GENETIC STRUCTURE OF WISCONSIN'S NATURALLY RECRUITING WALLEYE POPULATIONS

by

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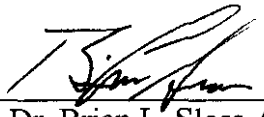

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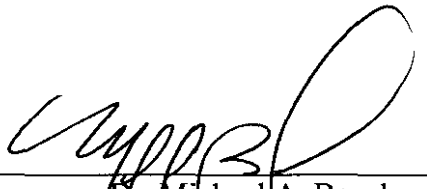

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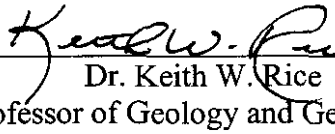

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ABSTRACT

Genetic diversity has been recognized as a vital component of fish management in Wisconsin. An explicit goal of the state's walleye management plan has been to preserve the genetic integrity of naturally recruiting walleye populations. A prerequisite to achieving this goal is understanding the distribution of genetic diversity within and among the State's walleye populations. My objectives were to 1) to determine whether there is significant genetic structure among Wisconsin's naturally recruiting walleye populations, and 2) if this resolved genetic structure was consistent with contemporary fisheries management zones employed for Wisconsin's walleye. Genetic diversity for these walleye populations was measured at 10 microsatellite loci and genetic structure was delineated through a process known as genetic stock identification (GSI). Genetic stock identification is a series of hierarchical tests consisting of genic differentiation, genetic distance, AMOVA, and pairwise F_{ST} comparisons to identify putative genetic units. Genetic diversity levels throughout the sampled populations were high (H_o = 0.7144, H_E = 0.7677) and comparable to other walleye studies (Wirth et al. 1999; Borer et al. 1999; Eldridge et al. 2002; Cena et al. 2006; Franckowiak et al. 2009) using a similar suite of microsatellite loci. Results however showed current fisheries management units were not consistent with this genetic structure. Delineation of genetic units using GSI identified 21 significant genetic units among the 26 sampled populations suggesting populations are primarily maintaining localized gene pools. Iterative analyses examining the ratio of among-group variance to within-group variance was performed to identify higher level genetic units (i.e., putative stocks). Eight putative genetic units, mostly consistent with geographic location of the populations and not with current watershed

regions, were identified using the ratio comparing among-group variance to within-group variance. Significant inbreeding coefficients were observed in half the sampled walleye populations. No relation was observed between inbreeding and population size or effective population size. A trend was observed where inbreeding predominately occurred in walleye populations from large systems; 81.5% (9/13) of all systems with a surface area > 500 ha showed significant inbreeding whereas 31.3% (4/13) of populations with a surface area of < 500 ha showed significant inbreeding. Several factors could account for these data including the preferential sampling in large systems of a single walleye spawning area, coupled with known philopatry of walleye, resulting in biased sampling of cohorts and/or related individuals. Current management strategies should be re-evaluated in light of these findings to better define management zones that can effectively conserve the genetic integrity of naturally recruiting walleye populations. This re-evaluation should weigh the cost of increasing the number of genetic units managed with the short- and long-term impacts on the genetic integrity of Wisconsin's walleye populations. A primary conflict between genetic structure and geographical location were the populations located in the Upper Chippewa River basin were more genetically similar to populations found in the Upper Wisconsin River basin. Geographical (glacial recession and stream capture) and anthropogenic (stocking across basin boundaries) are both reasonable explanations for this disruptive pattern. This issue requires further research to determine the biological reality of the resolved structure with strong implications for future management.

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INTRODUCTION

The current Wisconsin Department of Natural Resources (WDNR) walleye (*Sander vitreus*) management plan identifies seven specific management goals and 30 key issues necessary to sustain and improve statewide walleye populations (Hewett and Simonson 1998). At least three of these goals − to maintain the genetic integrity of walleye populations, to develop and employ biologically sound and cost effective propagation, and to develop an integrated propagation program with contributions from state, federal, tribal, and private entities − specifically demand knowledge and a better understanding of the genetic resources available within the overall walleye resource of the state. The remaining four goals – identify and protect critical habitat, provide diverse fishing opportunities, ensure accurate population status and trends data, and provide environmental education opportunities − would be aided and supported by the acquisition of comprehensive walleye genetic data. Therefore, understanding the amount and distribution of genetic diversity among the naturally recruiting walleye populations of Wisconsin represents a critical step in achieving the state's identified management goals for walleye.

Maintaining the genetic diversity of naturally recruiting populations is important to sustain walleye fisheries. The conservation of genetically distinct, locally-adapted populations of any species (i.e., spatial diversity) is important to maximize the adaptability and evolutionary potential for that species (Lande and Shannon 1996; Hughes et al. 1997; Hilborn et al. 2003; Luck et al. 2003). Predictions and estimations of historical and current population levels (Miller and Kapuscinski 1997), bottlenecks

(Stepien and Faber 1998; Cena et al. 2006; Allendorf and Luikart 2007), inbreeding and inbreeding depression (Hallerman 2003a; Cena et al. 2006; Allendorf and Luikart 2007), outbreeding depression (Hallerman 2003b; Cena et al. 2006), migration (Gharrett and Zhivotovsky 2003) , and mutation (Hallerman and Epifanio 2003) can be predicted through measures of genetic diversity within and among populations. Studies have demonstrated that genetic diversity is directly correlated with the fitness of a population. Reed and Frankham (2003) performed a meta-analysis on 34 published data sets and found a strong correlation between genetic diversity and fitness of populations. A study by Quattro and Vrijenhoek (1989) showed reduced fecundity, survival, and growth (i.e., fitness) in Sonoran topminnow (*Poeciliopsis occidentalis occidentalis*) was positively correlated to observed measures of genetic diversity. Another study conducted by Garant et al. (2004) found that female Atlantic salmon (*Salmo salar*) that mate with several different males, a characteristics that increases genetic diversity, also had more outbred offspring and demonstrate a positive correlation with reproductive success (i.e., fitness).

Genetic integrity is defined as the relative stability of the genetic diversity within a population over time (Allendorf and Luikart 2007). Different evolutionary phenomena can result in dynamic changes in the genetic characteristics of populations resulting in the spatial structuring of genetic diversity within and among populations and both short-term (i.e., a few generations) and long-term (i.e., hundreds of generations) temporal stability within populations (Allendorf and Luikart 2007). Genetic diversity in a population undergoes dynamic change under normal conditions related to the finite population size and changing environmental pressures leading to local adaptive change. However, the relative temporal stability of this spatial distribution of genetic diversity is a crucial

consideration in terms of managing a resource. It is theorized that most populations show relative stability in genetic diversity over time related to Hardy-Weinberg equilibrium (HWE) and genetic drift-mutation equilibrium (McClenaghan et al. 1985; Tessier and Bernatchez 1999) where the population's genetic diversity maintains equilibrium between the loss of genetic diversity through drift and the addition of new diversity through mutation. Given that populations of a species can have different sizes and can experience differing environmental conditions, stability of a population's genetic diversity over time will vary across a landscape. Only limited data exists on the temporal stability of genetic diversity in most inland fisheries species such as walleye (McClenaghan et al. 1985; Brown et al. 1996; Tessier and Bernatchez 1999; Hansen et al. 2002; Heath et al. 2002a; Franckowiak et al. 2009).

Sound, biologically-relevant management of a diverse and abundant resource, such as walleye, is challenging and requires the use of stock-based management. Stocks are a group(s) of organisms sharing a gene pool that is sufficiently discrete and nominally identifiable that it warrants discrete management (Bryan and Larkin 1972). As such, separate stocks tend to show similarities in terms of local adaptations, population dynamic measures (e.g., birthrate, mortality rates, growth rates, etc.), and other biological/ ecological features supporting the notion that stocks should be managed as biologically relevant units (Heidinger 1999; Van den Avyle and Hayward 1999). Long-term management of any species is dependent on understanding the number, distribution, and characteristics of all existing stocks thereby maintaining genetic and ecological integrity, diversity, and abundance. When overall productivity and evolutionary potential is a concern it becomes even more important to identify and acknowledge diverse and

subdivided populations (Shaklee and Currens 2003). Management of walleye on a stock basis will provide for this diversity and the evolutionary potential needed for the walleye's short-term and long-term viability.

Stock delineation in fisheries has been performed by estimating reproductive isolation, differentiating life history responses, morphological differentiating, and/or genetic differentiating of populations (Shaklee and Currens 2003; Dizon et al. 2007). Genetic stock identification (GSI) is a method for discriminating stocks and identifying genetic structure within and between populations (Shaklee and Currens 2003). The GSI approach provides a hierarchical classification of individuals into groups and identifies associations between those groups (Shaklee and Currens 2003). A study performed by Beacham et al. (1999) used microsatellite data and GSI to determine steelhead (*Oncorhynchus mykiss*) stock structure in the Columbia River drainage in British Columbia and Washington. Three distinct stocks of steelhead were resolved and mixed-stock analysis was conducted on the commercial fishery to provide for stock-based exploitation rates and effective management. Subsequently, management plans have used this stock model to account for within- and between-stock differences improving the effectiveness of steelhead management.

Despite being an important component of the Wisconsin Walleye Management Plan, limited basic research has been conducted to provide a framework to evaluate walleye genetic integrity and identify discrete genetic stocks. The contemporary spatial distribution of Wisconsin walleye genetic diversity has been inferred largely from a study by Fields et al. (1997). This study aimed to identify the genetic structure of walleye across the upper Midwest including 27 sample localities in Wisconsin. They interpreted

the geographic distribution of genetic variation with respect to historical/putative post-glacial colonization routes, historical range limits, gene flow, and possible impacts of previous stock transfers, and devised management units to preserve the remaining genetic diversity. As a result of their study, Fields et al. (1997) developed 16 separate management units for walleye throughout the upper Midwest with six genetic management zones (GMZs) located entirely, or in part, in Wisconsin's ceded territory of Wisconsin (Figure 1); a region of the state identified through the 1983 court ruling allowing tribal harvest rights in off-reservation water bodies (Staggs et al. 1990). This study represented a vital first step in identifying defendable GMZs within Wisconsin; however, several methodological limitations hamper the utility of the findings. A primary limitation of the study was the general lack of genetic diversity at the allozyme loci (seven loci with mean heterozygosity between 0.143-0.281 and mean number of alleles between 1.4-2.0) and the mitochondrial DNA locus (mean nucleotide diversity = 0.002123 and mean nucleotide divergence = 0.000714) used by the researchers. Furthermore, representative sample sizes ($n \leq 30$) for populations were low. The combination of such low levels of diversity coupled with modest sample sizes ($n \leq 30$) resulted in relatively low power to assess genetic structure (Ruzzante 1998). The resulting population structure/management zones conformed to a watershed boundary scenario consistent with *a priori* expectations and have been used by the WDNR since the completion of the study.

Several studies have recently addressed the temporal aspects of genetic integrity in Wisconsin walleye. Franckowiak et al. (2009) looked at the temporal stability of the walleye population in Escanaba Lake (Vilas Co.) and found the population demonstrated

relatively low effective population sizes compared to the overall census estimates for the population. This finding was attributed to a high degree of familial reproductive variance and predicted to cause unperceived threats to the genetic integrity of this population through increased genetic drift and susceptibility to inbreeding. Franckowiak et al. (2009) also showed periods of relative stability in the genetic characteristics of the population (i.e., genetic integrity) followed by complete disruption of the original genetic diversity in conjunction with a series of supplemental stockings. The disruption did not simply shift the genetic diversity measures but resulted in a complete replacement of the genetic characteristics of the original Escanaba Lake walleye population with that of the stocked fish. However, walleye in Escanaba Lake were founded in the 1950's through a series of stockings. Combined, Fields et al. (1997) and Franckowiak et al. (2009) helped identify gaps in understanding the genetic integrity of these populations; nevertheless, questions remain in relation to managing walleye populations for the maintenance of genetic integrity.

Imminent threats to the genetic composition of a population can be attributed to several factors but, particularly genetic drift and stocking. Genetic drift is a nonselective, random process of change in allele frequencies from generation to generation (Wright 1938, 1951) due to the finite nature of populations. Genetic drift leads to changes in allele frequencies, diversification among replicate populations from the same source (through fragmentation), and overall loss of genetic diversity (Frankham et al. 2002). Eventually, genetic drift will lead to the fixation of a single allele at a given locus and subsequently, the loss of alternative alleles in any given population. The probability of an allele becoming lost is dependent on its frequency and on population size. Larger

populations are less vulnerable to genetic drift due to higher probabilities that an allele will be passed between generations. Alternatively, smaller populations have a decreased probability that an allele will be passed between generations, resulting in decreased genetic diversity. This has become a concern for walleye populations located in Wisconsin, where many inland populations are considered to be small. In conservation biology, smaller populations are of genetic concern and preserving their diversity is important for evolutionary potential in the fishery.

The genetic composition of a population may also be impacted through supplemental stocking. The use of a non-native brood source can hinder the sustainability and viability of a population by disrupting gene pools and reducing overall population fitness through outbreeding depression. Outbreeding depression occurs when genetically divergent populations introgress into local gene pools resulting in offspring with lowered fitness (Hallerman 2003; Allendorf and Luikart 2007). This lowered fitness is thought to occur via two mechanisms: (1) disruption of coadapted gene complexes and subsequent recombination into less favorable (i.e., lowered fitness) complexes, and/or (2) when combinations of alleles from divergent populations result in an offspring ill adapted for either environment (Allendorf and Luikart 2007). Negative effects from outbreeding via stocking have been observed in several species including intensively managed species such as rainbow trout (Miller et al. 2004), pink salmon (*Oncorhynchus gorbuscha*; Gilk et al. 2004), largemouth bass (*Micropterus salmoides*; Phillipp et al. 2002; Goldberg et al. 2005), and walleye (Li et al. 1996a, 1996b).

Supplemental stocking can have the opposite intended effect by suppressing naturally produced year-classes. Li et al. (1996b) looked at catch-at-age data from over

200 lakes in Minnesota and observed how stocking affected the abundance of year-classes. Significant effects were observed on year-class strength both one-year after and one-year prior to the year that had been stocked. This reduction in abundance for both year-classes could result in a loss in genetic diversity. Future reproduction would be the result of fewer individuals from each of those year classes reducing genetic variation contributing to the population, lowering the overall genetic diversity.
Furthermore, future generations would also have a higher concentration of genetic variation contributed from the stocked individuals, reducing overall genetic variation and lowering genetic diversity. These findings have direct consequences for Wisconsin's walleye populations where a primary management technique is supplemental stocking.

Maintaining stock boundaries by stocking within a management zone reduces risk of outbreeding depression. Maintaining within management zones conserves genetic integrity and abundance, both top management goals for the state of Wisconsin. Disruption in coadaptive gene complexes is minimized when stocking is conducted within management zones retaining the sustainability and viability of a population, reducing risks of lowered fitness. Additionally, if successful introgression was to occur, lower risks exists that an ill adaptable offspring will result from the introgression, maintaining localized gene pools and adaptability.

Objectives

There is a clear need to better understand the genetic diversity and spatial stock structure of naturally recruiting walleye populations in Wisconsin. Development of a walleye genetic stock model for Wisconsin will provide essential information for

appropriate management decisions and collectively help to maintain walleye genetic diversity and integrity. The goal of this project was to elucidate the genetic diversity and relations among the naturally recruiting walleye populations in the ceded territory of Wisconsin to help apply improved management of Wisconsin's walleye resource. The specific objectives of this study were 1) to determine whether there is significant genetic structuring among Wisconsin's naturally recruiting walleye populations, and 2) if the resolved genetic structure was consistent with contemporary management zones employed for Wisconsin's walleye.

