	220 220	208 228	230 230
HWH-05	219 219	110 116	183 187	146 146	120 120	110 110	187 187	130 145	243 317	230 233	220 220	224 224	218 234
HWH-06	215 221	116 116	183 187	146 146	120 120	101 101	187 190	130 133	243 317	230 233	180 220	212 224	218 258
HWH-07	215 221	116 116	183 183	143 146	120 120	101 110	187 190	130 139	241 305	230 233	180 180	208 228	230 230
HWH-08	215 221	116 116	179 183	143 143	120 120	110 110	190 190	130 139	241 305	233 233	180 180	228 228	206 230
KUR-01	219 219	122 122	175 179	140 146	120 120	110 119	184 187	151 154	241 243	230 230	180 208	236 236	226 226
KUR-02	219 219	110 122	171 183	140 146	120 120	101 110	184 184	130 151	241 241	221 230	208 220	208 244	270 242
KUR-03	219 223	110 122	175 183	140 140	120 120	110 110	184 184	130 154	241 243	221 221	176 208	208 236	210 242
KUR-04	219 221	110 110	183 183	140 146	120 120	110 110	187 187	154 154	241 243	221 230	216 220	236 236	210 242
KUR-05	219 219	116 116	171 183	140 140	120 120	110 110	187 187	151 151	241 243	221 230	180 208	236 236	226 242
KUR-06	219 219	110 122	183 183	140 140	120 120	101 101	184 184	130 154	243 243	221 221	208 216	208 244	210 226
KUR-08	219 221	116 116	183 183	140 140	120 120	110 110	184 187	151 154	241 241	221 230	216 220	208 236	226 226
KUR-09	219 221	116 122	183 183	140 143	120 120	110 110	184 184	133 151	241 243	221 230	208 220	236 244	226 242
KUR-10	219 219	110 110	171 183	140 146	120 120	110 110	184 187	154 154	241 243	221 221	216 220	236 236	210 242
MAS-01	217 217	116 116	175 183	143 146	120 120	101 119	184 184	151 151	301 301	230 230	208 208	216 216	218 218
MAS-02	217 217	116 116	175 175	143 143	120 120	101 119	184 190	151 151	301 301	230 233	212 212	216 228	218 218
MAS-03	221 221	116 116	175 183	143 146	120 120	101 119	184 190	151 151	301 301	233 233	208 212	216 228	218 218
MAS-05	217 217	116 116	175 183	143 143	120 120	101 101	184 187	151 151	301 301	230 233	208 212	216 216	218 218
MAS-06	217 221	116 116	175 183	143 146	120 120	101 119	190 190	151 151	301 301	230 233	208 212	228 228	218 218
MAS-07	217 217	116 116	175 175	143 143	120 120	119 119	187 190	151 151	301 301	230 230	208 208	228 228	218 218
MAS-08	217 217	116 116	175 175	143 146	120 120	101 119	184 187	151 151	301 301	230 233	208 208	216 228	218 218
MAS-09	217 217	116 116	175 183	143 146	120 120	101 101	184 190	151 151	301 301	230 230	208 212	216 228	218 218
MAS-10	217 221	116 116	175 183	143 146	120 120	101 119	184 190	151 151	301 301	233 233	208 208	216 228	218 218

	Locus												
Sample	*SfoB52*	*SfoC24*	*SfoC28*	*SfoC38*	*SfoC79*	*SfoC86*	*SfoC88*	*SfoC113*	*SfoC115*	*SfoC129*	*SfoD75*	*SfoD91*	*SfoD100*
MPO-01	211 215	116 116	183 183	143 143	120 120	110 116	181 193	130 151	241 297	233 233	204 204	216 260	218 218
MPO-02	211 221	116 116	183 183	143 143	120 120	110 116	181 193	130 151	241 305	218 233	184 204	232 260	218 218
MPO-03	215 215	116 116	183 187	143 143	120 120	110 116	181 193	130 151	239 297	227 230	184 204	228 260	218 218
MPO-04	221 221	116 116	183 187	143 143	120 120	110 116	181 181	142 151	297 305	224 230	176 184	228 228	210 210
MPO-05	191 215	116 116	183 183	149 149	120 120	101 110	181 187	145 151	305 305	224 230	188 204	228 232	206 218
MPO-06	191 215	113 116	183 183	143 149	120 120	101 110	181 184	130 151	237 305	230 230	188 204	228 232	206 218
MPO-07	191 221	116 116	183 183	146 146	120 120	116 116	181 193	151 151	237 305	230 230	204 208	228 260	206 218
MPO-08	215 221	116 116	183 183	143 146	120 120	101 116	181 184	130 151	305 305	230 230	000 000	228 232	206 210
MPO-09	191 215	116 116	183 187	143 146	120 120	116 116	184 193	130 130	237 239	224 230	204 204	228 260	210 218
MPO-10	221 221	116 116	183 187	143 143	120 120	110 110	181 181	130 142	239 305	227 230	184 204	228 232	210 218
CRE-01	223 223	116 116	179 183	140 143	120 120	101 116	190 196	133 151	000 000	230 230	180 192	216 240	210 218
CRE-02	221 223	116 116	175 175	140 143	120 120	101 101	190 190	133 133	325 325	230 230	184 192	216 216	218 234
CRE-03	221 223	116 119	175 183	143 143	120 120	110 122	196 196	133 133	333 333	230 230	180 192	216 240	210 218
CRE-04	221 223	116 119	175 183	143 143	120 120	101 122	196 196	133 133	325 333	230 230	180 192	216 240	210 218
CRE-05	221 221	116 116	179 183	143 143	120 120	101 101	187 196	130 133	325 333	230 230	188 192	216 216	210 210
CRE-06	221 223	116 116	175 175	140 143	120 120	101 122	190 190	133 133	325 333	230 230	180 184	216 216	210 234
CRE-07	221 223	116 116	175 179	140 140	120 120	101 101	187 190	130 133	325 333	230 230	184 188	216 216	218 218
CRE-08	221 223	116 116	175 183	143 143	120 120	101 122	196 196	133 151	325 333	230 230	180 192	216 240	210 234
CRE-09	223 223	116 116	175 175	140 143	120 120	101 116	196 196	133 151	333 333	230 230	184 192	216 240	210 218
CRE-10	221 221	116 116	175 179	140 143	120 120	110 122	196 196	133 133	325 333	230 230	180 180	216 240	210 234
CRE-11	221 223	116 116	179 191	140 143	120 120	101 116	190 196	130 133	325 333	230 230	180 188	216 216	218 218
PRE-01	221 227	119 119	191 195	143 146	120 120	110 113	184 184	145 154	353 353	227 230	180 212	208 208	226 230
PRE-02	215 221	119 119	191 191	146 146	120 120	113 113	184 184	145 145	353 353	227 230	180 212	208 220	230 230
PRE-03	215 221	119 119	191 191	146 146	120 120	110 113	184 184	145 145	353 353	227 227	204 212	208 208	230 230
PRE-04	221 227	119 119	191 191	143 146	120 120	110 113	184 184	145 145	353 353	227 230	212 212	208 208	226 230
PRE-05	227 227	119 119	191 191	143 146	120 120	110 110	184 184	145 154	345 353	230 230	212 212	208 208	226 230
PRE-06	221 227	119 119	191 191	143 143	120 120	113 113	184 184	145 145	353 353	227 227	204 204	208 208	230 230
PRE-07	221 227	119 119	191 191	143 146	120 120	110 110	184 184	145 154	345 353	230 230	204 212	208 208	226 230
PRE-08	215 227	119 119	191 195	143 146	120 120	110 113	184 187	145 154	353 353	227 230	180 212	208 208	226 230