MATERIALS AND METHODS

Experimental Design

To address the objectives of this project, approximately 50 adult walleye were collected from 26 naturally recruiting populations found in the ceded territory of Wisconsin. A minimum sample size of 50 individuals was needed according to Ruzzante (1998) to accurately estimate key measures of population differentiation. Sampled individuals were genotyped using 10 microsatellite loci (Table 1) and measures of allelic diversity (A), allelic richness (A_r), observed (H_O) and expected (H_E) heterozygosity, and allele frequency distributions (described below) among populations were initially assessed to determine if the observed genetic diversity was sufficient to differentiate populations. Sufficient genetic diversity was determined by evaluating previous genetic structure studies (Douglas et al. 1999; Turgeon et al. 1999; Lu et al. 2001; Douglas and Brunner 2002). These studies generally concluded a mean H_O and H_E of ~ 0.60 was adequate polymorphism for genetic differentiation of populations. Furthermore, these same studies concluded 2 to 32 loci were necessary for adequate discrimination of genetic differences between and among populations. After ample genetic diversity was confirmed among a subset of sampled populations and loci of choice, GSI methods were performed on the genetic data based on Shaklee and Currens (2003).

Study Site

Sample sites were restricted to the ceded territory of Wisconsin (Figure 2). This region was chosen because it is thought to contain historical native stocks of walleye and

is the location of the majority of the naturally recruiting walleye stocks in the state. The WDNR annually monitors the ceded territory fish populations using three methods: spring adult and total population estimates, fall young-of-year relative abundance estimates, and creel surveys of angler catch and harvest. Walleye lakes considered naturally recruiting (NR) by the WDNR and thought to be native were the only lakes eligible for inclusion in this study. The final populations sampled for this study were selected through consultation with WDNR research and management biologists (Table 2). For all sampled populations, surface area and population estimates were collected when possible and compared using a simple linear regression in Microsoft Office Excel® 2007 (Microsoft Corporation 2007). Normality was checked and transformations were conducted through SPSS v. 16.0 (SPSS 2007). Surface areas were determined using the WDNR website (http://dnr.wi.gov/org/ water/fhp/lakes/Lakes1a.pdf) and population estimates were determined using the United States Geological Survey database website (http://infotrek.er.usgs.gov). Only the most recent population estimates (pre 2009) were used.

Sample Collection

Tissue samples were collected in spring 2007 and 2008 in conjunction with spring fyke-net and/or electrofishing surveys conducted by the WDNR to estimate the abundance of spawning walleye. Samples consisted of a fin-clip from either the pelvic, pectoral, anal, or caudal fins in conjunction with the UWSP Molecular Conservation Genetics Laboratory's (MCGL) standard operating procedure (Appendix 1) and in accordance with American Fisheries Society (AFS), American Society of Ichthyologists

and Herpetologists, and the American Institute of Fishery Research Biologists approved guidelines for the Use of Fishes in Research (available online at http://www.fisheries.org/afs/publicpolicy/ guidelines2004.pdf). Samples were placed in 95% ethanol in prelabeled vials for preservation. When available, archived scale samples collected no later than 2000 were considered representative of contemporary populations and included in the study. All sampled fish were measured for length (TL) and sex was recorded when possible.

DNA Extraction

DNA was extracted from each sample using the Promega Wizard® Genomic DNA purification kit (Promega Corp., Madison, WI) following a modified protocol for a 96-well format with re-hydration of the DNA in 100 µl of Tris-low-EDTA buffer solution (TLE; 10mM NaCl, 0.1 mM EDTA, pH 8.0). Extracted DNA was electrophoresed in a 1% agarose gel with EtBr and visualized using UV-light to ensure the presence of high molecular weight DNA. Comparisons were made with a known molecular weight ladder (Hyperladder™ I, Bioline USA Inc., Randolph, MA). DNA was subsequently quantified using a Nanodrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and normalized to a standard concentration of 20 ng/µl in 50 µl of TLE to ensure consistency in subsequent genotyping.

Genetic Analysis

Microsatellite DNA was chosen as the molecular marker for this study. Microsatellite DNA loci are useful for population studies because they generally provide

high-resolution data for prediction of genetic stock structure, gene flow between stocks, and overall genetic diversity of populations (Neff et al. 1999, Wilson and Gatt 2000). Microsatellites are genomic DNA sequences containing tandem arrays of short (2-5 bp) motifs of nucleotides such as ACACACACAC (Brown and Epifanio 2003). Variation at microsatellite loci is in the form of length variants wherein the motif (e.g., AC in the previous sentence) is duplicated or excised to form a different length variant or allele. The rate of microsatellite mutations is much higher than standard nucleotide mutations such that microsatellite loci have higher levels of variation than traditional markers (Lowe et al. 2004). They are thought to be randomly distributed throughout the nuclear genome making them an ideal marker for sampling genomic variation.

A total of 10 microsatellite loci were used to survey genetic variation within and among the sampled walleye populations (Table 1) based on the recommendations of Ruzzante (1998). Multilocus genotyping was conducted using the polymerase chain reaction (PCR) to isolate and amplify the individual loci in all sampled individuals. Loci were optimized for the MCGL equipment and placed into multiplex reactions where multiple loci were amplified in the same reaction (Table 3). Two multiplex reactions from Franckowiak et al. (2009) were used with the addition of two multiplex that were developed using the protocols of Henegariu et al. (1997) and Cena et al. (2006). Microsatellite fragment sizes were visualized on an ABI Prism™ 377XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) using an in-lane standard (GeneFlo™ 625, Chimerx Inc., Milwaukee, WI), and Genescan® genetic analysis software (Applied Biosystems, Inc. Foster City, CA). All genotypes were confirmed visually and

recorded in a master data sheet with the raw data consisting of observed genotypes and allele counts.

Data analysis

Estimates of genetic diversity.—Genetic diversity levels were determined using several different measures. The specific measures used were alleles/locus (A), allelic richness (A_r), expected heterozygosity (H_E), and observed heterozygosity (H_O). Microsatellite Toolkit v3.1 (Park 2001) was used to calculate population-specific allele frequencies, both H_O and H_E, and A. A rarefraction method was used to estimate A_r using HP-RARE v1.0 which accounts for unequal sample sizes (Leberg 2002; Kalinowski 2005). The number of private alleles per sampled population (i.e., alleles only found in a single population and no others) was also estimated, accounting for unequal sample sizes through rarefraction, using HP-RARE v1.0 (Leberg 2002; Kalinowski 2005).

Hardy-Weinberg equilibrium and gametic disequilibrium.—Hardy-Weinberg equilibrium (HWE) (Hardy 1908, Weinberg 1908) is a fundamental theorem of population genetics that allows for populations to be described by allele frequencies (Allendorf and Luikart 2007), thus, simplifying the task of describing the genetic characteristics of a population. Hardy-Weinberg equilibrium states that a population's genotypic frequencies should be stable from generation to generation if the population is sufficiently large, randomly breeding, and not experiencing mutation, migration, or natural selection (Guo and Thompson 1992, Frankham et al. 2002, Allendorf and Luikart 2007). As such, tests of HWE provide an initial determination whether a population is

panmictic or if there is significant violation of the aforementioned assumptions within the population. The initial step in spatial genetic studies is to determine if the populations conform to HWE expectations. Samples were tested for conformance to HWE using an exact Hardy-Weinberg test (Haldane 1954) with a Markov chain method (Guo and Thompson 1992) of 10,000 dememorization steps, 100 batches, and 10,000 iterations/batch as implemented in GENEPOP 3.4 (Raymond and Rousset 1995a, 1995b).

A problem that exists with highly polymorphic loci (i.e., microsatellites) is that high numbers of alleles (and corresponding genotypes) can lead to significant deviations from HWE based on exact tests due to cumulative effect of rare expected genotypes (Pamilo and Varvio-Aho 1984). Rare allele pooling has been suggested to resolve this problem (Hedrick 2000). Genotypes with a frequency of < 1.0 were pooled into one observed and one expected frequency value. The new observed and expected genotypes values were than tested using a chi-test square goodness of fit test in Microsoft Office Excel® 2007 (Microsoft Corporation 2007). A sequential Bonferroni correction was used (Rice 1989) with an initial $\alpha = 0.05$ to determine significance.

Another assumption for tests used in GSI is that all loci are segregating in an independent fashion (Mendel's 2nd law of independent assortment). If the alleles from one locus are not segregating independently of the alleles of another locus, the loci are said to show gametic disequilibrium. In this case, the loci cannot be considered independent for testing purposes and one of the loci should be dropped from the dataset. Tests for gametic disequilibrium were conducted in GENEPOP 3.4 (Raymond and Rousset 1995a, 1995b) using Fisher's exact test with a Markov chain method Markov chain method (Guo and Thompson 1992) of 10,000 dememorization steps, 100 batches,

and 10,000 iterations/batch as implemented in GENEPOP 3.4 (Raymond and Rousset 1995, 1995b) Significance was determined using sequential Bonferroni correction (Rice 1989) with an initial $\alpha = 0.05$.

Genetic stock identification and identification of management units.—Genetic stock identification (Shaklee and Currens 2003) was used to delineate the genetic stock structure of walleye in Wisconsin. The GSI process uses a series of hierarchical tests, where findings of one test were used to establish hypotheses for subsequent tests. Advantages of GSI include: (1) a direct examination of stock structure through statistical testing of the null hypothesis of a panmictic population, (2) its applicability to all species, (3) natural genetic variation comprises the data necessary for GSI studies, (4) genetic markers are typically unaffected by environmental conditions (i.e., permanent and not phenotypic), and (5) genetic markers can be used to test interbreeding (Shaklee and Bentzen 1998). Following the aforementioned tests of HWE and gametic disequilibrium, a series of hierarchical tests aimed at population differentiation were used to identify genetic units.

Genetic stock identification was conducted starting with an initial test of population differentiation was performed to test a global hypothesis of panmixia across all sampled walleye populations. This hypothesis was tested using the genic differentiation test employed in GENEPOP 3.4 (Raymond and Rousset 1995a, 1995b) that uses a Fisher's exact test to assess whether allele frequency distributions were the same in all sampled populations. Significance was determined through a Markov chain method employing 1,000 dememorization steps and 100 batches with 1,000 iterations each (Guo and Thompson 1992). A nominal α of 0.05 was used.

Following rejection of panmixia in the ceded territory, clustering of populations was done using a genetic distances among populations to develop subsequent hypotheses. Genetic distances are measures of genetic differences between populations based on allele frequencies (Frankham et al. 2002). Cavalli-Sforza and Edwards (1967) chord distance (D_c) was used to estimate genetic distances between all pairs of populations. Visualization of overall relations among populations was performed using an unrooted neighbor-joining (NJ) tree (Saitou and Nei 1987). The use of genetic distance in a clustering algorithm has been shown to efficiently resolve the correct tree topology in situations with relatively recent divergences (Bernatchez and Wilson 1998), such as walleye within Wisconsin, and has been recommended for use with microsatellite data (Takezaki and Nei 1996). Estimates of population pairwise D_c were conducted in PowerMarker v3.25 (Liu and Muse 2005) and an unrooted NJ tree was developed in TreeView v1.40 (Page 1996). Confidence in the topology was estimated using 5,000 bootstrap pseudoreplicates in PowerMarker. A majority rule consensus dendrogram was constructed using CONSENSE in the PHYLIP v3.5 package (Felsenstein 1993). Resolved relations/groups of populations were used to determine hypothetical population groups for subsequent analyses.

Identified groupings from the NJ tree were tested for significance using a hierarchical analysis of molecular variance (AMOVA)(Excoffier et al. 1992). AMOVA calculates a total molecular variance for all sampled individuals and determines the proportion of variance attributable to various hierarchical levels including among individuals within populations, and within and among *a priori*-defined groups of populations. The determination of stable genetic-groups is accomplished by

identification of *a priori* defined groups that provide significant among group variance while exhibiting non-significant within group variance. Significance in AMOVA is based upon the calculation of a pairwise matrix using selected genetic Φ-statistic, correlation statistics directly analogous to F-statistics (Wright 1931), derived from the variance components computed during AMOVA (Lowe et al. 2004). All AMOVA analyses were conducted in ARLEQUIN v3.11 (Excoffier et al. 2005) with significance computed by non-parametric permutation of the data set with 99,999 permutations (Lowe et al. 2004).

Stable population groups identified through AMOVA were subsequently tested for internal stability through F-statistics (F_{ST}; Wright 1931). The fixation index, F_{ST}, is a measure that quantifies the level of divergence between two populations/samples by assessing the heterozygote deficiency in the two samples versus what would be expected if no differences existed (Frankham et al. 2002, Allendorf and Luikart 2007). Due to the high level of allelic variation of microsatellites, a F_{ST} analog, θ, (Weir and Cockerham 1984) was used to determine the divergence among populations within groups. Theta ranges from zero, where all populations are fixed for a single allele, to one, where all populations are genetically unique. Tests of significance for θ test the null hypothesis that θ equals zero. ARLEQUIN v3.11 (Excoffier et al. 2005) was used to estimate θ for all population pairwise comparisons and for significance testing using 5,000 bootstrap pseudoreplicates and a sequential Bonferroni correction (Rice 1989).

The reliance on a gene pool definition of a stock will likely result in the overestimation of genetic groups when the GSI process is used on insular fish populations with limited real opportunity for gene flow, such as walleye in relatively

isolated lakes. To account for this overestimation, a novel approach was used where hypotheses for putative groups (2-9) were examined by comparing the ratio of between group variance (V_a) to the variance within groups (V_b). If this ratio is less than one, the amount of genetic variance within putative groups is greater than that between groups; an unreasonable recommendation for genetic management units. Initial tests were performed on hypothesized groupings resulting from the D_c-based NJ tree. Subsequent tests followed, breaking these groups into smaller clusters until a minimum V_a/V_b ratio of one was observed; indicative of equal variance among and within putative groups. Once this occurred, two additional steps were performed to determine the increase in the ratio achieved through the addition of 1-2 more genetic units. All tests were performed in ARLEQUIN v3.11 (Excoffier et al. 2005) as described previously.

Evaluation of contemporary management units with AMOVA. — Contemporary walleye stock management units employed by the WDNR were assessed to determine if consistency exists among the genetic structure Wisconsin's naturally recruiting walleye populations and current fisheries management units. If they do not significantly account for among group genetic variance, the biological defensibility of such units is questionable. An AMOVA was performed on management unit (watershed) groupings by first grouping all populations from each contemporary unit into a single group and then testing for differences among groups (Figure 1). Significance levels for AMOVA were computed by non-parametric permutation of the data set with 99,999 permutations (Lowe et al. 2004). All AMOVA tests were performed in ARLEQUIN v3.11 (Excoffier et al. 2005). Following the standard AMOVA calculations, the current management units

were also examined in terms of the V_a/V_b ratio to compare results with the genetic-based management units described above.

Inbreeding.—A critical concern for any genetic management plan is inbreeding within a population. Inbreeding is the mating of individuals related by ancestry (Frankham et al. 2002). Inbreeding within the sampled walleye populations was measured using the inbreeding coefficient (F_{IS}; Wright 1951). The inbreeding coefficient is the probability that two alleles at a locus from one individual share common ancestry (Allendorf and Luikart 2007) and ranges from zero to one where zero represents a randomly breeding population and one represents a completely inbred population (i.e., no heterozygosity). Tests of significance for F_{IS} test the null hypothesis that F_{IS} equals zero. Population-specific F_{IS} values were estimated using ARLEQUIN v3.11 (Excoffier et al. 2005) with significance determined by with a nominal $\alpha = 0.05$ and a sequential Bonferroni correction for multiple tests (Rice 1989).

Inbreeding should be inversely related to N_e and overall population size. Comparisons of F_{IS} were made with N_e, surface area, and census size, where possible, with simple linear regression Data analysis in Microsoft Office Excel® 2007 (Microsoft Corporation 2007). Transformations in the data were used to account for any outliers or failures in normality (Dytham 2003). Normality was checked and transformations (Log_{10}) were conducted through SPSS v.16.0 (SPSS 2007). For comparisons, negative F_{IS} values were considered zero since a negative value implies an excess of heterozygotes and, thus, no inbreeding (Allendorf and Luikart 2007).