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							Locus						
Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
PRE-09	215 221	119 119	191 191	143 143	120 120	113 113	184 184	145 145	353 353	227 227	204 212	208 220	230 230
PRE-10	215 221	119 119	191 191	143 146	120 120	110 110	184 187	145 145	353 353	227 227	204 204	208 208	230 230
PRE-11	215 227	119 119	191 191	146 146	120 120	110 110	184 184	145 154	345 353	230 230	212 212	208 208	226 230
HAV-01	219 221	116 119	179 179	140 143	120 120	119 125	184 184	130 145	241 349	230 230	176 180	220 224	218 218
HAV-02	219 223	116 119	175 179	140 143	120 120	125 125	184 184	145 151	241 345	230 230	180 212	208 224	218 230
HAV-03	219 221	119 119	175 179	140 143	120 120	116 119	184 184	148 151	241 317	230 230	176 212	208 224	218 218
HAV-04	219 221	116 119	175 191	140 146	120 120	125 125	184 184	151 151	241 349	230 230	180 212	208 208	218 218
HAV-08	219 221	119 119	179 179	140 143	120 120	101 125	184 184	130 151	353 353	230 230	176 176	208 208	218 230
HAV-09	219 221	119 119	191 191	143 146	120 120	125 125	184 184	148 151	241 343	230 230	176 180	208 208	218 230
HAV-10	219 219	119 119	179 191	140 146	120 120	116 125	184 184	148 151	241 317	230 230	176 180	208 220	218 230
COO-01	203 215	113 119	167 183	143 143	120 123	101 110	181 187	130 151	305 305	224 230	176 188	216 220	218 230
COO-02	191 221	116 116	187 187	140 143	120 120	101 110	184 187	130 133	235 235	224 233	184 224	228 232	230 234
COO-03	215 223	116 119	183 187	143 143	120 123	101 116	184 187	130 130	235 249	233 233	208 224	232 232	218 234
COO-04	203 221	116 116	179 187	143 143	120 123	101 119	184 187	130 133	237 241	233 233	212 224	220 232	222 230
COO-05	191 203	116 119	179 187	143 143	120 120	101 113	187 187	130 133	235 241	233 233	212 224	228 236	222 222
COO-06	215 223	116 119	167 183	143 143	120 120	101 101	187 190	151 154	235 305	233 233	184 212	228 232	206 218
COO-07	215 223	113 116	179 183	143 143	120 123	116 119	184 187	151 151	305 305	224 230	224 224	228 236	218 218
COO-08	203 223	116 119	183 183	146 149	120 120	113 116	184 187	130 130	235 249	230 230	208 224	228 232	222 234
COO-09	203 215	116 116	183 183	143 149	120 123	113 119	184 187	130 130	235 249	230 230	208 224	228 232	222 230
COO-10	203 223	116 119	183 187	143 143	120 123	113 116	184 187	130 130	249 305	230 233	208 224	228 232	218 234
COO-11	215 223	116 119	171 187	140 143	120 123	101 110	184 190	145 151	235 337	224 224	208 212	224 232	230 234
BMB-01	221 221	116 119	171 191	140 143	120 120	110 110	184 184	154 154	345 345	230 233	176 208	204 208	218 226
BMB-02	221 221	116 119	191 191	140 140	120 120	110 110	184 184	154 157	241 345	230 233	176 208	204 208	218 218
BMB-03	221 221	116 119	191 195	140 143	120 120	110 113	184 184	154 157	241 345	230 230	176 208	204 204	218 218
BMB-04	221 221	116 119	191 191	140 143	120 120	110 110	184 184	154 157	345 345	230 233	176 176	204 204	218 218
BMB-05	221 221	116 122	171 171	140 143	120 120	101 110	184 184	154 154	305 305	000 000	208 208	208 208	226 226
BMB-06	221 221	116 119	191 191	140 143	120 120	110 116	184 184	154 157	241 337	230 230	208 212	204 204	218 218
BMB-07	221 221	116 119	171 191	140 143	120 120	110 110	184 184	154 157	337 345	230 233	208 212	204 208	218 226

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Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
											Locus		
BMB-08	221 221	116 119	171 191	140 140	120 120	101 116	184 184	154 157	337 345	230 230	208 212	204 208	218 218
BMB-09	221 223	116 119	171 187	140 143	120 120	110 116	184 184	154 154	337 337	230 230	176 212	204 208	218 218
BMB-10	221 221	119 119	167 191	143 143	120 120	101 110	184 184	151 154	337 337	230 230	208 224	204 208	218 226
BMB-11	221 221	119 119	179 187	143 143	120 120	101 110	184 184	154 154	337 337	227 230	208 224	204 208	218 218
LSB-01	221 221	116 119	179 179	143 146	120 120	101 110	184 184	130 133	305 313	230 230	176 176	000 000	230 234
LSB-02	215 221	119 119	179 179	143 143	120 120	101 101	184 184	130 130	305 305	230 233	176 180	000 000	218 218
LSB-03	215 219	116 119	167 179	143 143	120 120	101 110	184 184	130 130	305 349	230 230	176 176	000 000	218 218
LSB-04	219 219	116 119	167 179	143 143	120 120	101 101	184 193	133 145	305 345	230 233	176 176	000 000	230 230
LSB-05	215 219	116 119	179 179	143 146	120 120	101 110	184 184	130 130	305 305	230 233	176 176	000 000	230 234
LSB-06	219 223	116 119	179 179	143 146	120 120	101 110	184 184	133 145	243 305	230 230	176 180	000 000	218 230
LSB-07	219 221	116 119	179 179	143 146	120 120	101 101	184 184	145 151	243 305	230 230	176 180	000 000	218 234
LSB-08	215 223	116 116	167 179	143 146	120 120	101 101	184 184	130 151	243 305	230 230	176 212	000 000	218 218
LSB-09	221 221	113 116	167 179	143 143	120 120	101 101	184 184	130 145	235 305	230 230	176 176	000 000	230 234
LSB-10	219 221	113 116	179 179	143 143	120 120	101 101	184 184	130 142	305 305	230 233	176 176	000 000	218 230
HIB-01	221 223	116 116	167 175	140 143	120 120	110 119	184 190	130 130	239 349	230 230	204 204	212 216	234 234
HIB-02	223 223	116 116	175 175	143 143	120 120	110 110	184 187	130 151	345 349	224 230	204 220	220 220	222 234
HIB-03	215 221	116 116	175 179	143 143	120 120	119 128	190 190	130 151	249 353	224 230	200 204	216 216	234 234
HIB-04	221 221	116 116	167 175	140 146	120 120	110 128	184 190	130 151	353 353	230 230	200 204	212 220	234 234
HIB-05	223 223	116 116	167 175	143 146	120 120	110 110	184 187	130 151	239 239	224 230	176 180	220 220	234 234
HIB-06	221 223	116 116	167 175	143 143	120 120	101 110	190 190	133 133	239 349	230 230	220 220	212 220	222 234
HIB-07	221 221	116 116	175 179	140 146	120 120	119 119	187 190	133 151	241 349	230 230	204 204	220 220	222 234
HIB-08	221 223	116 119	179 179	143 143	120 120	119 119	184 190	130 151	349 357	230 230	204 204	216 220	222 234
HIB-09	221 225	116 116	175 179	143 146	120 120	110 128	184 193	130 133	239 345	224 230	204 220	216 236	234 234
HIB-10	221 223	116 116	175 179	143 146	120 120	110 128	184 187	130 151	239 349	224 230	200 204	212 216	234 234
CBT-01	215 215	116 116	175 175	143 146	120 120	119 119	184 184	130 130	345 345	230 230	196 204	204 204	218 238
CBT-02	215 221	116 116	175 175	143 143	120 120	119 119	184 184	130 142	345 345	230 233	176 196	204 204	234 238
CBT-03	000 000	000 000	175 191	000 000	120 120	113 119	184 184	130 130	345 345	230 233	204 204	204 204	218 238
CBT-04	221 221	116 116	203 203	143 146	120 120	101 119	184 184	130 130	345 345	230 233	176 180	204 204	218 234