Effective population size.—A key measure in conservation genetics is the effective population size (N_e; Bartley et al. 1992; Caballero 1994), defined as the number

of breeding individuals in an idealized population (i.e., one that meets HWE assumptions) that would show the same amount of genetic variation due to genetic drift as the population under consideration (Frankham et al. 2002; Allendorf and Luikart 2007). An oversimplified, but useful, definition of N_e is the number of successful breeders contributing to the population within a generation; a crucial determinant for genetic diversity because all individuals in a population rarely contribute equally to the next generation. As such, N_e is usually less than the census population size (N_c). Two key factors reducing N_e compared to N_c are fluctuations in population size (Frankham 1995) and unequal sex ratios (Frankham et al. 2002), both concerns in walleye management. Furthermore, high variance of reproductive success can reduce the N_e relative to N_c and has been suggested in at least one Wisconsin walleye population by Franckowiak et al. (2009). The N_e of all sampled populations was estimated using a point estimation technique based on the linkage/gametic disequilibrium estimator of Hill (1981) and Bartley et al. (1992) in NeEstimator v1.3 (Peel et al. 2004). Confidence intervals (95%) were calculated based on Waples (1991).

Effective population size may be correlated to inbreeding and overall population size. As such, comparisons of N_e were made with F_{IS}, surface area, and census size where possible using simple linear regression found in Microsoft Office Excel® 2007 (Microsoft Corporation 2007). Normality was checked and transformations (Log_{10}) were conducted through SPSS v. 16.0 (SPSS 2007). For comparisons, N_e estimates of infinity (∞) were dropped from subsequent analysis since it has been suggested ∞ represents an estimate that was not able to produce a finite number due to insufficient genetic data (Peel et al. 2004).

RESULTS

A total of 1,289 walleye were collected from 26 populations (Table 4; Figure 2). Samples represented populations in 13 northern counties in the ceded territory of Wisconsin and all six fisheries management zones were represented. Sample sizes were ≥45 individuals/population except for three populations: Blaisdell Lake (N=36), St. Croix River (N=39), and Dowling Lake (N=29) (Table 4). A total of 17 populations were collected in spring 2007, one was collected in fall 2007, and 8 were collected in spring 2008. Mean surface area of lakes sampled was 923.53 ± se ha (range 40.47 – 6,070.50 ha; Table 5). Mean population census size was 10,971 ± se walleye/lake (range 743 – 80,202; Table 5). A significant relation was found between surface area and population estimates; a regression analysis was performed comparing $\log_{10}$ (surface area) and $\log_{10}$ (population estimates) for sampled populations with a resulting $R^2 = 0.7272$, slope = 0.8240, and $p < 0.0001$ (SE = 0.30; Figure 3).

Data Analysis

Genetic diversity measures.—All samples were initially analyzed at 13 microsatellite loci (Table 3). Three loci (Svi-5, Svi-8, Svi-18) were dropped from the study due to inconsistent amplification. Data from the remaining 10 loci were used for subsequent analyses.

High levels of diversity were observed across all populations (Table 4). The observed mean allelic diversity (A) was 9.5 with a range of 8.4 (GL) to 10.7 (RCL). When unequal sample sizes were considered, allelic richness (A_r) was consistent across

all populations (Table 4). Mean A_r was 6.79 with a range of 6.13 (GL) to 7.28 (WIL). Mean private alleles across all populations was 0.96 with a range of 0.27 (BN) to 1.91 (BCL; Table 4). Levels of heterozygosity were high across all populations with mean H_O of 0.7144 (range 0.6580 [EC] to 0.8138 [DOW]) and mean H_E of 0.7677 (range 0.7372 [BD] to 0.7977 [LCO]).

Hardy-Weinberg equilibrium and gametic disequilibrium.—Initial tests of HWE showed 33% (86/260) of comparisons were significantly different than those expected under HWE based on an initial α-level of 0.05. Following sequential Bonferroni correction ($\alpha = 0.00019231$) with rare allele pooling, 99.6% (259/260) of comparisons were in HWE. Because only one locus/population comparison departed from HWE all loci and all sampled populations were considered to conform to HWE.

Independent segregation of alleles was demonstrated in 83.7% (455/550) of the comparisons using a gametic disequilibrium test ($\alpha = 0.05$). Following sequential Bonferroni correction (Rice 1989), only 98% (539/550) of locus/population comparisons exhibited independent segregation. No consistent patterns in loci were observed suggesting the significant deviations were due to sampling error and not overall lack of independence due to physical linkage of the loci on a chromosome (Ohta 1982; Allendorf and Luikart 2007). All loci and populations were therefore considered to be in gametic equilibrium for all subsequent analyses.

Genetic stock identification.—Walleye populations were not panmictic as significant genetic differences were observed when all sampled populations based on a test of genic differentiation ($p<0.00001$). A subsequent pairwise population test of genic

differentiation showed that 100% (325/325) of comparisons showed significant differences between populations.

Significant structure was present among the walleye populations sampled as evidenced by the unrooted NJ tree of D_c (Figure 4). Two initial *a priori* groups, consistent with an east/west split in the populations (Figure 5), were used in the hierarchical GSI tests. An AMOVA of this hypothesis showed significant ($p < 0.00001$) among-group variance (1.12% of total variance) and significant ($p < 0.00001$) within-group variance (2.57%; Figure 5). Despite the significant among-group variance, the significant within-group variance dictated further testing was needed since significant differences exist within our two initial groups. Because of the high number of populations in each group, two independent series of tests were conducted to further delineate groups. Two series of AMOVA tests using only populations from Group I and Group II separately were conducted to minimize the influence of among group interference.

Fifteen stable genetic units (gene pools), out of 16 total samples, were resolved in Group I using the hierarchical GSI approach. This 15 unit genetic model resulted in significant among-group variance (3.35% of total variance; $p = 0.00801$) and non-significant (0.06% variance; $p = 0.35316$) within-group variance (Figure 6). Low clustering of groups was observed with only CHF and LCO clustering as a single unit (Figure 6).

Six stable genetic units, out of 10 total samples, were identified in Group II following GSI. This six group model showed significant among-group variance (1.26% variance; $p = 0.00160$) and non-significant (0.26%; $p = 0.10030$) within-group variance

(Figure 7). Three multi-sample units were recovered including a BFL/EC unit, a PF/TFL unit, and a MC/SPL/LAV unit. The three remaining populations, BN, GF, and KL, resolved as individual sample units.

The majority (94.9%) of pairwise F_{ST} comparisons were significantly different from zero consistent with the high number of genetic units resolved in the GSI analysis (Table 6). Values for F_{ST} ranged from 0.00036 (BFL and EC) to 0.10581 (STL and DOW) with only 18 pairwise comparisons being non-significant following a sequential Bonferroni correction (Rice 1989). Non-significant population comparisons were identified within the previously discussed 21 genetic groups and not among groups indicating gene flow within but not among groupings.

The suggestion of 21 genetic units out of 26 sampled populations purposed that each population was maintaining a unique localized gene pool and each population warranted management as such. Due to certain constraints, it was deemed that individual population management would be impracticable and a higher order grouping was needed to determine genetic structure. To identify the higher order genetic structure consisting of related gene pools, further analyses balancing the within versus between group variance ratio were performed. The second hypothesis based on the unrooted NJ tree of D_c (Figure 4) consisted of six groups, two eastern WI groups, one northern WI group, and three western (Figure 8). An AMOVA on this hypothesis resulted in significant within- (1.58% variance; $p > 0.00001$) and among-group (1.92% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 1.22. Since this hypothesis had reached the determined criteria for stable genetic units (i.e., minimum $V_a/V_b = 1.00$), three subsequent hypotheses were tested examining the effects of increasing the number of units on the V_a/V_b ratio.

The third hypothesis based on the unrooted NJ tree of D_c (Figure 4) consisted of seven groups, two eastern WI groups, one northern WI group, and four western (Figure 9). An AMOVA on this hypothesis resulted in significant within- (1.37% variance; $p > 0.00001$) and among-group (2.18% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 1.59 (Figure 9).

The fourth hypothesis based on the unrooted NJ tree of D_c (Figure 4) consisted of eight groups (two eastern WI groups, one northern WI group, and five western WI groups; Figure 10). An AMOVA on this hypothesis resulted in significant within- (2.33% variance; $p > 0.00001$) and among-group (1.16% variance; $p > 0.00001$) variance and a Va/Vb ratio of 2.00 (Figure 10).

The fifth hypothesis based on the unrooted NJ tree of D_c (Figure 4) consisted of a nine groups (three eastern WI groups, one northern WI groups, and five western WI groups; Figure 11). An AMOVA on this hypothesis resulted in significant within- (2.28% variance; $p > 0.00001$) and among-group (1.15% variance; $p > 0.00001$) variance with a V_a/V_b ratio of 1.98 (Figure 11). Following hypothesis testing a V_a/V_b ratio vs. number of populations was plotted. Stabilization of the V_a/V_b ratio was observed at eight genetic groups (Figure 12).

Following a review of stocking records (Appendix 3), it was determined that the gene pools of some populations had potentially been compromised (LCO, SIK, BCL, WIL, STL); therefore, genetic structure was re-analyzed after omitting these five populations. A total of 21 populations were subdivided into groups until the V_a/V_b ratio of = 1.00 was reached. Once that threshold was reached five subsequent hypotheses were tested examining the effects of increasing the number of units on the Va/V_b ratio.

The first hypothesis based on the 21 populations scenario consisted of three groups, one western WI groups and two eastern WI groups (Figure 13). An AMOVA of this hypothesis resulted in significant within- (1.85% variance; $p > 0.00001$) and among-group (1.72% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 0.93 (Figure 13).

The second hypothesis based on the 21 populations scenario consisted of four groups, two eastern WI groups, two western WI groups (Figure 14). An AMOVA on this hypothesis resulted in significant within- (1.72% variance; $p > 0.00001$) and among-group (1.67% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 0.97 (Figure 14).

The third hypothesis based on the 21 populations scenario consisted of seven groups (two eastern WI groups, one northern WI group, and four western (Figure 15). An AMOVA on this hypothesis resulted in significant within- (1.11% variance; $p > 0.00001$) and among-group (2.19% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 1.97 (Figure 15). Since this hypothesis had reached the determined criteria for stable genetic units (i.e., minimum $V_a/V_b = 1.00$), two subsequent hypotheses were tested examining the effects of increasing the number of units on the V_a/V_b ratio.

The fourth hypothesis based on the 21 populations scenario consisted of eight groups (three eastern WI groups, one northern WI group, and four western WI groups; Figure 16). An AMOVA on this hypothesis resulted in significant within- (1.08% variance; $p > 0.00001$) and among-group (2.09% variance; $p > 0.00001$) variance and a V_a/V_b ratio of 1.94 (Figure 16).

The fifth hypothesis based on the 21 populations scenario consisted of a nine groups (four eastern WI groups, one northern WI groups, and four western WI groups; Figure 17). An AMOVA on this hypothesis resulted in significant within- (2.05%

variance; $p > 0.00001$) and among-group (1.04% variance; $p > 0.00001$) variance with a V_a/V_b ratio of 1.97(Figure 17). Following hypothesis testing, a V_a/V_b ratio vs. number of populations was plotted. Stabilization of the V_a/V_b ratio was observed at two locations: 3-4 genetic groups and 7-9 genetic groups (Figure 18).

Evaluation of contemporary management units with AMOVA.—Contemporary management units constructed by the WDNR were not consistent with resolved genetic structure. The AMOVA of the six contemporary genetic management units (Figure 19) resulted in significant among-group variance (0.64% of total variance; $p = 0.00397$) and significant within-group variance (2.62% variance; $p < 0.00001$). The ratio of V_a/V_b was 0.24 showing that nearly four times more genetic variance is explained by the differences among populations within groups than between the six groups.

Inbreeding coefficients and effective population size.—Significant inbreeding was observed in half (13/26) the sampled walleye populations in this study following sequential Bonferroni correction. The mean F_{IS} among all populations was 0.0680 (range -0.0057 (Dowling Lake) - 0.1342 (Eagle Chain); (Table 5). An apparent trend was observed between surface area and F_{IS} with 87.5% (7/8) sampled populations greater than 500 ha exhibiting significant inbreeding following sequential Bonferroni correction and only 31.3% (5/16) of sampled populations < 500 ha showing significant inbreeding (Table 5). A simple linear regression of $\log_{10}$ (surface area) to F_{IS} failed to show a significant relation with an $R^2 = 0.15$, slope = 0.0263, and $p = 0.0621$ (SE = 0.04; Figure 20).

Effective population size estimates varied widely among populations. Estimates ranged from 34.4 individuals (DOW) to infinity (∞) (PF, ML, TFL) with a mean (minus

the three ∞ populations) of 384.4 (Table 5). Confidence intervals (95%) varied across all populations containing wide ranges (Table 5). Since it has been suggested that N_e is correlated to surface area and population size, comparisons between N_e, surface area, and population estimates were made. A significant correlation was observed between $\log_{10}$ (surface area) and Log_{10} (N_e). A simple linear regression showed a $R^2 = 0.2290$, slope = 0.3376, and p = 0.02 (SE = 0.37; Figure 21). A simple linear regression of $\log_{10}$ (population size) and $\log_{10}$ (N_e) failed to show a significant relation with a $R^2 = 0.13$, slope = 0.2849, and p = 0.1623 (SE = 0.38; Figure 22).

The intensity and rate of inbreeding is inversely proportional to a population's N_e (Hedrick 2000). No significant relation was observed in this study between $\log_{10}$ (N_e) and estimates of inbreeding. A simple linear regression of both variables showed a $R^2 = 0.11$, slope = 0.0347, and p = 0.1230 (SE = 0.04; Figure 23).

DISCUSSION

Genetic Diversity and Marker Utility

Genetic diversity levels of walleye stocks at the microsatellite loci used in this study were high both within and among Wisconsin's walleye populations. Microsatellite diversity levels in Wisconsin's walleye also exhibited slightly higher variation than populations located in northern Minnesota (Borer et al. 1999; Eldridge et al. 2002) and Quebec (Wirth et al. 1999), but similar to populations found in Ontario (Cena et al. 2006) and in a separate population in Wisconsin (Escanaba Lake) (Franckowiak et al. 2009). Additionally, this data set also met specific criteria (H_O and $H_E \sim 0.60$) for appropriate diversity described in past studies (Douglas et al. 1999; Turgeon et al. 1999; Lu et al. 2001; Douglas and Brunner 2002). Diversity levels were deemed to be high and consistent with studies demonstrating that diversity levels were adequate for population delineation and appropriate to determine genetic stock structure of Wisconsin's walleye.

Genetic Structure of Wisconsin Walleye

Significant genetic differences occurred among sampled walleye populations suggesting genetic structure is present among Wisconsin's walleye populations. These findings were consistent with previous studies examining population differentiation of walleye across landscapes (McInerny et al. 1991; Billington 1992; Stepien and Faber 1998; McParland et al. 1999; Strange and Stepien 2007) and in previous walleye studies conducted in Wisconsin (Fields et al. 1997. For example, Strange and Stepien (2007) were able to distinguish genetic differences among major spawning populations found in

Lake Erie and significant genetic differences among riverine spawning populations and reef spawning populations using a suite of 10 microsatellite loci. Fields et al. (1997) examined the genetic diversity of Wisconsin walleye at allozyme loci and concluded, despite low levels of genetic diversity, significant differences existed among the populations sufficient to suggest six genetic management units existed in Wisconsin's ceded territory. Microsatellites are known to contain higher levels of genetic diversity when compared to allozymes (Vignal et al. 2002; Allendorf and Luikart 2007. The walleye populations included in this study suggested individual populations in Wisconsin constituted mostly unique gene pools. This is consistent with the highly insular nature of Wisconsin walleye populations and has been observed in other studies of fish genetic structure (Kamonrat 1996; Lafontaine and Dodson 1997; Elmer et al. 2008; Guy et al. 2008) including the closely related pikeperch (*Sander lucioperca*) in Finland (Björklund et al. 2007) and white sucker (*Catostomus commersoni*) in the northeastern U.S. (Lafontaine and Dodson 1997).

Among all these studies, the levels of divergence among walleye populations in Wisconsin in this study were most consistent with the high genetic divergence in pikeperch populations observed in the Fennoscandian region between Finland and Sweden (Björklund et al. 2007). Using microsatellites and assignment testing, slight gene flow between southern populations was found but overall, most populations were isolated and a degree of differentiation existed within regions. Long-term isolation and genetic drift caused by geographic barriers (i.e., isolation by distance) was proposed as the predominant means for genetic differentiation. These same factors are common in Wisconsin walleye and likely play an important role in interpopulation divergence

(McInerny et al. 1991; Billington 1992; Stepien and Faber 1998; McParland et al. 1999; Strange and Stepien 2007; Zhao et al. 2008).

The life history of Wisconsin walleye coupled with landscape/biological factors and anthropogenic changes to that landscape are clearly responsible for the isolation of populations over time and space. A primary characteristic of most of the sampled walleye populations in this study was that they were landlocked, groundwater drainage lakes that have little to no migration through connecting water systems. Furthermore, these populations were typically smaller (median surface area = 223.8 ha) and demonstrated lower N_e (median = 212.7) when compared to other studies (Cena et al. 2006; Franckowiak et al. 2009). Smaller populations are more influenced by genetic drift (Hedrick 2000; Frankham et al. 2002; Allendorf and Luikart 2007) than larger populations, causing populations to diverge separately. Along with that, as populations increase in geographic distance gene flow will decrease and genetic drift will increase (Relethford 1996) leading to populations differing genetically over time.

The reproductive characteristics of walleye likely contributed to the observed genetic differentiation between populations. Studies have shown that walleye exhibit philopatry when spawning (Crow 1962; Olson et al. 1978; Jennings et al. 1996; Stepien and Faber 1998; Strange and Stepien 2007) which can genetically separate populations over time. Crow (1962) used physical tagging of spawning walleye to demonstrate philopatry within walleye populations found in the Muskegon River (MI) system by identifying individual walleye returning to the same spawning grounds year after year with limited variation between tagging locations and recapture locations. A smaller philopatric population will be more rapidly influenced by genetic drift (Hedrick 2000;

Hallerman et al. 2002; Allendorf and Luikart 2007) causing the population to form genetically unique gene pools that differ from surrounding populations. Additionally, reproductive life characteristics, such as females being highly fecund (~50,000 eggs; Niemuth et al. 1972; Becker 1983), simultaneous fertilization of a single female by several males (sperm competition), and a variety of spawning habitats (Priegel 1970, Becker 1983), likely shape the gene pool of a population and can contribute to the divergence observed between these populations. High fecundity and potential sperm competition increase the chance of a population being dominated by few individuals altering the gene pool. The combination of landscape and life history characteristics is a dominant force isolating walleye populations and resulting in high levels of among population genetic divergence.

In addition to natural landscape and biological factors, anthropogenic events throughout Wisconsin have likely influenced watersheds/basins and, subsequently, the genetic structure of Wisconsin's walleye populations (Lande 1998). Historically, the aquatic landscape of Wisconsin's ceded territory likely exhibited some connectivity among populations through the presence of wetland complexes and seasonal flooding. However, transformations of land use associated with hydrological patterns (i.e., floodplains, wetlands) have changed throughout time not only in Wisconsin but across the entire landscape of North America (National Research Council 1992). In Wisconsin, anthropogenic activities have largely controlled the hydrological patterns resulting in reinforcement of the natural isolating mechanisms of walleye populations. For example, historic wetlands have been reduced by 50% in Wisconsin (Dahl 1990) and studies have shown that degradation to wetlands has the potential to disrupt aquatic communities

(Niemi et al. 1990). These events further isolate populations by limiting migration or habitat availability, reducing or eliminating connectivity among populations resulting in further genetic divergence among walleye populations.

Genetic Diversity and Contemporary Management Units

Overall contemporary management units failed to account for the resolved genetic diversity among Wisconsin's naturally recruiting walleye populations studied here and therefore are not consistent with the stated management objectives of conserving/ preserving the genetic integrity of native populations. For example, inconsistencies exist in the microsatellite-resolved Lake Superior genetic unit (LSGU) versus the contemporary management unit (Figure 19). The LSGU contains one population (ML) that resides in the contemporary management unit known as the Upper Chippewa. If continued management relies on contemporary management units, supplemental stocking could result in the disruption of ML's gene pool. This loss of genetic integrity will potentially have a negative effect on the production of this walleye population.

Several populations showed similar fundamental disagreements between the genetic data and their predicted resolution based on contemporary management units (Figure 19). A consistent contradiction in the watershed distribution of genetic diversity was the resolution of the Upper Chippewa River watershed populations (LCO, CHF, BD, ML, PF, SPL, BCL, TFL). Two possible explanations for this pattern were genetic impacts of supplemental stocking and/or geological processes resulting in changing watershed boundaries.

Supplemental stocking particularly within the Upper Chippewa River basin populations and has potentially changed the genetic pattern of populations. Despite the initial study criteria that only non-stocked populations be included in this study, over half (14/26) of the WDNR-selected populations have had a recorded stocking event since 1972 (Appendix 3). Given the high level of genetic divergence among the sampled walleye populations and the introgressive behavior of fish following cross-basin stocking events (Campton and Johnston 1985; Taggart and Ferguson 1986; Allendorf and Leary 1988; Franckowiak et al. 2009), alteration of the original gene pools in at least some of these populations is plausible. Further, complicating matters, historical stockings were often from the most geographically proximate hatchery and not often based on management units. For inst upper Chippewa River headwater populations that resolved with the upper Wisconsin River populations are more geographically proximate to the Art Oehmcke State Fish Hatchery in Woodruff, WI (Eastern) as opposed to the proper contemporary management unit hatchery, Tommy G. Thompson State Fish Hatchery in Spooner, WI (Western). The Oehmcke hatchery uses upper Wisconsin River brood sources stocking Oehmcke walleye into Upper Chippewa populations could result in genetic impacts (including homogenization or complete genetic replacement of the original Upper Chippewa River fish) to the upper Chippewa walleye populations (Franckowiak et al. 2009).

Although contemporary management zones were inconsistent with genetic structure, geographic consistency was observed within genetic structure with only a few anomalies (WIL, BCL, and SIK) within the sampled populations (Figure 24). Potential genetic changes caused by stocking might explain these anomalies. A closer look into

the stocking records for Willow Flowage (WIL) revealed walleye stocking events occurred in 1974, 1975, and 1994. The source of brood stocking is unknown but these stockings could have disrupted the gene pool and changed the genetic configuration of this population. Genetic outbreeding and total genepool replacement has been documented in many species, including walleye (Campton and Johnston 1985; Taggart and Ferguson 1986; Allendorf and Leary 1988; Philipp et al. 2002; Franckowiak et al. 2009). A study conducted by Franckowiak et al. (2009) looked at the temporal stability of the genetic diversity of walleye in Escanaba Lake, WI and found that there has been almost total replacement of the original gene pool through supplemental stocking. Big Crooked Lake (BCL) also had three similar stocking events with three stockings occurring from 1972-1973. Furthermore, the origin of this population is sketchy and walleye in this system are suspected to be non-native (Steve Newman, WDNR-Woodruff, personal communication). This system is located in the northeastern portion of the state were water bodies are generally dominated by either walleye or smallmouth bass (*Micropterus dolomieui*). Historically, it was thought BCL was a smallmouth bass fishery and walleye did not exist or if found were native within the lake. Likewise, the origin of the Siskiwit Lake (SIK) walleye population is questionable. No recorded stocking events have occurred since 1972, however, this population was thought to have been stocked annually from the early 1950's until 1972 and is likely a non-native population (Dennis Pratt, WDNR-Superior, personal communication). Therefore, the discordant resolution of BCL and SIK is likely the result of walleye introductions with further supplemental stockings. Founding populations have demonstrated a higher survival rate when stocked (Threinen 1955; Laarman 1978). Combined with annual

stocking, it is probable that inconsistencies between geographic location and genetic structure would exist.

An additional key factor in understanding the genetic relatedness of these three outlier populations is their close genetic association with Lac Courte Oreilles (LCO); a historical broodsource for Wisconsin walleye propagation. Given the suspect history of these populations and their grouping with LCO, it is reasonable to believe that LCO, at one time, was likely used as a stock source for these populations. These stockings likely introgressed into the gene pool of these populations resulting in the observed inconsistency between geographic location and genetic structure among populations. Despite the presence of stocking records for these systems (Appendix 3), the source of walleye used for stocking and the completeness of the records is questionable or unknown.

Another population of concern was the St. Louis River (ST) population in the Northwest corner of Wisconsin. Investigation into this population showed a possible corruption of the gene pool through supplemental stocking (Appendix 3). Preliminary data showed that this population was closely related to a Lake Winnebago strain of walleye (data not shown), further supporting the idea that this population's original gene pool may have been compromised.

Excluding these five populations, potential genetic impacts through supplemental stocking is thought to be low among the remaining 21 populations represent the historic genetic diversity and structure of Wisconsin's walleye. Supplemental stocking of walleye into NR sites has shown low success rates and thus reducing potential for introgression (survival and recruitment)(Threinen 1955; Laarman 1978; Mathias et al.

1992; Johnson et al. 1996; Li et al. 1996a; Brooks et al. 2002; Jennings et al. 2005). For example, Jennings et al. (2005) evaluated supplemental stocking success in 23 northern Wisconsin lakes over a four year period and found that the survival rate of supplementally stocked fish was 3.4%, resulting in little overall contribution to the year-class strength of the population. These low contributions allow the native walleye populations to retain most of their genetic diversity and spatial genetic structure. Li et al. (1996a) found that stocking in lakes with natural reproduction occurring had no increased effect on population abundance, thus demonstrating that survival of supplemental stocked fish was likely low.

A primary determinant of genetic diversity in Wisconsin walleye is due to the historical connectivity of water bodies observed within larger watershed basins due to historical hydrological patterns produced by Wisconsin's glacial receding. The recession of the Wisconsonian glaciation (~12,000 years ago) is the predominant geological event that produced the myriad of lakes and streams found in the ceded territory of Wisconsin today (Pielou 1991). Walleye were thought to have survived the glacial period in one of three glacial refugia at the time: the Atlantic refugia, the Mississippi refugia, and the Missouri refugia (Billington and Hebert 1988; Ward et al. 1989; Billington 1992). Past studies have shown that glacial refugia are key elements in shaping the genetic structure of walleye (Billington 1992; Stepien and Faber 1998; McParland et al. 1999; Strange and Stepien 2007; Zhao et al. 2008). Billington et al. (1992) used mitochondrial DNA variation to identify and manage stocks of walleye throughout their native range (Western Montana to Eastern Ohio). Results showed that geographically similar populations were also genetically similar and related to their glacial refugia. Stepien and Faber (1998)

looked at walleye populations at different geographical scales in Lake Erie and Lake St. Clair (spawning sites, lake basins, lakes, and putative glacial refugia), and found that populations in the unglaciated tip of southern Ohio were genetically different from populations found in the Missouri, Mississippi, and Atlantic glacial refugia further supporting that genetic stock structure is explained by historical landscape of the region. The walleye that founded Wisconsin populations likely are from the Mississippi refugia (Billington 1992). As the glaciers receded, the meltwater at the glacial front was the avenue of colonization for walleye populations. Over time, migration routes become dry stranding established walleye populations which followed these migration routes, isolating them into the east and west regions of Wisconsin. Isolated populations through genetic drift allow the east and west regions to independently diverge from one another resulting in genetically divergent east/west split for Wisconsin's walleye populations. This would explain why a few populations found in the Upper Chippewa headwaters are genetically most similar to the Upper Wisconsin River populations and consistent with glacial predictions despite the contradiction with current watershed boundaries which likely were established post-glacially.

Post-glacial geological events may also have influenced some of the current genetic structure observed in the Upper Wisconsin River and Upper Chippewa headwaters. Stream capture is the diversion of the head-waters of one stream into the channel of another stream (Wetzel 2001). This diversion may be caused by erosion, breaching of a stream, damming, geological-upshift, glaciations, etc. Observed inconsistencies between genetic structure and watershed boundaries has been demonstrated in other studies where contemporary rearrangement of the landscape has

been suggested as not reflecting the historical hydrological structure of the region (Strange 1998; Poissant et al. 2005; Burridge et al 2006). For example, Strange (1998) looked at mitochondrial DNA variation between Johnny darters (*Etheostoma nigrum*) in eastern Kentucky and showed that, both phenotypically and genetically, fish from the upper fork of the Cumberland River were more similar to fish from the Kentucky River than they were to their closest relative, the sympatric Cumberland Johnny darter (*Etheostoma susanae*). Stream capture was the proposed mechanism for this genetic resolution where geological uplift resulted in the diversion of the historical headwaters of the Kentucky River (where Johnny darters occurred) into the upper Cumberland River basin where the related Cumberland Johnny darter had allopatrically evolved. Headwater regions and major tributaries for both the Chippewa and Wisconsin Rivers are in close proximity (e.g., < 21 kilometers) from each other and elevation for the area reaches <500 meters throughout the area (WDNR Surface Water Data Viewer). The combination of proximity and elevation make it possible that a small shift in landscape has the potential to alter historical water patterns, in turn, changing watershed boundaries.

Genetic Units for Wisconsin Walleye

Despite the high degree of distinctiveness among sampled populations, geographical patterns of genetic diversity were readily apparent in the data consistent with higher level groups of populations. The resolution of an east/west split in sampled populations was consistent with geographic location of these populations and showed a mostly consistent pattern with known hydrology of the region with two primary basins dominating the sampled region: the Wisconsin River basin and the Chippewa/Upper

Mississippi River basin. Geographic and watershed consistency in genetic structure is expected in fish populations due to isolation by distance (Wright 1943, Hedrick 2000) within a region and the connectivity of water systems within a given basin. The resolution of two groups consistent with current geography suggested the higher order genetic structure of walleye in Wisconsin was largely influenced by hydrology and/or geological processes of the recent past.

Results of GSI suggested 6-9 contemporary genetic units could provide reasonable protection of genetic integrity within and among resolved units while at the same time; consider agency programmatic efforts necessary to manage specific management zones when all populations are considered. To predict contemporary genetic zones, a modified approach to interpreting AMOVA results was adopted. An AMOVA provides estimates of the within group variance (V_a) and among group variance (V_b). A management zone framework where the ratio of V_a/V_b is < 1 was considered inconsistent with the specified goal of maintaining genetic integrity of walleye populations because this represented a scenario where there is more variance among populations within the groups then between the groups. However, if a V_a/V_b ratio of >1 existed, more variance was explained by differences among groups than among populations within groups; a reasonable goal for identifying functional genetic management units. In essence, when the ratio was >1, populations within a given group were more similar to one another than populations among groups.

Stocking potentially compromised five populations (BCL, WIL, LCO, SIK, STL; Appendix 3) which were dropped from analysis. Genetic structure of remaining populations demonstrated thresholds were reached at both 3-4 genetic units and at 7-9

genetic units (Figure 18). These two thresholds demonstrate hierarchical levels of genetic structure within the naturally recruiting walleye populations of Wisconsin. Correlation between geographic location of these populations and genetic structuring was observed. For example, the three genetic units seem to have an east, west, and northern structuring (Figure 13) while the four genetic units demonstrates the same geographic structuring with the addition of an isolating grouping within the eastern unit (Figure 14). This isolated grouping is closely related to the Eagle River in the eastern part of the ceded territory. This type of pattern may result from two possible phenomena: stocking and glaciations.

Genetic Dynamics of Wisconsin Walleye: Effective Population Size, Census Size, and inbreeding.

The observed N_e estimates and N_e: N_c ratios were consistent with biological characteristics exhibited in walleye (i.e., high fecundity, unequal sex ratio at mating, intermittent recruitment, etc.). High fecundity coupled with iteroparity produces high family-size variance within a population. A single female is capable of producing well over > 50,000 eggs annually (Colby and Smith 1967; Niemuth et al. 1972; Becker 1983; Lester et al. 2000). However, differential reproductive success in the population leads to unequal proportions of individuals contributing to recruitment of the population and the gene pool, in turn limiting genes being passed down from generation to generation, which increase risk of inbreeding within the population.