	Locus												
Sample	*SfoB52*	*SfoC24*	*SfoC28*	*SfoC38*	*SfoC79*	*SfoC86*	*SfoC88*	*SfoC113*	*SfoC115*	*SfoC129*	*SfoD75*	*SfoD91*	*SfoD100*
CBT-05	215 221	116 116	191 203	143 143	120 120	119 119	184 184	130 130	345 345	230 230	196 196	204 204	218 218
CBT-06	215 221	116 116	175 203	143 143	120 120	119 119	184 184	130 130	345 345	230 230	176 180	204 232	234 238
CBT-07	215 221	116 119	191 191	143 146	120 120	119 119	184 184	130 130	305 345	230 230	180 204	204 204	218 238
CBT-08	221 221	116 116	191 203	143 146	120 120	101 119	184 184	130 130	345 345	230 233	176 180	204 204	218 234
CBT-09	221 221	116 116	175 203	143 146	120 120	101 119	184 184	130 130	345 345	230 230	196 204	232 232	218 218
FLA-01	217 221	110 116	175 179	140 146	120 120	101 119	184 193	130 148	297 303	221 230	176 200	248 248	214 222
FLA-02	217 221	110 116	175 179	143 146	120 120	116 119	184 193	130 148	297 303	230 230	176 176	240 248	214 222
FLA-03	217 221	110 113	179 179	143 143	120 120	101 110	181 193	133 133	297 303	230 230	176 200	232 232	218 218
FLA-04	211 219	116 125	175 183	140 140	120 120	101 119	184 184	154 154	297 303	236 236	176 200	212 232	214 214
FLA-05	219 221	110 125	175 183	140 143	120 120	101 119	184 184	148 148	297 303	230 236	200 200	232 248	214 214
FLA-06	217 221	110 116	175 175	000 000	120 120	101 119	184 184	130 148	297 303	221 230	176 200	248 248	214 230
FLA-07	219 221	110 110	175 183	140 143	120 120	101 110	184 184	148 154	237 297	236 236	176 176	232 248	214 214
FLA-08	217 221	110 116	175 179	140 143	120 120	101 116	190 193	130 151	243 303	230 230	176 176	248 248	214 222
FLA-09	217 219	113 119	179 179	140 146	120 120	116 116	193 193	148 154	243 301	230 230	176 200	208 216	218 222
FLA-10	217 219	110 125	175 179	143 143	120 120	119 119	193 193	145 148	243 301	230 230	176 200	236 240	214 214
FLA-11	217 219	110 113	175 179	140 143	120 120	119 119	184 184	148 154	237 297	230 230	176 176	216 216	214 214
FLA-12	219 221	110 116	179 179	143 143	120 120	101 116	193 193	133 151	297 305	221 230	200 200	208 212	214 234
FLA-13	217 221	110 125	175 179	143 143	120 120	101 119	184 184	130 133	305 305	230 230	184 200	212 248	222 234
KRU-01	221 221	113 119	187 191	143 146	120 120	110 116	184 190	133 133	239 305	224 230	180 180	236 260	234 234
KRU-02	217 221	119 119	175 191	146 146	120 120	110 116	190 190	130 133	305 305	230 236	180 180	212 236	206 230
KRU-03	219 221	119 119	167 171	146 146	120 123	110 110	184 184	130 130	305 305	233 236	180 224	212 260	230 238
KRU-04	219 219	113 119	179 191	143 146	120 120	116 116	184 193	133 133	241 305	230 230	180 180	212 236	214 230
KRU-05	221 221	113 119	167 191	143 146	120 123	110 110	184 193	130 130	305 305	230 233	180 180	220 248	218 230
KRU-06	217 221	113 119	167 175	140 143	120 123	110 110	184 193	130 133	239 305	230 233	176 180	232 248	218 218
KRU-07	221 221	113 116	179 187	140 143	120 123	110 110	181 184	130 133	239 305	230 233	180 204	212 220	218 234
KRU-08	217 219	113 116	175 179	143 143	120 120	110 110	181 184	133 154	237 301	221 230	176 204	208 220	230 230
KRU-09	217 221	116 119	191 191	146 146	120 120	110 110	184 184	130 133	239 305	224 224	176 180	212 260	230 230
KRU-10	221 221	113 119	175 175	143 143	120 123	110 119	181 184	133 148	239 305	230 230	180 180	260 260	218 234

TABLE A1.1.—Continued.

Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
							Locus						
TUR-01	000 000	116 122	175 179	000 000	120 120	110 119	000 000	133 151	000 000	230 233	180 180	000 000	210 218
TUR-02	221 221	110 110	175 175	146 146	120 120	110 125	184 184	151 151	239 239	221 230	176 212	208 212	214 230
TUR-03	221 221	110 113	175 179	143 146	120 120	110 125	181 187	130 151	239 241	218 233	176 204	208 208	238 238
TUR-04	217 221	110 110	167 167	140 143	120 120	101 110	181 193	151 151	239 321	230 230	204 212	212 228	230 230
TUR-05	219 223	110 113	175 183	143 146	120 120	113 116	184 187	133 145	237 239	230 233	204 212	208 212	234 238
TUR-06	217 225	110 110	175 175	140 146	120 120	110 110	181 184	148 151	241 341	230 233	180 184	208 212	218 238
TUR-07	219 221	110 113	175 175	143 146	120 120	110 119	184 193	130 151	241 341	230 230	176 212	216 236	214 226
TUR-08	219 221	113 113	175 179	143 143	120 120	110 125	181 184	130 151	241 341	230 233	180 204	208 212	230 238
TUR-09	217 221	113 119	175 179	143 146	120 120	110 125	184 193	151 151	241 243	230 230	196 196	212 212	230 234
TUR-10	219 219	110 119	171 175	140 143	120 120	110 125	184 184	151 151	239 241	230 233	204 212	204 260	226 238
SOH-02	219 221	116 122	179 179	143 143	120 120	101 101	181 184	130 130	239 241	230 230	176 200	220 228	222 238
SOH-03	219 221	113 119	179 179	140 143	120 120	101 101	184 184	130 133	239 337	230 230	176 208	212 212	222 246
SOH-04	219 221	122 122	175 179	143 149	120 120	101 101	184 193	130 133	301 305	224 230	180 180	212 212	238 238
SOH-05	219 221	113 113	167 179	143 146	120 120	101 101	184 184	133 133	241 305	230 233	184 204	208 228	230 238
SOH-06	219 221	113 113	179 179	140 143	120 120	101 116	184 184	133 133	241 305	230 233	176 184	212 212	222 238
SOH-07	219 219	119 122	179 179	143 143	120 120	116 116	184 184	130 130	241 241	233 233	176 200	212 212	222 222
SOH-08	215 219	113 122	179 179	140 143	120 120	101 116	184 184	133 133	241 241	230 233	176 184	212 212	222 222
SOH-09	219 221	119 122	179 179	143 143	120 120	101 116	184 184	130 133	241 241	233 233	184 220	212 260	222 222
SOH-10	219 219	119 119	179 179	143 143	120 120	101 116	184 184	130 133	241 241	230 230	176 184	220 228	218 226
SOH-12	219 221	113 113	179 179	140 143	120 120	101 116	184 184	133 133	241 305	230 233	200 200	212 228	222 222
ROC-01	215 219	116 122	167 183	140 143	120 120	101 125	190 190	151 151	239 239	230 230	176 188	232 232	218 218
ROC-02	000 000	116 116	175 175	140 140	120 120	101 125	190 196	136 151	239 239	230 233	176 176	000 000	218 238
ROC-03	219 219	116 122	167 179	140 143	120 120	101 116	190 196	136 148	241 241	230 230	176 176	224 232	218 234
ROC-04	215 219	116 122	179 183	140 143	120 120	116 125	196 196	148 151	241 241	233 233	176 188	224 224	218 234
ROC-05	219 219	110 122	179 183	140 143	120 120	116 119	190 190	130 151	239 239	230 233	176 176	224 224	218 218
ROC-06	219 219	116 122	175 183	140 143	120 120	101 101	190 196	130 133	239 305	230 230	176 188	220 224	238 238
ROC-07	219 219	122 122	000 000	140 143	120 120	000 000	190 196	000 000	239 239	230 233	176 176	220 224	234 238
ROC-08	219 219	116 116	175 179	140 143	120 120	000 000	196 196	148 151	241 241	233 233	176 176	212 224	218 234
ROC-09	219 221	116 116	175 183	140 140	120 120	125 125	190 190	133 148	239 297	233 233	176 176	224 224	218 234

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TABLE A1.1.—Continued.

Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
ROC-10	219 219	116 116	179 183	140 143	120 120	116 125	196 196	151 151	239 241	233 233	176 188	224 224	218 234
OAK-01	219 219	116 119	179 179	143 143	120 120	101 119	184 184	142 142	301 305	230 230	180 180	236 236	238 238
OAK-02	223 223	116 119	179 197	143 143	120 120	101 101	184 184	142 142	301 305	230 230	180 180	236 236	238 238
OAK-03	223 223	116 122	179 179	143 143	120 120	101 119	184 184	142 142	301 301	230 230	180 180	236 236	238 238
OAK-04	219 223	116 122	179 179	143 143	120 120	101 110	184 184	142 151	301 305	230 230	180 220	236 236	222 238
OAK-05	219 223	116 122	179 179	143 143	120 120	110 119	181 184	142 142	301 305	230 230	180 220	212 236	238 238
OAK-06	219 223	116 116	179 179	143 143	120 120	101 119	184 184	142 142	239 305	230 230	204 220	236 236	222 238
OAK-07	219 223	116 122	179 179	143 143	120 120	101 119	184 184	142 151	239 305	230 230	180 220	236 236	222 238
OAK-08	219 223	116 122	179 183	143 143	120 120	101 119	184 184	142 142	301 305	230 230	180 204	236 236	238 238
OAK-09	219 223	116 116	179 179	143 143	120 120	101 119	184 184	142 142	301 301	230 230	180 204	236 236	238 238
OAK-10	219 223	000 000	179 179	143 143	120 120	101 119	181 184	142 151	239 305	230 230	180 220	212 236	238 238
HAC-01	199 203	113 116	183 183	143 146	120 123	101 113	187 187	145 151	241 309	233 233	188 204	232 236	218 218
HAC-02	217 219	113 113	179 179	149 149	120 120	101 101	184 190	151 151	241 243	230 233	176 200	232 236	238 238
HAC-03	219 219	113 113	179 179	149 149	120 120	101 101	184 190	148 151	243 243	230 230	200 200	232 236	238 238
HAC-04	217 223	116 116	179 179	143 149	120 120	101 119	184 184	139 142	301 305	230 230	180 212	236 236	222 238
HAC-06	217 219	113 113	179 179	143 149	120 120	101 101	184 193	151 151	241 243	230 233	176 200	236 236	238 238
HAC-07	217 219	113 113	175 187	143 149	120 123	101 110	190 193	133 148	241 301	230 230	176 200	212 216	222 238
HAC-08	221 221	110 122	175 187	143 149	120 123	101 101	190 190	133 133	239 313	230 233	220 220	232 232	238 238
HAC-09	217 219	110 113	179 179	143 149	120 120	101 101	184 193	133 151	241 243	230 233	200 200	232 236	238 238
HAC-10	217 223	110 119	179 191	143 149	120 120	000 000	184 184	130 142	305 305	230 230	180 212	212 236	222 238
HAC-11	217 223	110 119	179 179	143 149	120 120	119 119	184 184	130 142	305 305	230 230	180 212	236 236	222 238
PTH-01	203 221	113 116	183 187	140 143	120 120	101 101	181 184	145 151	241 309	224 233	200 220	216 228	218 218
PTH-02	211 223	113 119	179 183	140 143	120 120	110 116	181 187	133 151	237 243	230 233	180 204	232 280	206 206
PTH-03	221 221	116 116	179 183	146 146	120 120	101 110	181 190	145 151	309 337	230 233	192 212	216 240	206 218
PTH-04	203 215	116 116	183 183	143 143	120 120	110 116	181 184	145 151	241 241	224 227	188 204	220 232	218 218
PTH-05	215 215	113 116	183 187	143 143	120 120	101 101	181 187	145 151	305 305	230 233	204 212	260 280	206 218
PTH-06	211 223	116 116	179 183	140 143	120 123	110 110	190 193	151 151	243 333	221 233	184 184	216 232	218 222
PTH-07	221 227	113 116	179 187	146 146	120 120	110 110	184 190	133 145	305 305	233 233	188 224	232 256	218 218
PTH-08	215 215	113 116	179 183	143 146	120 120	101 110	184 187	145 151	241 305	221 233	204 212	216 232	206 218

TABLE A1.1.—Continued.