Additionally, unequal sex ratios, sperm competition (differential fertility among sperm from different males) and multiple fertilizations by a single male contribute to

naturally low N_e and N_e: Nc ratios found in naturally recruiting walleye populations. Both sperm competition and multiple fertilizations by a single male could result in the potential for unequal proportions of individuals fertilizing eggs, resulting in disproportionate contribution of genes being passed from generation to generation. A disproportionate contribution allows few individuals to dominate and gene pool lowering N_e and N_e: N_c ratios for the overall population (Geiger et al. 1997; Jones and Hutchings 2002).

The population dynamics of walleye show variable year-to-year recruitment caused by several broad-scale environmental factors (Busch et al. 1975; Forney 1976; Chevalier 1977; Madenjian et al. 1996; Hansen et al. 1998). This variability in year-to-year recruitment presents an situation where strong year-classes can dominate recruitment over several years. This allows one year-class to be a primary contributor to the gene pool. Because Ne is composed of the genetic material passed from generation to generation, if a single year-class contributes genetic material to the gene pool at a higher proportion from one generation to the next, low N_e and N_e: N_c ratios will result. A low N_e: N_c ratio was present in Escanaba Lake, Wisconsin, where naturally recruiting walleye experience variable year-to-year walleye recruitment (Franckowiak et al. 2009). However, Franckowiak et al. (2009) found that a low N_e/N_c ratio was maintained through time in a naturally recruiting walleye population, signifying that this population continued to persist over time with a low N_e without showing signs of reduced fitness. Franckowiak et al. (2009) came to the conclusion that low N_e may be a common biological factor among Wisconsin's naturally recruiting walleye populations.

Significant inbreeding was observed in half the sampled walleye populations in this study. Inbreeding within walleye has been shown or suggested in previous studies (Cena et al. 2006; Franckowiak et al. 2009). Cena et al. (2006) found correlations between lake parameters (e.g., surface area, surface area, growing degree days, hatchery supplementation) and genetic diversity that suggested inbreeding is occurring in some walleye populations. Observations were based on the population genetic diversity being significantly correlated to these lake parameters, possibly due to habitat stability and population size. Both factors have been found to have a negative effect on the genetic diversity that has led to inbreeding depression where lack of habitat suitability could lead to only a small portion of the population contributing recruitment year-to-year and smaller population size leading to a higher risk of a population being inbred (Allendorf and Luikart 2007).

Interestingly, a conflicting trend from the Cena et al. (2006) study was observed in the current study where inbreeding coefficients increased as surface area increased; however, a simple linear regression showed no significant relation between the two. Predictably, F_{IS} should decrease with larger surface area because of the positive relation between surface area and population size. However, larger surface area systems may have multiple spawning subpopulations, resulting in increased F_{IS} values if sampling was only taken from a single subpopulation within the system. Multiple populations within a larger system are plausible in walleye since individuals/populations have been shown to exhibit philopatry and/or natal homing (Jennings et al. 1996; Stepien and Faber 1998; Wilson and Gatt 2000). Stepien and Faber (1998) looked at walleye phylogeographic structure in the Great Lakes and found consistent male and female genotypes year-to-year

at spawning sites suggesting philopatry and possible natal homing. Furthermore, life history characteristics of walleye allow individuals to dominate a gene pool of populations. The high fecundity of walleye (Niemuth et al. 1972; Becker 1983) make it possible for an entire cohort to be produced from a few individuals, increasing the chances a population could become inbred. If this pattern were to persist, genetic diversity would be lost and the subpopulations would become inbred with a high risk of inbreeding depression. Nevertheless, the lack of significance in HWE tests suggested this was not the sole reason for the significant F_{IS} values.

A Wahlund effect was possibly occurring in some of these populations resulting in significant inbreeding coefficients. A Wahlund effect indicates inbreeding due to a deficit in heterozygosity by sampling of multiple populations or subpopulations in one sample set (Wahlund 1928; Hartl and Clark 1997; Frankham et al. 2002; Allendorf and Luikart 2007). Multiple populations result in differing allele frequencies. If a single sample set was to be composed of multiple populations than different allele frequencies would result in heterozygote deficiencies indicating possible inbreeding. This may have been true with some of the sample sets on our larger water bodies. These larger bodies of water have the potential to accommodate multiple populations due to the natural life characteristics of walleye. Within larger water systems (e.g., Lake Erie) it has been shown that multiple distinct populations can exist in an open water system (Stepien and Faber 1998; Strange and Stepien 2007). Even though Lake Erie is astronomically larger in comparison to these sampled populations, the walleye within this system still experience the same life history characteristics (i.e., homing) that would lead to distinctly different populations as inland smaller systems. Therefore it is reasonable to believe

within inland water systems, especially in larger inland systems, that multiple distinct subpopulations could occur. Further support lies in the relationship between surface area and F_{IS}. Even though, no significant relationship was found, there did look to be a slight correlation indicating higher F_{IS} values being associated with larger surface areas (87.5% of populations > 500 ha significant). This is contradictory to what one would expect but not unrealistic. Again due to the biological characteristics (i.e., philopatry) of walleye multiple populations maybe supported in these systems producing a Wahlund effect associated with these larger surface area populations.

Overall, genetic diversity levels for these walleye populations were considered high and comparable to other naturally recruiting walleye populations found in the region. Genetic structure for naturally recruiting walleye populations in Wisconsin was found in the ceded territory of Wisconsin. This genetic structure looked to be consistent with geographic location and not with the watershed regions for these populations. This was thought to be due to two hypotheses: 1) supplemental stocking and 2) post-glacial events. In addition, supplemental stocking looked to have possibly genetically influence a small portion of the sampled populations. Furthermore, inbreeding looked to be a concern for a many of these populations. This was demonstrated by the significant F_{IS} values and also was consistent with the Franckowiak et al. (2009) study. Further understanding of the genetic structure and inbreeding occurring in Wisconsin's naturally recruiting walleye populations will help to provide the appropriate management techniques needed to help maintain and sustain future walleye populations for the future.

MANAGEMENT IMPLICATIONS

A primary goal of the Wisconsin Walleye Management Plan (Hewett and Simonson 1998) is to maintain the genetic integrity of naturally recruiting walleye populations found in Wisconsin. Highly distinct localized gene pools were observed for most of the 26 sampled walleye populations in the study. If these lakes have maintained isolated gene pools, management should be directed to reduce potential disruption of those gene pools and conserve this contemporary genetic diversity. To reduce this risk, proper management techniques need to be applied.

A central finding of this study is that, in general, walleye populations were genetically diverse and thus could potentially contain numerous local adaptations across the Wisconsin landscape. As such, there is no single broodsource that should or could be used to eliminate risks associated with any supplemental stocking and regardless success rate of supplemental stocking (i.e., recruitment) is usually low (Threinen 1955; Laarman 1978; Jennings et al. 1996). This coupled with genetic drift occurring in these small populations will continue to diverge these populations from one another. The most sound scientific approach for augmenting naturally recruiting walleye populations while protecting the population's genetic integrity would be to fortify the population's habitat and/or community dynamics to ensure more consistent recruitment. If supplemental stocking is still deemed necessary, a local source, such as shore-side rearing, may lower genetic risks to these divergent populations; however, this approach is not without risk (i.e., swamping the gene pool with a few individuals).

Refining management zones that consider the genetic structure of the ceded territory would reduce genetic risks, but not eliminate these risks based on the high level of population divergence observed in inland lakes in this study. Management units based, at least in part, on genetic structure have the potential to more accurately depict biological characteristics (i.e., population/ recruitment estimates) leading to more appropriate management decisions (Knaepkens et al. 2002; Jónsdóttir et al. 2002; Shikano and Taniguchi 2002; Cena et al. 2006). For example, Jónsdóttir et al. (2002) looked at two separate spawning sites of Atlantic cod (*Gadus morhua*) off southern Iceland and found the population spawning groups showed genetic differentiation and differences in life history traits (i.e., growth). These differences indicated the two populations should be treated as separate stocks for appropriate and effective. A primary limitation to any management program is the difference between the financial needs to effectively manage a resource and the available finances given for management. Therefore, genetic units must be developed minimizing the risk of genetic loss but also considering management limitations (i.e., number of units).

There were a total of 7-9 management units out of 26 sampled populations identified for the ceded territory with all populations considered. These populations were consistent with geographic location of population with a few exceptions (WIL, BCL, and SIK) where stocking was concluded as a primary source for genetic grouping. If these lakes have maintained unique genetic gene pools, management should be directed to reduce potential disruption of those gene pools. To reduce this risk, proper management techniques need to be applied. Supplemental stocking should only be applied to these

lakes when necessary and within their genetic units described here. Following this would maintain their current genetic diversity and conserve their genetic integrity.

Out of the 7-9 genetic units identified, the majority of evidence supported an eight unit scenario (i.e., NJ tree, AMOVA, V_a/V_b ratio, F-statistics) when all populations were considered. These eight units were found to be inconsistent with current management units but consistent with geographical location for the majority of lakes. Restructuring current boundaries based on a combination of demographic data and genetic data will help fulfill the goal of the Wisconsin Walleye Management Plan (Hewett and Simonson 1998) in conserving the genetic integrity of Wisconsin's naturally recruiting walleye populations while also potentially filling a gap in understanding biological parameters such as recruitment and growth.

A hierarchical grouping was observed at 3-4 genetic units when five populations were eliminated due to possible contamination through supplemental stocking, suggesting a smaller number of management units. Limiting the number of genetic units would help to improve efficiency by managing these populations through a lower number of stocks, improving time and decreasing money associated with management. Unfortunately, a risk exists in considering only 3-4 genetic units. Under-representing the genetic structure of Wisconsin's naturally recruiting walleye populations introduces the risk of compromising the current genetic structure, risking a top goal in the Wisconsin Walleye Management plan. However, if keeping genetic units for management at a minimum is a priority for the WDNR, then management through these 3-4 management units would reduce total number of genetic units to manage.

FUTURE RESEARCH

Genetic Stock Identification of a Non-stocked Species Focused in Upper Wisconsin and Upper Chippewa Region

Resolved genetic units were not consistent with the current management units found in the ceded territory. There were two supported causes for this discrepancy: 1) supplemental stockings occurred across stock boundaries thus altering the genetic structure, and 2) a geological event(s) has largely influenced the genetic diversity of these populations. Examining the genetic structure of a sympatric, non-stocked fish species (e.g., rock bass *Ambloplites rupestris* or log perch *Percina caprodes*) would help to resolve whether this inconsistency is natural (i.e., geologic) or human-mediated (i.e., stocking). This information would provide crucial information for the development of genetic-based management zones for the state.

Comparison between Muskellunge and Walleye Genetic Stock Structure

In Wisconsin, walleye and muskellunge (*Esox masquinongy*) are typically found and distributed in similar water systems (Becker 1983). For this reason, walleye and muskellunge in the ceded territory are managed by similar units and techniques. If these two species were historically located in similar water systems and management has been conducted in a similar fashion, then the genetic structure of the two species should be similar. Preliminary data on the stock structure of muskellunge in the ceded territory (B. Spude, UWSP, unpublished data) has shown similar patterns in genetic structure among the two species. By comparing these two data sets, multispecies management units can

be defined that likely explain the genetic diversity patterns for the majority of fish species in Wisconsin.

Genetic Diversity and Biological Characteristics of Walleye

Several studies have documented how genetic diversity may be correlated with fitness (Heath et al. 2002; Knaepkens et al. 2002; Borrell et al. 2004). A key component to management would be to better understand the population dynamics and habitat characteristics for these populations. A better understanding of the role that genetic diversity plays in these biological parameters, could lead to the application of more appropriate management techniques to reduce loss of fitness and sustain the population for the future.

Inbreeding

Inbreeding may be a concern for some naturally recruiting walleye populations in Wisconsin. Further understanding key biological parameters (i.e., habitat, forage abundance, etc.), reproductive behavior, and the temporal stability of populations should provide links to predicting factors influencing inbreeding. Ecological studies on the physiology and recruitment of walleye should provide key data to determine whether subsequent inbreeding depression is a concern in many of these populations.

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Table 1. Microsatellite loci, primer sequence, and locus-specific descriptive characteristics previously developed and used for walleye (Wirth et al. 1999, Borer et al. 1999, Eldridge et al. 2002).

Locus	Primer Sequence (5'-3')	Allele size (bp)	Number of Alleles	Reference
Svi-2	F:CAA CCA GAC CCA ATC CCT TG R:GGG CCG AGT ATA TCA GTT AAC	192-208	9	Eldridge et al. 2002
Svi-4	F:ACA AAT GCG GGC TGC TGT TC R:GAT CGC GGC ACA GAT GTA TTG	102-118	4	Eldridge et al. 2002
Svi-6	F:AGT CGA CAT ATT ATG TAG AGT GC R:GAT CAA CTG TGG AGG ATG AGC	136-173	18	Eldridge et al. 2002
Svi-7	F:GAA ACC TTA CAA AAG CCT GG R:TTA TCT GCA CTT CTA CAG GC	163-173	N/A	Eldridge et al. 2002
Svi-9	F:GGA TCT GTA AAC TTG TCA AAT GGA R:ACG GAT TGG TAA AAC TAC AGA A	330-370	21	Wirth et al. 1999
Svi-L9	F:TAC TGT TCA CTT ATC TAT CC R:TGT ATG TGT GTG TGT TCA TGT	243-297	13	Wirth et al. 1999
Svi-17	F:GCG CAC TCT CGC ATA GGC CCT G R:CGT TAA AGT CCT TGG AAA CC	101-113	5	Borer et al. 1999
Svi-20	F:CAA GTG CGC AAT GGT GCA TTA C R:GAA TGA AGA AAT GCA CCC ATG C	144-193	18	Eldridge et al. 2002
Svi-26	F:CGA ACT ACT TAT CTT CTG GC R:GTA AGT GTG AAT CAG CCA GAC	156-189	16	Eldridge et al. 2002
Svi-33	F:CAG GAC TGC TGT GTA TAG ACT TG R:GAT ATA GCT TTC TGC TGG GGT C	90-102	6	Borer et al. 1999

Table 2. Walleye populations used to assess the genetic structure of Wisconsin walleye, their abbreviations, county, and management units.

Lake	Abbreviation	County	Management Unit
St. Louis	STL	Douglas	Lake Superior
Amnicon Lake	AL	Douglas	Lake Superior
Millicent Lake	LM	Bayfield	Lake Superior
Moen Chain	MC	Oneida	Upper Wisconsin
Butternut	BN	Forest	Green Bay
Clam Lake	CL	Burnett	St. Croix
Gordon Lake	GL	Ashland	Lake Superior
Gile Flowage	GF	Iron	Lake Superior
Pixley Flowage	PF	Price	Upper Chippewa
Blaisdell Lake	BD	Sawyer	Upper Chippewa
Moose Lake	ML	Sawyer	Upper Chippewa
Big Fork Lake	BFL	Oneida	Upper Wisconsin
Kawaguesaga Lake	KL	Oneida	Upper Wisconsin
Eagle Chain	EC	Vilas	Upper Wisconsin
Spider Lake	SPL	Iron	Upper Chippewa
Turtle-Flambeau	TFL	Iron	Upper Chippewa
Little Arbor Vitae	LAV	Vilas	Upper Wisconsin
Red Cedar lake	RCL	Barron	Lower Chippewa
St. Croix	STC	Polk	St. Croix
Dowling lake	DOW	Douglas	Lake Superior
Duck Lake	DUC	Barron	Lower Chippewa
Siskiwit Lake	SIK	Bayfield	Lake Superior
Big Crooked Lake	BCL	Vilas	Upper Chippewa
Willow Flowage	WIL	Oneida	Upper Wisconsin
Chippewa Flowage	CHF	Sawyer	Upper Chippewa
Lac Courte Oreilles	LCO	Sawyer	Upper Chippewa

Table 3. PCR reaction recipes, fluorescent labels, and thermocycler temperature profiles for all multiplexes used and developed (* developed by Franckowiak et al. 2009). 10x buffer refers to 10x PCR Buffer B without $MgCl_2$ (Thermo Fisher Scientific Inc., Waltham, MA), dNTP mix, 25mM magnesium chloride solution ($MgCl_2$), and 0.5 U of *Taq* DNA polymerase.

Locus	Multiplex	10x buffer	dNTPs	$MgCl_2$	Primer Forward (Conc.)	Primer Reverse (Conc.)	Label
Svi-2	A*	1x	0.60mM	1.50mM	0.08µM	0.08µM	Fam
Svi-4					0.06µM	0.06µM	Fam
Svi-6b					0.17µM	0.17µM	Ned
Svi-7					0.20µM	0.2µM	Hex
Svi-26	B*	1x	1.00mM	1.50mM	0.30µM	0.30µM	Fam
Svi-17					0.30µM	0.30µM	Ned
Svi-33					0.30µM	0.30µM	Hex
Svi-L9	C	1x	1.00mM	2.40mM	0.24µM	0.24µM	Fam
Svi-18					0.10µM	0.10µM	Ned
Svi-20					0.30µM	0.30µM	Hex
Svi-5	D	1x	1.00mM	2.20mM	0.20µM	0.20µM	Fam
Svi-8					0.24µM	0.24µM	Ned
Svi-9					0.24µM	0.24µM	Ned

A 94°C for 2.0 min. 1 series of 31 cycles each at 94°C for 30 s, then 60°C annealing for 1.0 min. 72°C for 2.0 min then a final elongation of 72°C for 40.0 min.

B 94°C for 5.0 min. 1 series of 35 cycles each at 94°C for 1.0 min, then 52°C annealing for 1.0 min. 72°C for 1.0 min then a final elongation of 72°C for 45.0 min.

C 94°C for 5.0 min. 2 series of 35 cycles each at 94°C for 1.0 min, then 53°C annealing for 1.0 min. 72°C for 1.0 min then a final elongation of 72°C for 45.0 min.

D 94°C for 1.5 min. 2 series of 35 cycles each at 94°C for 1.0 min, then 53°C annealing for 1.5 min. 72°C for 1.5 min then a final elongation of 72°C for 5.0 min.

Table 4. Summary statistics for all 26 sampled populations. Abbreviations for populations are found in Table 2. Allelic diversity (A), allelic richness (A_r) observed heterozygosity (H_O), expected heterozygosity (H_E), and standard deviations (SD) are all represented below.

Pop.	Sample size	No. of loci	H_E	H_E SD	H_O	H_O SD	A	A_r	A SD	Private Alleles
STL	50	10	0.7534	0.0326	0.6834	0.0209	9.8	6.91	3.61	1.35
AL	50	10	0.7583	0.0198	0.6951	0.0206	10.0	6.64	3.62	0.58
LM	50	10	0.7518	0.0105	0.7067	0.0204	9.2	6.37	2.86	0.59
MC	50	10	0.7738	0.0171	0.6800	0.0209	9.8	6.93	2.90	0.86
BN	50	10	0.7614	0.0214	0.6947	0.0206	9.2	6.65	3.71	0.27
CL	50	10	0.7570	0.0256	0.7064	0.0205	9.1	6.71	2.81	1.62
GL	50	10	0.7373	0.0154	0.7620	0.0190	8.4	6.13	2.27	0.28
GF	50	10	0.7467	0.0246	0.6700	0.0211	9.1	6.60	2.64	1.16
PF	50	10	0.7602	0.0174	0.6680	0.0211	9.4	6.65	3.34	0.61
BD	36	10	0.7372	0.0271	0.7000	0.0242	8.6	6.57	1.84	0.87
ML	61	10	0.7717	0.0167	0.6869	0.0188	9.9	6.88	3.75	0.46
BFL	50	10	0.7726	0.0174	0.7220	0.0200	9.2	6.43	3.33	1.42
KL	50	10	0.7463	0.0226	0.6735	0.0211	10.2	6.79	3.43	1.46
EC	50	10	0.7589	0.0239	0.6580	0.0212	9.3	6.53	3.47	0.50
SPL	50	10	0.7681	0.0183	0.7260	0.0199	10.0	6.93	2.83	0.83
TFL	50	10	0.7485	0.0191	0.6790	0.0211	9.8	6.72	3.33	1.00
LAV	50	10	0.7709	0.0186	0.7453	0.0195	9.9	7.02	3.63	0.79
RCL	73	10	0.7825	0.0151	0.6777	0.0175	10.7	7.07	3.43	1.33
STC	39	10	0.7662	0.0263	0.6729	0.0238	9.1	6.92	2.85	1.80
DOW	29	10	0.7704	0.0246	0.8138	0.0229	8.9	7.04	2.08	1.37
DUC	48	10	0.7787	0.0188	0.7940	0.0185	9.4	6.80	2.84	0.64
SIK	47	10	0.7750	0.0181	0.7583	0.0202	9.2	6.80	2.66	0.93
BCL	50	10	0.7957	0.0178	0.7704	0.0191	9.9	7.25	2.73	1.91
WIL	49	10	0.7842	0.0206	0.7117	0.0206	10.2	7.28	2.35	1.10
CHF	62	10	0.7972	0.0142	0.7565	0.0172	10.4	7.06	3.81	0.60
LCO	45	10	0.7977	0.0100	0.7613	0.0207	9.0	6.94	2.11	0.54
Mean	50	10	0.7662	0.0198	0.7144	0.0204	9.5	6.79	3.01	0.96

Table 5. The surface area, population estimates, N_e, 95% confidence intervals (CI) for the N_e estimates, F_{IS} values, and p-values for F_{IS} values for all populations. An asterisk (*) signifies significance following sequential Bonferroni correction. Abbreviations for populations are found in Table 2.

Population	Surface area (hectares)	Population Estimates (year)	N_e	Lower CI	Upper CI	N_e/N_c	F_{IS}	P-value
STL	-	-	164.2	110.3	303.5	-	0.0913	0.00006*
AL	172.4	1,239	198.3	127.4	415.1	0.16	0.0838	0.00021*
LM	74.1	1,974	279.3	151.4	1,322.2	0.14	0.0594	0.00794
MC	104.4	1,160	201.1	128.0	433.6	0.17	0.1223	0.00000*
BN	522.9	1,703	176.8	114.2	363.0	0.10	0.0881	0.00035*
CL	488.5	743	764.0	234.0	∞	1.03	0.0691	0.00372
GL	57.5	-	235.2	132.5	838.2	-	-0.0339	0.91436
GF	1,369.5	6,093	1,452.1	176.8	∞	0.24	0.1016	0.00134*
PF	135.2	-	∞	396.1	∞	-	0.1223	0.00000*
BD	144.1	-	125.4	79.8	267.8	-	0.0511	0.04947
ML	675.9	4,674	∞	568.2	∞	-	0.1107	0.00000*
BFL	279.2	4,040	270.4	149.7	1,094.0	0.07	0.0661	0.02443
KL	271.2	3,495	1330.0	294.8	∞	0.38	0.0955	0.00073*
EC	231.5	2,855	303.3	160.6	1,790.9	0.11	0.1342	0.00000*
SPL	142.6	943	271.0	155.8	886.3	0.29	0.0553	0.01193
TFL	5,481.7	57,697	∞	403.8	∞	-	0.0918	0.00063*
LAV	216.1	4,360	332.1	173.7	2,309.0	0.08	0.0332	0.47216
RCL	745.1	3,733	325.2	203.6	745.3	0.09	0.1337	0.00000*
STC	-	-	153.0	95.3	352.5	-	0.1229	0.00001*
DOW	62.3	-	34.4	28.2	43.3	-	-0.0574	0.97397
DUC	40.5	-	175.8	113.1	365.6	-	-0.0196	0.81353
SIK	133.6	-	212.7	122.8	673.9	-	0.0227	0.88242
BCL	155.1	1,724	58.4	48.5	72.2	0.03	0.0328	0.60798
WIL	2,552.0	13,324	188.8	122.8	3,81.6	0.01	0.0933	0.00084*
CHF	6,070.5	80,202	1,460.7	369.3	∞	0.02	0.0515	0.00738
LCO	2,039.3	7,526	128.8	85.6	2,43.7	0.02	0.0471	0.10734*
Median	223.8	3,614	212.7	141.1	424.3	0.11	0.07645	0.26081
Mean	923.5	10,971	384.4	182.6	722.1	0.18	0.06800	–

Table 6. Pairwise F_{ST} values (above diagonal) and their corresponding p-values (below diagonal). P-value < 0.00001 indicates a significant comparison. Non-significant p-values following sequential Bonferroni correction are marked in bold.

	LCO	CHF	WIL	BCL	SIK	DUC	DOW	STC	RCL	LAV	TFL	SPL	EC
LCO	*	**0.00093**	0.00877	0.01315	0.01609	0.02882	0.03545	0.02667	0.01887	0.01898	0.02378	0.01169	0.04101
CHF	**0.00200**	*	0.01480	0.01237	0.01792	0.01874	0.02151	0.01693	0.01740	0.02714	0.02713	0.02628	0.04258
WIL	0.00001	0.00001	*	0.01125	0.01658	0.03624	0.04044	0.02272	0.03201	0.02961	0.02344	0.02323	0.02674
BCL	0.00001	0.00001	0.00001	*	0.02571	0.04161	0.04060	0.03536	0.04243	0.03339	0.03233	0.03103	0.04656
SIK	0.00001	0.00001	0.00001	0.00001	*	0.03347	0.03173	0.03506	0.01763	0.01939	0.01089	0.02074	0.02895
DUC	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.02904	0.05183	0.02432	0.06593	0.05689	0.06525	0.07369
DOW	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.03812	0.02939	0.05404	0.05319	0.06401	0.06398
STC	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.02850	0.04458	0.04026	0.04008	0.04543
RCL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.04220	0.03845	0.04470	0.04536
LAV	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	**0.00777**	**0.00135**	0.02872
TFL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.00508**	*	**0.00951**	0.02490
SPL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.03415**	**0.00108**	*	0.03174
EC	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*
KL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.00169**	**0.00200**	**0.00031**	0.00001
BFL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.09585**
ML	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
BD	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
PF	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.00092**	**0.14585**	0.00001	0.00001
GF	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.00354**	**0.00523**	0.00001	0.00001
GL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
CL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
BN	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.00046**	0.00001	0.00001
MC	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	**0.11508**	**0.00169**	**0.00892**	0.00001
LM	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
AL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
STL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001

Table 6. Continued.

	KL	BFL	ML	BD	PF	GF	GL	CL	BN	MC	LM	AL	STL
LCO	0.02399	0.03073	0.01830	0.05062	0.02523	0.02997	0.03520	0.03625	0.02089	0.01720	0.01837	0.02227	0.06684
CHF	0.02887	0.03275	0.02073	0.04489	0.03157	0.03247	0.03248	0.03129	0.02715	0.02398	0.02321	0.02312	0.06473
WIL	0.02619	0.02263	0.02543	0.04983	0.03057	0.02646	0.03638	0.03323	0.02070	0.02221	0.02883	0.03383	0.06409
BCL	0.03278	0.04105	0.04102	0.04855	0.03437	0.03931	0.05438	0.05234	0.02558	0.03083	0.04538	0.04978	0.07595
SIK	0.02178	0.02206	0.01111	0.03456	0.01565	0.02140	0.01765	0.02372	0.01013	0.01554	0.01235	0.01399	0.05737
DUC	0.06654	0.06205	0.03758	0.05227	0.05843	0.06977	0.03658	0.04507	0.05083	0.05237	0.04566	0.03538	0.09308
DOW	0.06117	0.06139	0.03511	0.05805	0.06003	0.06527	0.03896	0.03973	0.04465	0.05137	0.04251	0.03690	0.10581
STC	0.04808	0.03888	0.03349	0.06694	0.04856	0.03656	0.04529	0.02214	0.04336	0.04083	0.03572	0.03866	0.07001
RCL	0.04927	0.04098	0.01769	0.05497	0.04567	0.04144	0.02502	0.03532	0.03614	0.03209	0.01865	0.01195	0.07698
LAV	**0.00523**	0.02276	0.01433	0.05282	**0.00559**	**0.00745**	0.03312	0.03820	0.01074	**0.00130**	0.01304	0.02559	0.05970
TFL	**0.00825**	0.01858	0.01401	0.03946	**0.00446**	**0.00781**	0.02304	0.03414	**0.00491**	**0.00589**	0.01624	0.02455	0.05786
SPL	**0.00599**	0.02118	0.02114	0.05494	0.01042	0.01225	0.03693	0.04041	0.01708	**0.00595**	0.01441	0.03035	0.05860
EC	0.03106	**0.00036**	0.02772	0.06530	0.03822	0.01353	0.03621	0.04317	0.02506	0.02168	0.04037	0.03782	0.06048
KL	*	0.02397	0.02091	0.05094	0.00979	0.01285	0.03607	0.04426	0.01232	**0.00342**	0.02001	0.03380	0.07132
BFL	0.00001	*	0.02349	0.05605	0.02918	0.00964	0.03452	0.03615	0.02181	0.01386	0.02822	0.03025	0.04670
ML	0.00001	0.00001	*	0.03248	0.01707	0.01107	0.01190	0.02129	0.01232	0.01317	0.00642	**0.00426**	0.06291
BD	0.00001	0.00001	0.00001	*	0.04125	0.04608	0.04122	0.04065	0.03637	0.04293	0.04517	0.04697	0.06475
PF	0.00001	0.00001	0.00001	0.00001	*	0.01420	0.03506	0.03780	0.01165	0.01334	0.01794	0.02927	0.05745
GF	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.02912	0.03483	0.01035	**0.00966**	0.02086	0.02521	0.05310
GL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.02774	0.02157	0.02152	0.01555	0.01442	0.08411
CL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.03649	0.03474	0.03334	0.02791	0.06324
BN	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.01036	0.01788	0.02639	0.06751
MC	**0.00862**	0.00001	0.00001	0.00001	0.00001	**0.00046**	0.00001	0.00001	0.00001	*	0.00864	0.02126	0.05676
LM	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.00747	0.06251
AL	0.00001	0.00001	**0.00031**	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*	0.07054
STL	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	*

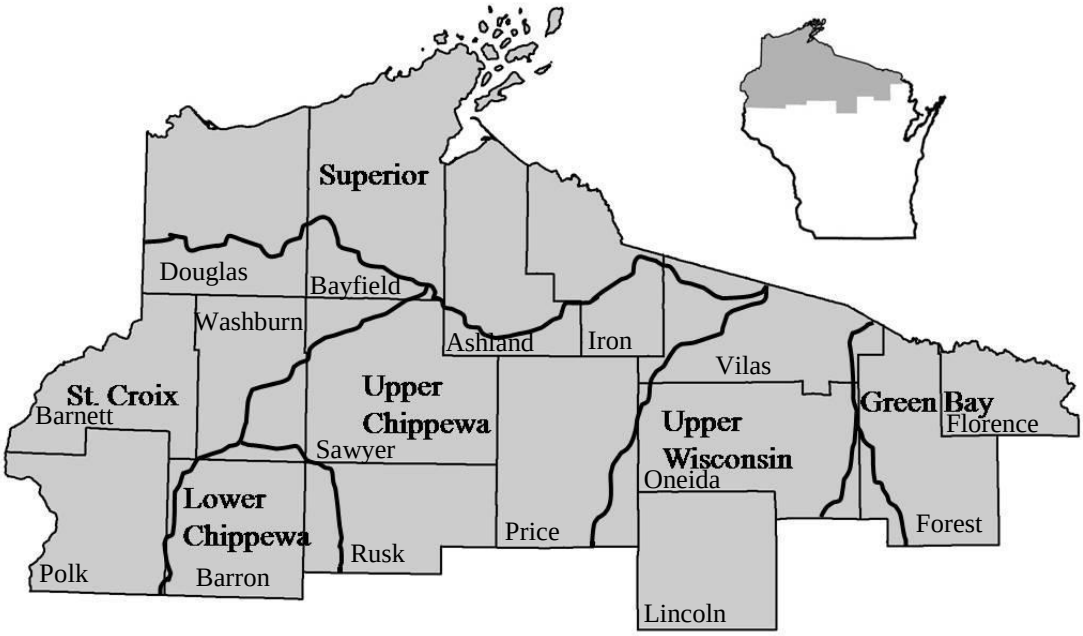

Figure 1. Map of the ceded territory of Wisconsin based on the 1983 Consent Decree (Staggs 1990). Black lines represent borders for contemporary management units based on Fields et al. (1997).