							Locus						
Sample	SfoB52	SfoC24	SfoC28	SfoC38	SfoC79	SfoC86	SfoC88	SfoC113	SfoC115	SfoC129	SfoD75	SfoD91	SfoD100
PTH-09	221 221	113 116	179 187	143 143	120 120	101 110	190 190	145 151	305 333	233 233	188 204	232 232	218 218
PTH-10	221 221	113 116	187 187	140 149	120 120	113 119	181 190	133 145	235 235	233 236	188 196	232 240	218 226
PTH-11	199 221	116 119	179 187	140 143	120 120	101 110	187 193	130 133	243 337	233 233	192 204	216 232	206 230
PTH-12	221 221	113 113	179 183	143 143	120 120	101 113	181 184	145 145	243 305	221 227	192 212	220 232	218 222
PTH-13	215 221	116 119	179 179	143 143	120 120	110 116	181 190	142 151	243 305	227 233	188 204	232 232	206 234
PTH-14	211 221	113 116	183 199	146 146	120 120	110 110	190 193	145 145	241 241	230 233	184 212	228 232	206 222
PTH-15	215 227	116 116	183 187	143 146	123 123	110 119	181 190	130 145	333 337	233 236	176 204	240 256	206 206
PTH-16	221 221	113 116	179 187	143 143	120 123	110 113	184 190	145 151	305 309	218 233	180 192	216 220	206 230
PTH-17	207 221	113 116	187 187	140 140	120 120	113 119	181 190	130 151	305 305	233 233	176 224	216 224	218 230
PTH-18	215 215	116 116	179 183	143 143	120 120	101 101	181 184	142 145	237 249	233 233	188 204	216 232	206 234
PTH-19	207 215	113 116	183 187	143 143	120 123	110 116	184 187	130 145	241 243	230 233	188 188	236 280	206 230
PTH-20	203 203	116 116	183 183	140 143	120 120	101 119	187 187	133 145	305 309	221 221	192 204	224 236	218 218

TABLE A1.2.—Allele frequencies, sample size (*N*), observed heterozygosity by locus (*H*), mean heterozygosity, and mean number of alleles per locus at 13 microsatellite DNA markers in brook trout from 23 collections from New Jersey. See Table 1 for collection abbreviations. Allele frequencies in bold italics indicate private alleles (found only in one collection).

							Collection					
Allele	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB
SfoB52												
N	9	9	11	8	9	9	10	11	11	7	11	11
191	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.091	0.000
199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.273	0.000
207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
211	0.000	0.111	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000
215	0.000	0.056	0.000	0.375	0.000	0.000	0.350	0.000	0.273	0.000	0.273	0.000
217	0.000	0.000	0.000	0.063	0.000	0.778	0.000	0.000	0.000	0.000	0.000	0.000
219	0.278	0.222	0.091	0.188	0.778	0.000	0.000	0.000	0.000	0.571	0.000	0.000
221	0.500	0.444	0.045	0.375	0.167	0.222	0.350	0.500	0.364	0.357	0.091	0.955
223	0.222	0.167	0.864	0.000	0.056	0.000	0.000	0.500	0.000	0.071	0.273	0.045
225	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
227	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.364	0.000	0.000	0.000
H	0.444	0.889	0.273	0.750	0.444	0.222	0.700	0.636	0.909	0.857	1.000	0.091
SfoC24												
N	9	9	11	8	9	9	10	11	11	7	11	11
110	0.167	0.000	0.000	0.063	0.389	0.000	0.000	0.000	0.000	0.000	0.000	0.000
113	0.056	0.000	0.045	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.091	0.000
116	0.556	0.000	0.591	0.938	0.278	1.000	0.950	0.909	0.000	0.214	0.591	0.409
119	0.222	1.000	0.227	0.000	0.000	0.000	0.000	0.091	1.000	0.786	0.318	0.545
122	0.000	0.000	0.136	0.000	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.045
125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.889	0.000	0.636	0.125	0.444	0.000	0.100	0.182	0.000	0.429	0.727	0.818
SfoC28												
N	9	9	11	8	9	9	10	11	11	7	11	11
167	0.056	0.222	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.045
171	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.045	0.273
175	0.389	0.500	0.182	0.125	0.111	0.667	0.000	0.500	0.000	0.214	0.000	0.000
177	*0.056*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
179	0.278	0.056	0.727	0.188	0.056	0.000	0.000	0.227	0.000	0.500	0.136	0.045
183	0.222	0.000	0.091	0.563	0.667	0.333	0.800	0.227	0.000	0.000	0.409	0.000
187	0.000	0.222	0.000	0.125	0.000	0.000	0.200	0.000	0.000	0.000	0.318	0.091
191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.909	0.286	0.000	0.500
195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.045
197	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.889	0.778	0.545	0.875	0.556	0.667	0.400	0.727	0.182	0.571	0.727	0.636

Allele	Collection										
	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
						SfoB52					
N	10	10	8	13	10	9	10	9	10	10	20
191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.025
203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.100
207	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.050*
211	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.075
215	0.200	0.050	0.375	0.000	0.000	0.000	0.050	0.111	0.000	0.000	0.250
217	0.000	0.000	0.000	0.346	0.200	0.167	0.000	0.000	0.000	0.350	0.000
219	0.350	0.000	0.000	0.269	0.200	0.278	0.600	0.833	0.450	0.300	0.000
221	0.350	0.500	0.625	0.346	0.600	0.444	0.350	0.056	0.000	0.100	0.400
223	0.100	0.400	0.000	0.000	0.000	0.056	0.000	0.000	0.550	0.150	0.050
225	0.000	0.050	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
227	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
H	0.700	0.600	0.500	1.000	0.500	0.667	0.800	0.333	0.700	0.800	0.550
						SfoC24					
N	10	10	8	13	10	10	10	10	9	10	20
110	0.000	0.000	0.000	0.462	0.000	0.500	0.000	0.050	0.000	0.200	0.000
113	0.100	0.000	0.000	0.115	0.350	0.300	0.400	0.000	0.000	0.500	0.325
116	0.500	0.950	0.938	0.231	0.150	0.050	0.050	0.600	0.611	0.150	0.600
119	0.400	0.050	0.063	0.038	0.500	0.100	0.250	0.000	0.111	0.100	0.075
122	0.000	0.000	0.000	0.000	0.000	0.050	0.300	0.350	0.278	0.050	0.000
125	0.000	0.000	0.000	*0.154*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.800	0.100	0.125	0.923	0.800	0.600	0.500	0.500	0.778	0.500	0.650
						SfoC28					
N	10	10	9	13	10	10	10	9	10	10	20
167	0.200	0.200	0.000	0.000	0.150	0.100	0.050	0.111	0.000	0.000	0.000
171	0.000	0.000	0.000	0.000	0.050	0.050	0.000	0.000	0.000	0.000	0.000
175	0.000	0.500	0.389	0.423	0.250	0.600	0.050	0.278	0.000	0.100	0.000
177	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
179	0.800	0.300	0.000	0.462	0.150	0.200	0.900	0.278	0.900	0.650	0.300
183	0.000	0.000	0.000	0.115	0.000	0.050	0.000	0.333	0.050	0.100	0.375
187	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.100	0.300
191	0.000	0.000	0.278	0.000	0.300	0.000	0.000	0.000	0.000	0.050	0.000
195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
197	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.050*	0.000	0.000
199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.025*
203	0.000	0.000	*0.333*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.400	0.800	0.556	0.692	0.800	0.600	0.200	0.889	0.200	0.300	0.750

						Collection						
Allele	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB
						SfoC38						
N	9	9	11	8	9	9	10	11	11	7	11	11
140	0.000	0.000	0.045	0.000	0.722	0.000	0.000	0.364	0.000	0.429	0.091	0.500
143	1.000	0.722	0.864	0.375	0.056	0.667	0.650	0.636	0.455	0.357	0.773	0.500
146	0.000	0.278	0.045	0.625	0.222	0.333	0.200	0.000	0.545	0.214	0.045	0.000
149	0.000	0.000	0.045	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.091	0.000
H	0.000	0.333	0.182	0.250	0.556	0.667	0.300	0.545	0.545	1.000	0.364	0.636
						SfoC79						
N	9	9	11	8	9	9	10	11	11	7	11	11
120	0.833	0.944	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.682	0.000
123	0.167	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.318	0.000
H	0.333	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.636	0.000
						SfoC86						
N	9	9	11	8	9	9	10	11	11	7	11	11
101	0.611	0.722	0.500	0.375	0.167	0.556	0.150	0.545	0.000	0.071	0.364	0.182
110	0.222	0.000	0.045	0.563	0.778	0.000	0.400	0.091	0.545	0.000	0.136	0.636
113	0.000	0.167	0.045	0.000	0.000	0.000	0.000	0.000	0.455	0.000	0.182	0.045
116	0.056	0.000	0.000	0.063	0.000	0.000	0.450	0.136	0.000	0.143	0.182	0.136
119	0.111	0.111	0.409	0.000	0.056	0.444	0.000	0.000	0.000	0.143	0.136	0.000
122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.227*	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.643	0.000	0.000
128	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.333	0.333	0.818	0.500	0.222	0.667	0.700	0.727	0.364	0.571	0.909	0.636
						SfoC88						
N	9	9	11	8	9	9	10	11	11	7	11	11
181	0.167	0.000	0.000	0.000	0.000	0.000	0.550	0.000	0.000	0.000	0.045	0.000
184	0.667	0.444	0.636	0.000	0.611	0.444	0.150	0.000	0.909	1.000	0.364	0.000
187	0.111	0.333	0.000	0.438	0.389	0.167	0.050	0.091	0.091	0.000	0.500	0.000
190	0.056	0.111	0.318	0.438	0.000	0.389	0.000	0.318	0.000	0.000	0.091	0.000
193	0.000	0.111	0.000	0.125	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000
196	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.591	0.000	0.000	0.000	0.000
H	0.556	0.444	0.455	0.750	0.333	0.778	0.800	0.364	0.182	0.000	0.909	0.000
						SfoC113						
N	9	9	11	8	9	9	10	11	11	7	11	11
124	0.000	*0.056*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
130	0.278	0.056	0.000	0.500	0.167	0.000	0.400	0.136	0.000	0.143	0.545	0.000
133	0.000	0.222	0.545	0.125	0.056	0.000	0.000	0.727	0.000	0.000	0.136	0.000
136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
139	0.000	0.000	0.000	0.313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
142	0.111	0.278	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000
145	0.111	0.278	0.000	0.063	0.000	0.000	0.050	0.000	0.773	0.143	0.045	0.000
148	0.111	0.000	0.182	0.000	0.000	0.000	0.000	0.000	0.000	0.214	0.000	0.000
151	0.389	0.056	0.273	0.000	0.333	1.000	0.450	0.136	0.000	0.500	0.227	0.045
154	0.000	0.056	0.000	0.000	0.444	0.000	0.000	0.000	0.227	0.000	0.045	0.682
157	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.273*
H	0.778	0.667	0.727	0.875	0.667	0.000	0.800	0.545	0.455	0.857	0.545	0.636

TABLE A1.2.—Extended.

Allele	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
						Collection					
						SfoC38					
N	10	10	8	12	10	9	10	10	10	10	20
140	0.000	0.150	0.000	0.333	0.100	0.167	0.200	0.600	0.000	0.000	0.200
143	0.750	0.600	0.688	0.542	0.450	0.444	0.700	0.400	1.000	0.400	0.575
146	0.250	0.250	0.313	0.125	0.450	0.389	0.050	0.000	0.000	0.050	0.200
149	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.550	0.025
H	0.500	0.600	0.625	0.583	0.500	0.778	0.600	0.800	0.000	0.800	0.400
						SfoC79					
N	10	10	9	13	10	10	10	10	10	10	20
120	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	1.000	0.850	0.875
123	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.150	0.125
H	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.000	0.300	0.150
						SfoC86					
N	10	10	9	13	10	10	10	8	10	9	20
101	0.800	0.050	0.167	0.346	0.000	0.050	0.650	0.313	0.500	0.722	0.300
110	0.200	0.450	0.000	0.077	0.750	0.500	0.000	0.000	0.100	0.056	0.400
113	0.000	0.000	0.056	0.000	0.000	0.050	0.000	0.000	0.000	0.056	0.100
116	0.000	0.000	0.000	0.192	0.200	0.050	0.350	0.250	0.000	0.000	0.100
119	0.000	0.300	0.778	0.385	0.050	0.100	0.000	0.063	0.400	0.167	0.100
122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.375	0.000	0.000	0.000
128	0.000	*0.200*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.400	0.600	0.444	0.769	0.300	0.900	0.500	0.750	0.900	0.333	0.700
						SfoC88					
N	10	10	9	13	10	9	10	10	10	10	20
181	0.000	0.000	0.000	0.038	0.150	0.222	0.050	0.000	0.100	0.000	0.275
184	0.950	0.350	1.000	0.538	0.550	0.500	0.900	0.000	0.900	0.500	0.200
187	0.000	0.200	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.100	0.175
190	0.000	0.400	0.000	0.038	0.150	0.000	0.000	0.500	0.000	0.250	0.275
193	0.050	0.050	0.000	0.385	0.150	0.167	0.050	0.000	0.000	0.150	0.075
196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.000	0.000
H	0.100	0.800	0.000	0.308	0.700	0.778	0.200	0.400	0.200	0.500	0.900
						SfoC113					
N	10	10	9	13	10	10	10	9	10	10	20
124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
130	0.500	0.450	0.944	0.192	0.400	0.150	0.400	0.111	0.000	0.100	0.100
133	0.150	0.200	0.000	0.154	0.500	0.100	0.600	0.111	0.000	0.200	0.125
136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.111*	0.000	0.000	0.000
139	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
142	0.050	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.850	0.150	0.050
145	0.200	0.000	0.000	0.038	0.000	0.050	0.000	0.000	0.000	0.050	0.425
148	0.000	0.000	0.000	0.346	0.050	0.050	0.000	0.222	0.000	0.100	0.000
151	0.100	0.350	0.000	0.077	0.000	0.650	0.000	0.444	0.150	0.350	0.300
154	0.000	0.000	0.000	0.192	0.050	0.000	0.000	0.000	0.000	0.000	0.000
157	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.700	0.800	0.111	0.769	0.600	0.600	0.400	0.778	0.300	0.700	0.850