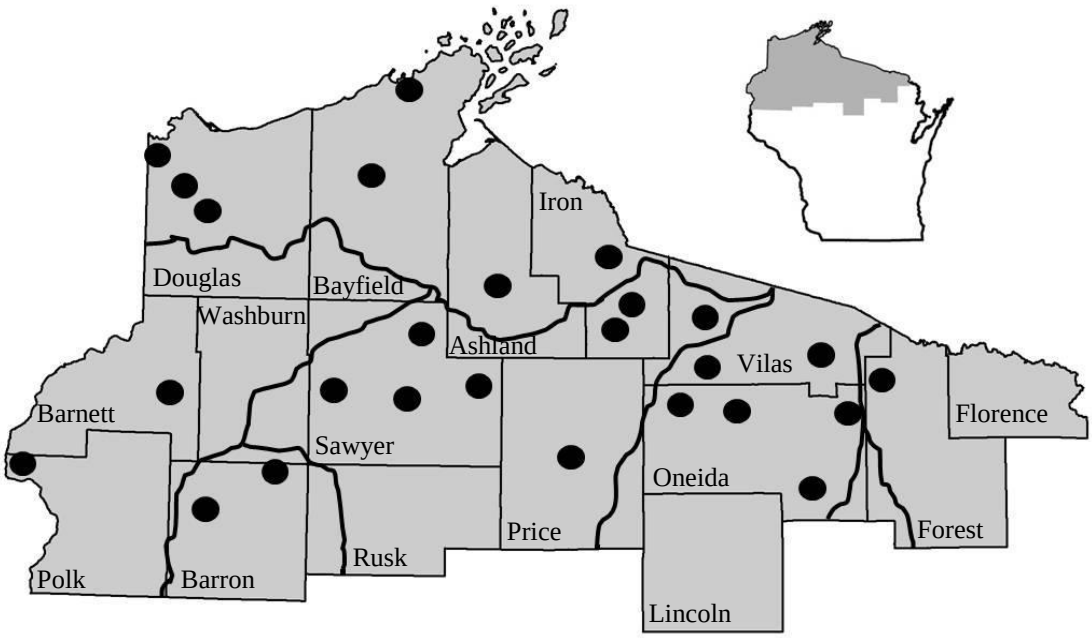

Figure 2. Map of the sampled walleye populations. Black lines represent borders for contemporary management units based on Fields et al. (1997). Black dots represent sampled walleye populations.

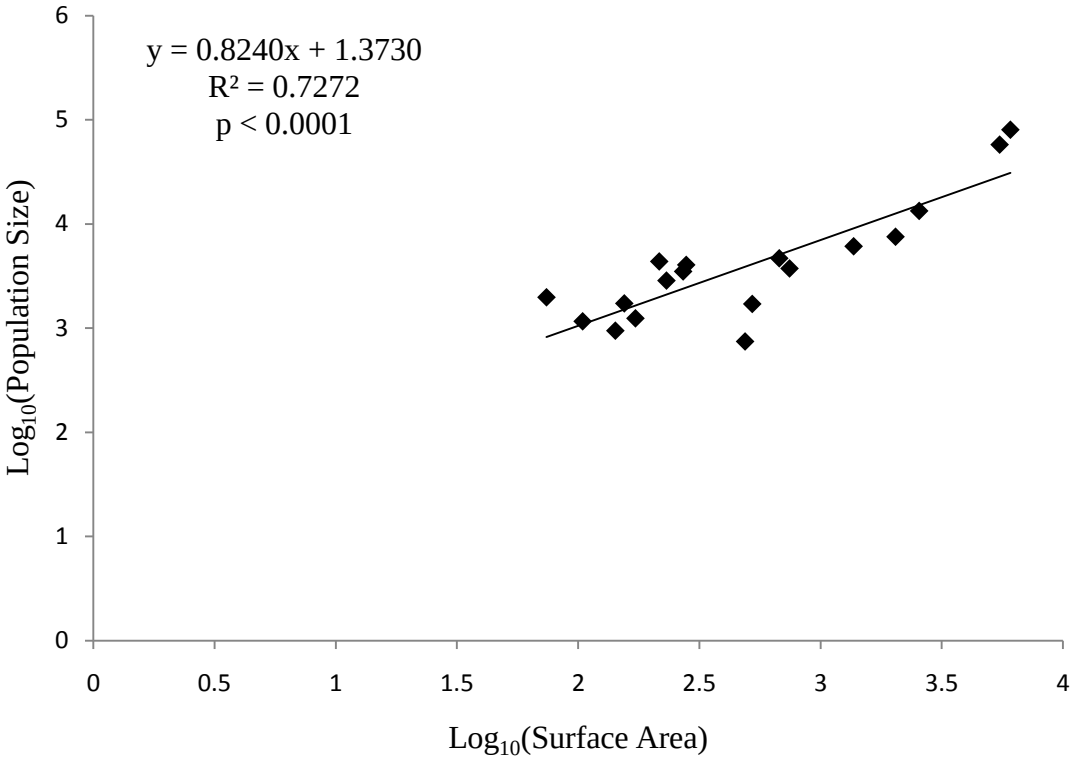

Figure 3. Relation of surface area and population size in sampled population. Slope, R^2, and p-value shown in top left corner. Diamonds represent sampled populations.

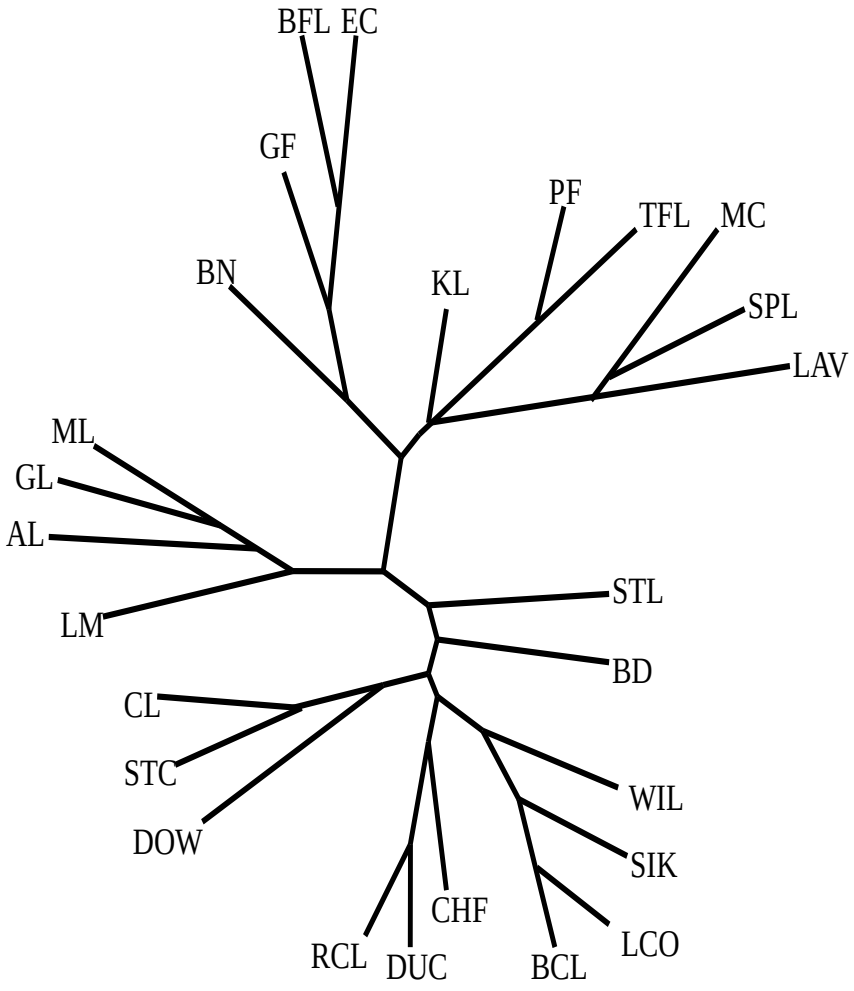

Figure 4. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c). Abbreviations for populations are found in Table 2.

	Sum of Squares	Variance Components	% of Variance
Among Groups (V_a)	67.650	0.04371	1.12
Within Groups (V_b)	334.509	0.10044	2.57

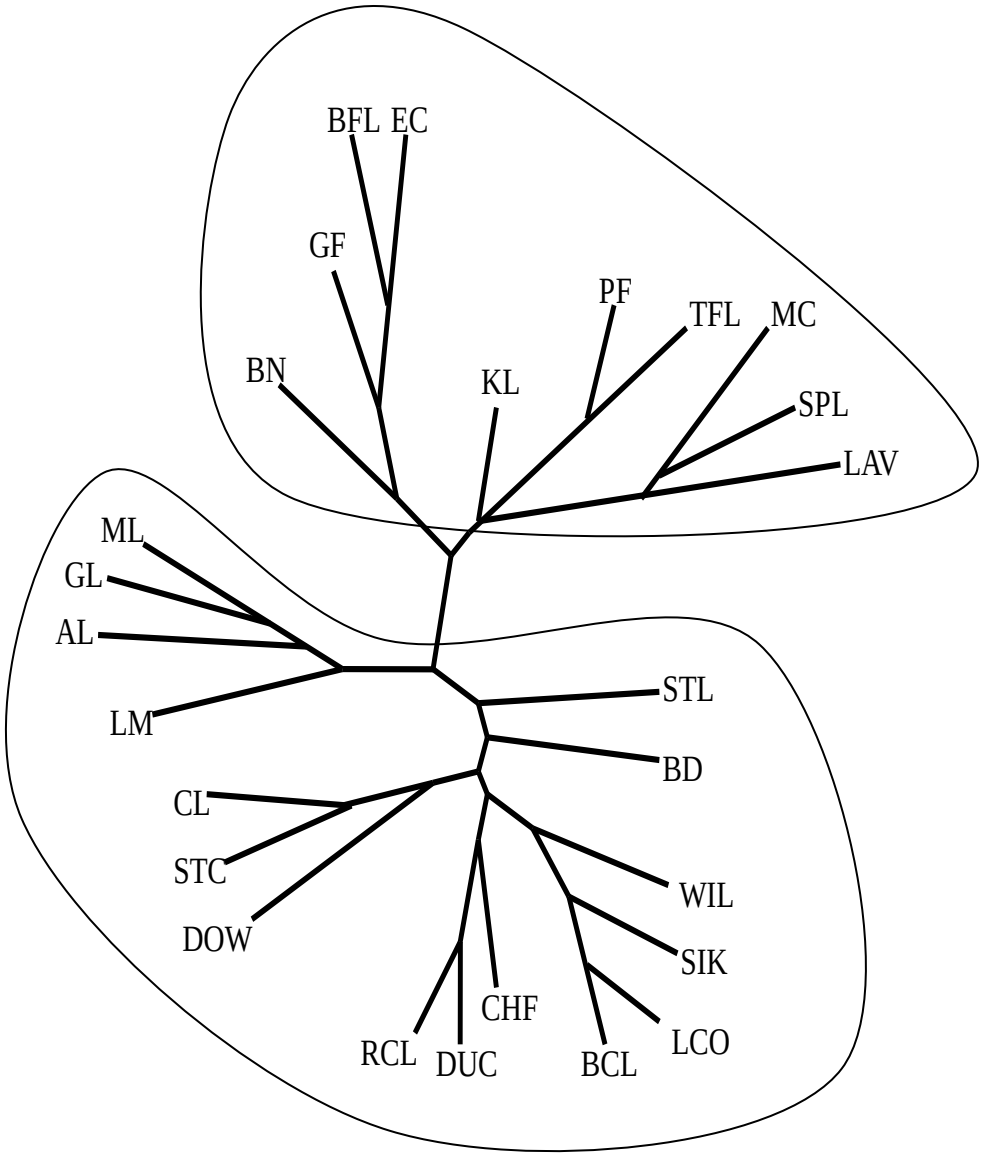

Figure 5. Hypothesis one: an east/west split among sampled populations. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance Components	% of Variance
Among Groups (V_a)	248.999	0.10852	3.35
Within Groups (V_b)	4.187	0.02502	0.06

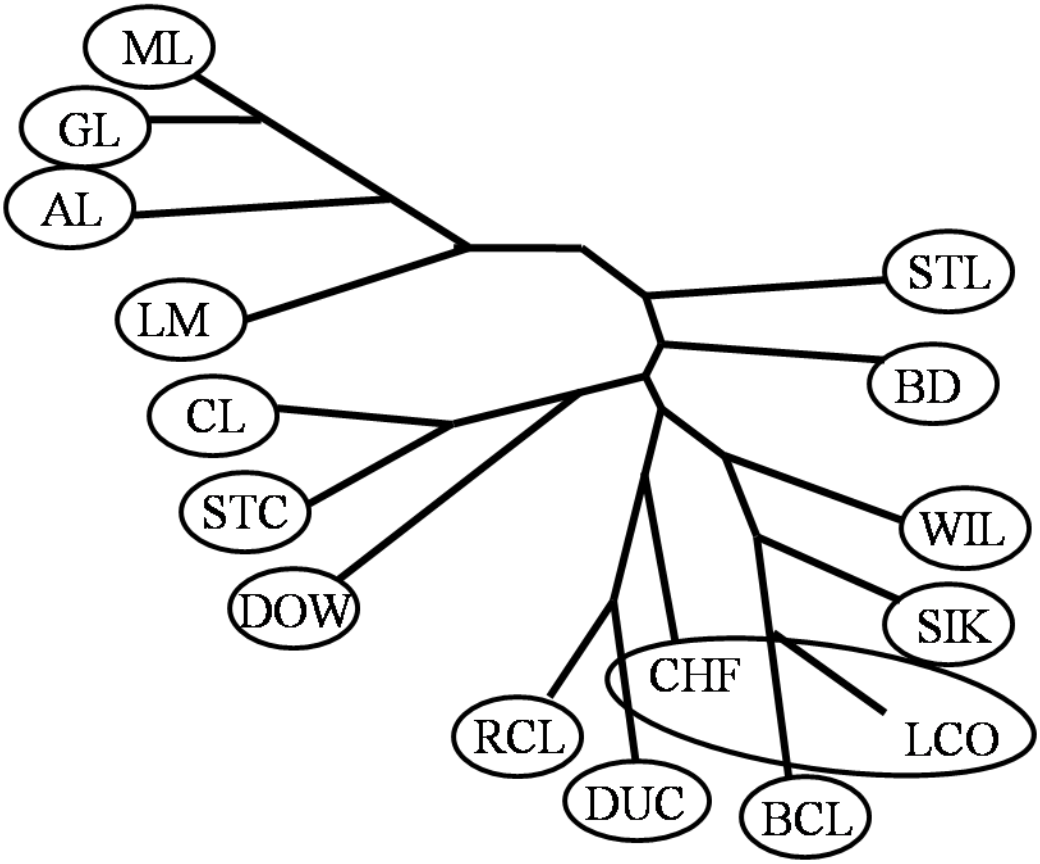

Figure 6. Group I. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance Components	% of Variance
Among Groups (V_a)	62.346	0.04828	1.26
Within Groups (V_b)	18.977	0.00976	0.26