TABLE A1.2.─Continued.

Allele	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB
							SfoC115					
N	9	9	11	8	9	9	10	10	11	7	11	11
235	0.000	0.000	0.182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.364	0.000
237	0.000	0.056	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.045	0.000
239	0.222	0.111	0.227	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.000	0.000
241	0.278	0.056	0.091	0.250	0.556	0.000	0.100	0.000	0.000	0.429	0.091	0.136
243	0.500	0.333	0.364	0.250	0.444	0.000	0.000	0.000	0.000	0.000	0.000	0.000
249	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.182	0.000
297	0.000	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.000	0.000	0.000	0.000
301	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
303	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
305	0.000	0.278	0.000	0.188	0.000	0.000	0.450	0.000	0.000	0.000	0.273	0.091
309	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
317	0.000	0.000	0.000	0.313	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000
321	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
325	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.450	0.000	0.000	0.000	0.000
329	0.000	*0.056*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.550	0.000	0.000	0.000	0.000
337	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.409
341	0.000	0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
343	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.071*	0.000	0.000
345	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.136	0.071	0.000	0.364
349	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000
353	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.864	0.143	0.000	0.000
357	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.667	1.000	1.000	1.000	0.667	0.000	0.800	0.700	0.273	0.857	0.727	0.455
							SfoC129					
N	9	9	11	8	9	9	10	11	11	7	11	10
218	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000
221	0.389	0.444	0.273	0.000	0.611	0.000	0.000	0.000	0.000	0.000	0.000	0.000
224	0.111	0.222	0.000	0.063	0.000	0.000	0.150	0.000	0.000	0.000	0.227	0.000
227	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.545	0.000	0.000	0.050
230	0.500	0.333	0.682	0.500	0.389	0.556	0.550	1.000	0.455	1.000	0.318	0.750
233	0.000	0.000	0.045	0.438	0.000	0.444	0.150	0.000	0.000	0.000	0.455	0.200
236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.556	0.444	0.364	0.750	0.556	0.444	0.600	0.000	0.364	0.000	0.364	0.500

TABLE A1.2.—Extended.

Allele	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
						Collection					
						SfoC115					
N	10	10	9	13	10	9	10	10	10	10	20
235	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
237	0.000	0.000	0.000	0.077	0.050	0.056	0.000	0.000	0.000	0.000	0.050
239	0.000	0.300	0.000	0.000	0.250	0.333	0.100	0.550	0.150	0.050	0.000
241	0.000	0.050	0.000	0.000	0.050	0.333	0.600	0.350	0.000	0.250	0.175
243	0.150	0.000	0.000	0.115	0.000	0.056	0.000	0.000	0.000	0.250	0.150
249	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025
297	0.000	0.000	0.000	0.346	0.000	0.000	0.000	0.050	0.000	0.000	0.000
301	0.000	0.000	0.000	0.077	0.050	0.000	0.050	0.000	0.450	0.100	0.000
303	0.000	0.000	0.000	*0.269*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
305	0.650	0.000	0.056	0.115	0.600	0.000	0.200	0.050	0.400	0.250	0.300
309	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.100
313	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
317	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
321	0.000	0.000	0.000	0.000	0.000	*0.056*	0.000	0.000	0.000	0.000	0.000
325	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
329	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075
337	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.075
341	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000
343	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
345	0.050	0.100	0.944	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
349	0.050	0.300	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
353	0.000	0.150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
357	0.000	*0.050*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.700	0.800	0.111	0.923	0.700	0.889	0.600	0.300	0.800	0.700	0.700
						SfoC129					
N	10	10	9	13	10	10	10	10	10	10	20
218	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.025
221	0.000	0.000	0.000	0.115	0.050	0.050	0.000	0.000	0.000	0.000	0.125
224	0.000	0.250	0.000	0.000	0.150	0.000	0.050	0.000	0.000	0.000	0.050
227	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075
230	0.800	0.750	0.778	0.692	0.500	0.600	0.550	0.450	1.000	0.700	0.125
233	0.200	0.000	0.222	0.000	0.200	0.300	0.400	0.550	0.000	0.300	0.550
236	0.000	0.000	0.000	0.192	0.100	0.000	0.000	0.000	0.000	0.000	0.050
H	0.400	0.500	0.444	0.308	0.700	0.700	0.500	0.300	0.000	0.400	0.700

Allele	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB
						Collection						
						SfoD75						
N	9	9	11	8	9	9	9	11	11	7	11	11
176	0.278	0.389	0.273	0.063	0.056	0.000	0.056	0.000	0.000	0.429	0.045	0.273
180	0.111	0.056	0.455	0.438	0.111	0.000	0.000	0.364	0.136	0.357	0.000	0.000
184	0.000	0.056	0.091	0.000	0.000	0.000	0.222	0.182	0.000	0.000	0.091	0.000
188	0.167	0.000	0.045	0.000	0.000	0.000	0.111	0.136	0.000	0.000	0.045	0.000
192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.318	0.000	0.000	0.000	0.000
196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
204	0.000	0.056	0.000	0.000	0.000	0.000	0.556	0.000	0.318	0.000	0.000	0.000
208	0.278	0.278	0.000	0.000	0.333	0.667	0.056	0.000	0.000	0.000	0.227	0.455
212	0.000	0.167	0.000	0.000	0.000	0.333	0.000	0.000	0.545	0.214	0.182	0.182
216	0.000	0.000	0.000	0.000	*0.222*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.000	0.000	0.500	0.278	0.000	0.000	0.000	0.000	0.000	0.000	0.000
224	0.000	0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.409	0.091
H	0.889	0.667	0.818	0.500	1.000	0.444	0.778	0.909	0.545	0.857	0.909	0.818
						SfoD91						
N	9	9	11	8	9	9	10	11	11	7	11	11
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.591
208	0.000	0.000	0.000	0.313	0.222	0.000	0.000	0.000	0.909	0.643	0.000	0.409
212	0.000	0.056	0.182	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.056	0.389	0.000	0.000	0.000	0.500	0.050	0.727	0.000	0.000	0.045	0.000
220	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.143	0.091	0.000
224	0.000	0.222	0.000	0.313	0.000	0.000	0.000	0.000	0.000	0.214	0.045	0.000
228	0.111	0.000	0.045	0.250	0.000	0.500	0.450	0.000	0.000	0.000	0.318	0.000
232	0.111	0.167	0.091	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.409	0.000
236	0.222	0.111	0.227	0.000	0.611	0.000	0.000	0.000	0.000	0.000	0.091	0.000
240	0.056	0.000	0.455	0.000	0.000	0.000	0.000	0.273	0.000	0.000	0.000	0.000
244	0.056	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000
248	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
252	0.056	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
256	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
260	0.056	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000
280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	1.000	0.667	0.909	0.625	0.556	0.556	0.900	0.545	0.182	0.571	0.909	0.636

TABLE A1.2.—Extended.

Allele	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
						Collection					
						SfoD75					
N	10	10	9	13	10	10	10	10	10	10	20
176	0.800	0.050	0.222	0.538	0.150	0.150	0.300	0.800	0.000	0.150	0.050
180	0.150	0.050	0.222	0.000	0.700	0.200	0.100	0.000	0.600	0.150	0.050
184	0.000	0.000	0.000	0.038	0.000	0.050	0.250	0.000	0.000	0.000	0.075
188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.050	0.200
192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125
196	0.000	0.000	0.278	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.025
200	0.000	0.150	0.000	0.423	0.000	0.000	0.200	0.000	0.000	0.350	0.025
204	0.000	0.550	0.278	0.000	0.100	0.250	0.050	0.000	0.150	0.050	0.250
208	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
212	0.050	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.150	0.125
216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
220	0.000	0.200	0.000	0.000	0.000	0.000	0.050	0.000	0.250	0.100	0.025
224	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.050
H	0.400	0.600	0.778	0.538	0.500	0.800	0.800	0.400	0.700	0.700	0.900
						SfoD91					
N	0	10	9	13	10	9	10	9	10	10	20
204	0.000	0.000	0.833	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
208	0.000	0.000	0.000	0.077	0.050	0.333	0.050	0.000	0.000	0.000	0.000
212	0.000	0.200	0.000	0.115	0.250	0.389	0.600	0.056	0.100	0.100	0.000
216	0.000	0.300	0.000	0.115	0.000	0.056	0.000	0.000	0.000	0.050	0.200
220	0.000	0.450	0.000	0.000	0.150	0.000	0.100	0.111	0.000	0.000	0.075
224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.667	0.000	0.000	0.050
228	0.000	0.000	0.000	0.000	0.000	0.056	0.200	0.000	0.000	0.000	0.050
232	0.000	0.000	0.167	0.192	0.050	0.000	0.000	0.167	0.000	0.300	0.350
236	0.000	0.050	0.000	0.038	0.150	0.056	0.000	0.000	0.900	0.550	0.050
240	0.000	0.000	0.000	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.075
244	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
248	0.000	0.000	0.000	0.385	0.100	0.000	0.000	0.000	0.000	0.000	0.000
252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
260	0.000	0.000	0.000	0.000	0.250	0.056	0.050	0.000	0.000	0.000	0.025
280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	***0.075***
H	0.000	0.600	0.111	0.615	0.900	0.778	0.500	0.444	0.200	0.600	0.900

TABLE A1.2.─Continued.

Allele	FOR	VCB	IND	HWH	KUR	MAS	MPO	CRE	PRE	HAV	COO	BMB
											Collection	
						SfoD100						
N	9	9	11	8	9	9	10	11	11	7	11	11
206	0.000	0.000	0.000	0.125	0.000	0.000	0.200	0.000	0.000	0.000	0.045	0.000
210	0.000	0.056	0.409	0.000	0.222	0.000	0.250	0.409	0.000	0.000	0.000	0.000
214	0.000	0.222	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
218	0.444	0.333	0.318	0.250	0.000	1.000	0.550	0.409	0.000	0.714	0.273	0.773
222	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.000
226	0.278	0.111	0.000	0.000	0.389	0.000	0.000	0.000	0.273	0.000	0.000	0.227
230	0.000	0.000	0.000	0.375	0.000	0.000	0.000	0.000	0.727	0.286	0.227	0.000
234	0.056	0.111	0.136	0.125	0.000	0.000	0.000	0.182	0.000	0.000	0.227	0.000
238	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.000	0.000	0.000	0.000	*0.333*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
246	0.000	0.000	0.136	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	*0.125*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	*0.056*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
274	0.056	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
282	*0.056*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.667	0.778	0.818	0.750	0.778	0.000	0.600	0.727	0.545	0.571	0.818	0.273
						Means and SEs						
H	0.615	0.547	0.580	0.596	0.521	0.342	0.575	0.508	0.350	0.549	0.734	0.472
SE	0.079	0.083	0.085	0.085	0.069	0.087	0.080	0.080	0.070	0.098	0.58	0.080
Alleles	4.31	4.46	3.62	3.23	3.00	1.69	3.46	2.54	2.00	2.77	4.54	2.85
SE	0.67	0.62	0.31	0.30	0.30	0.17	0.35	0.29	0.16	0.39	0.35	0.39

TABLE A1.2.—Extended.

Allele	LSB	HIB	CBT	FLA	KRU	TUR	SOH	ROC	OAK	HAC	PTH
						Collection					
						SfoD100					
N	10	10	9	13	10	10	10	10	10	10	20
206	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.325
210	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000
214	0.000	0.000	0.000	0.577	0.050	0.100	0.000	0.000	0.000	0.000	0.000
218	0.450	0.000	0.500	0.115	0.250	0.100	0.050	0.500	0.000	0.100	0.425
222	0.000	0.200	0.000	0.192	0.000	0.000	0.550	0.000	0.150	0.200	0.075
226	0.000	0.000	0.000	0.000	0.000	0.100	0.050	0.000	0.000	0.000	0.025
230	0.350	0.000	0.000	0.038	0.400	0.250	0.050	0.000	0.000	0.000	0.100
234	0.200	0.800	0.222	0.077	0.200	0.100	0.000	0.300	0.000	0.000	0.050
238	0.000	0.000	0.278	0.000	0.050	0.300	0.250	0.200	0.850	0.700	0.000
242	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
246	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
282	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.600	0.400	0.778	0.538	0.600	0.800	0.500	0.700	0.300	0.400	0.650
						Means and SEs					
H	0.475	0.554	0.353	0.613	0.623	0.684	0.469	0.507	0.391	0.541	0.677
SE	0.071	0.071	0.080	0.079	0.046	0.064	0.063	0.072	0.093	0.052	0.060
Alleles	2.92	3.38	2.23	4.15	4.23	4.92	3.69	3.00	2.15	4.54	6.08
SE	0.42	0.43	0.23	0.45	0.41	0.46	0.47	0.32	0.22	0.50	0.76