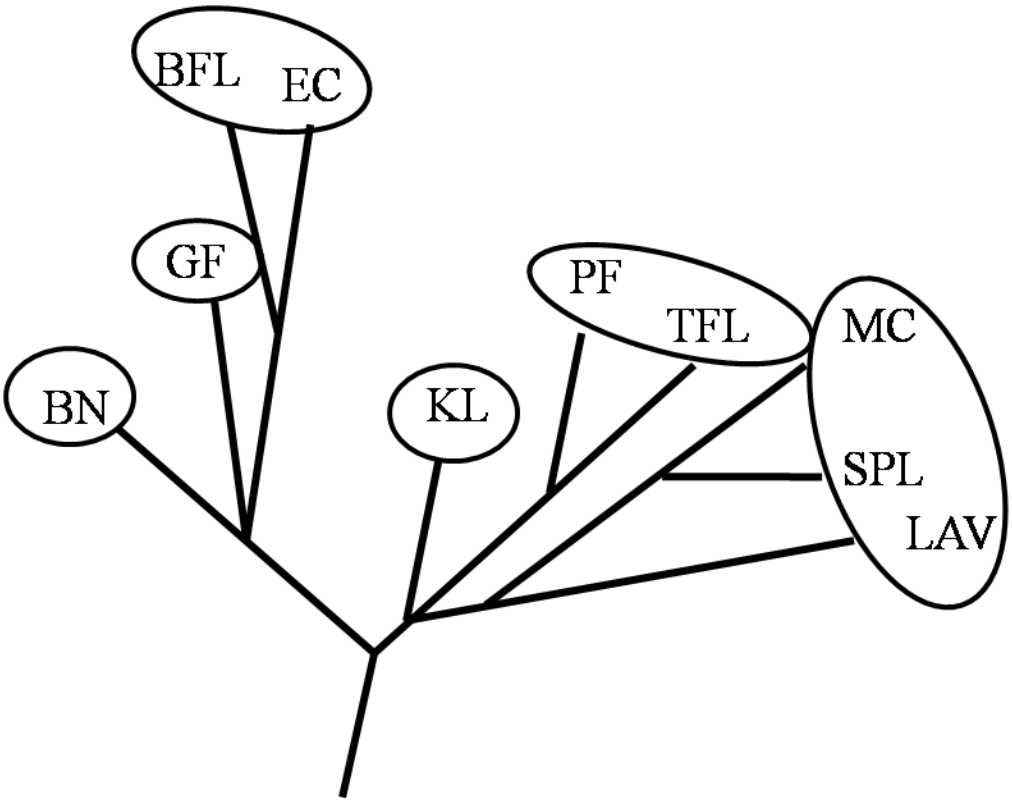

Figure 7. Group II. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance Component	% of Variance
Among Groups (V_a)	199.383	0.07490	1.92
Within Groups (V_b)	202.776	0.06170	1.58

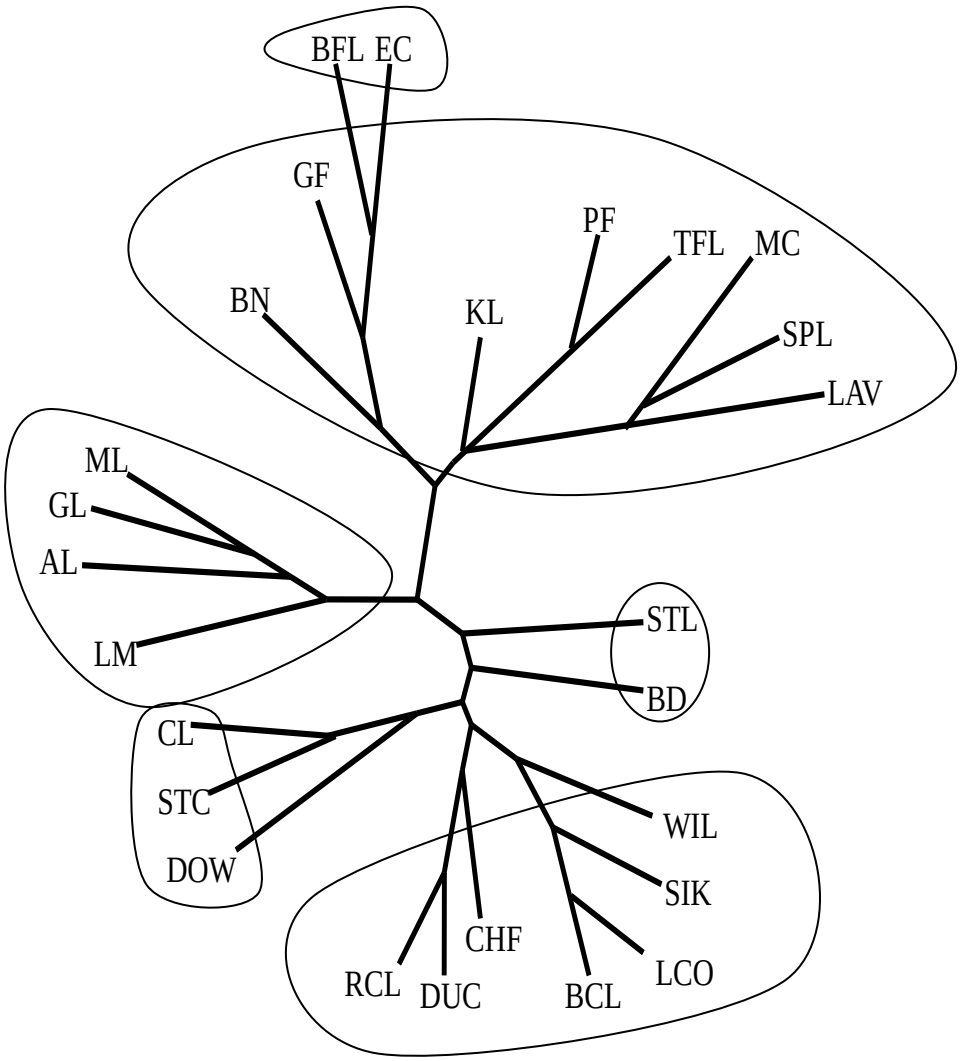

Figure 8. Hypothesis two, six group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance Component	% of Variance
Among Groups (V_a)	224.370	0.08496	2.18
Within Groups (V_b)	177.789	0.05340	1.37

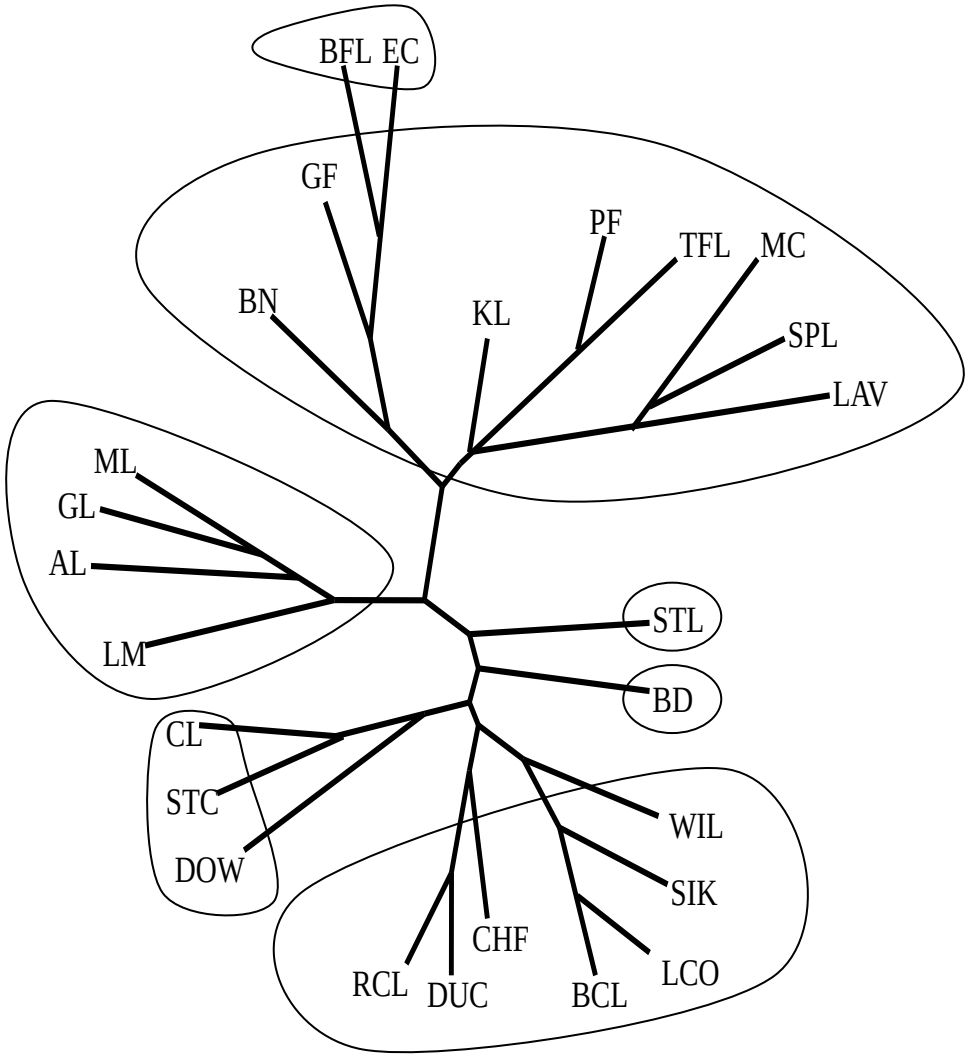

Figure 9. Hypothesis three, seven group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance Component	% of Variance
Among Groups (V_a)	249.270	0.09074	2.33
Within Groups (V_b)	152.889	0.04526	1.16

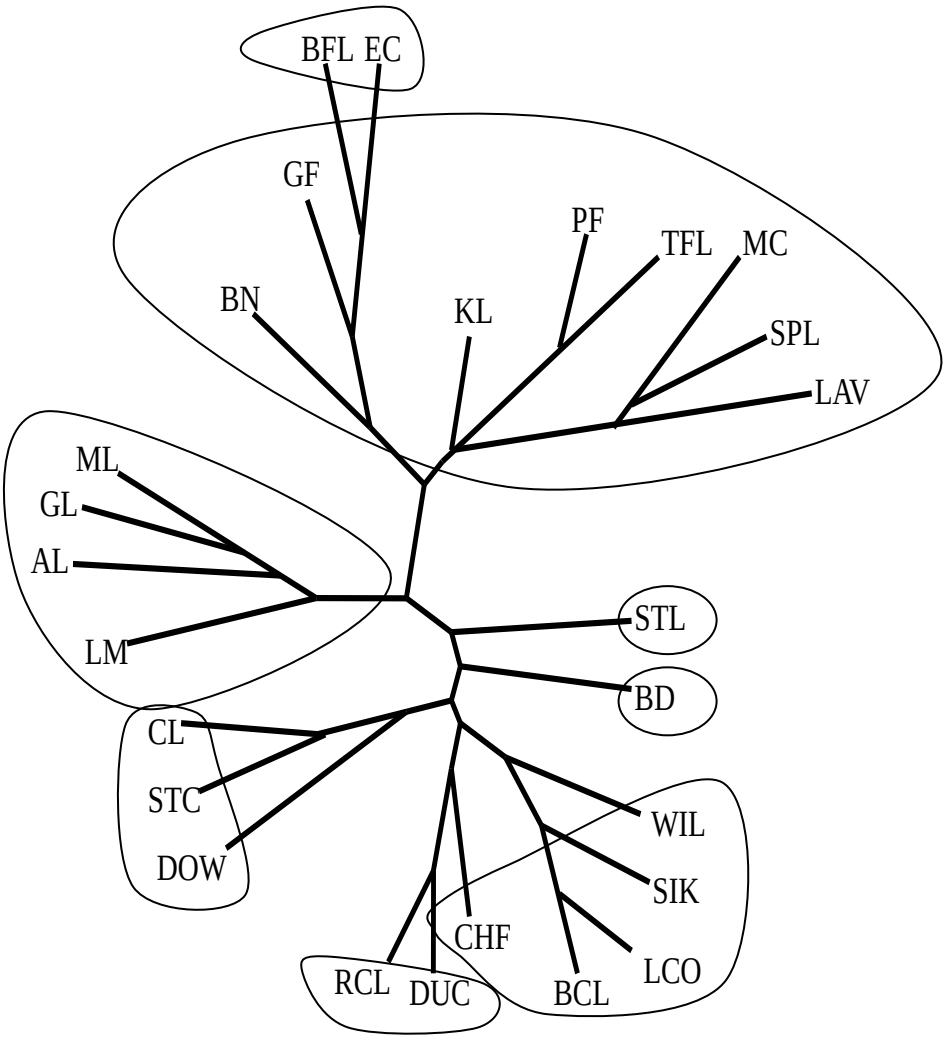

Figure 10. Hypothesis four, eight group scenario. AMOVA analysis: sum of squares, percent of variation, and p-values in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c). Black concave segments represent separate genetic groupings of populations.

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	258.237	0.08875	2.28
Within Groups (V_b)	143.922	0.04499	1.15

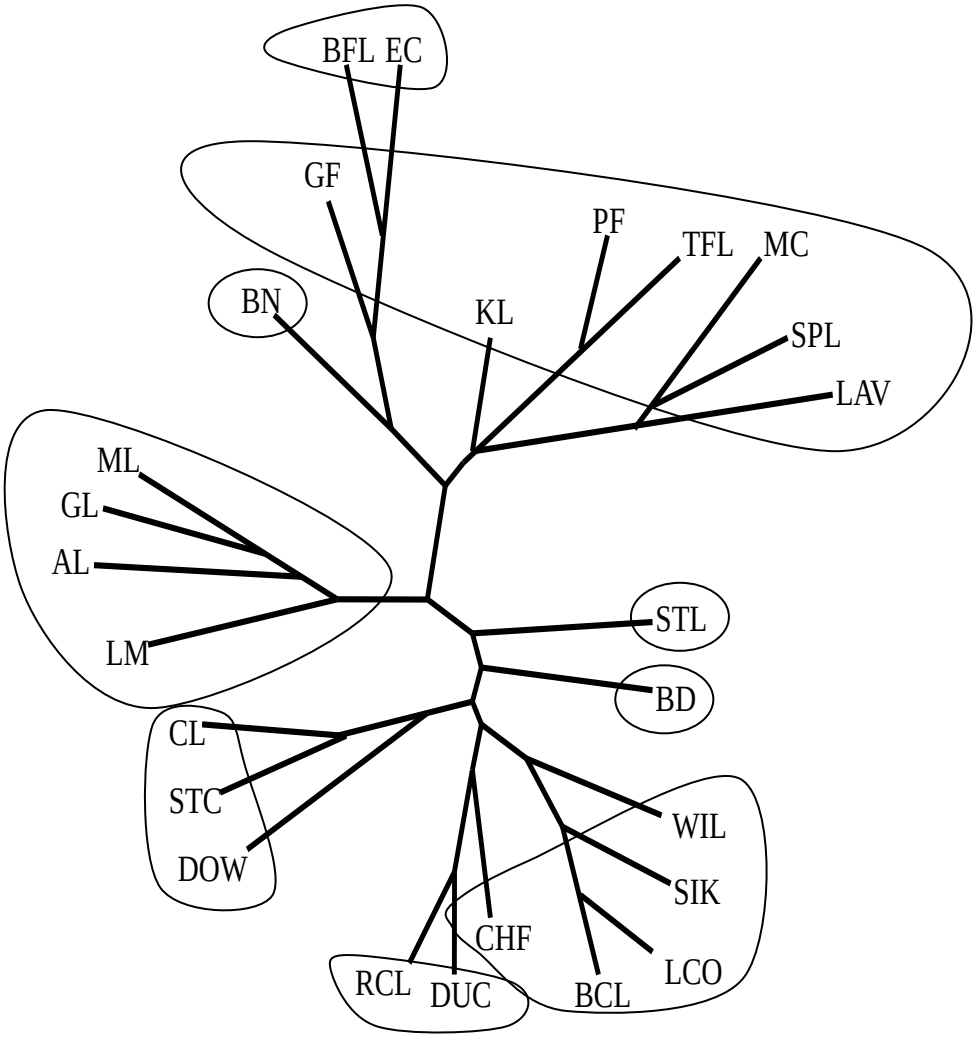

Figure 11. Hypothesis five, nine group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

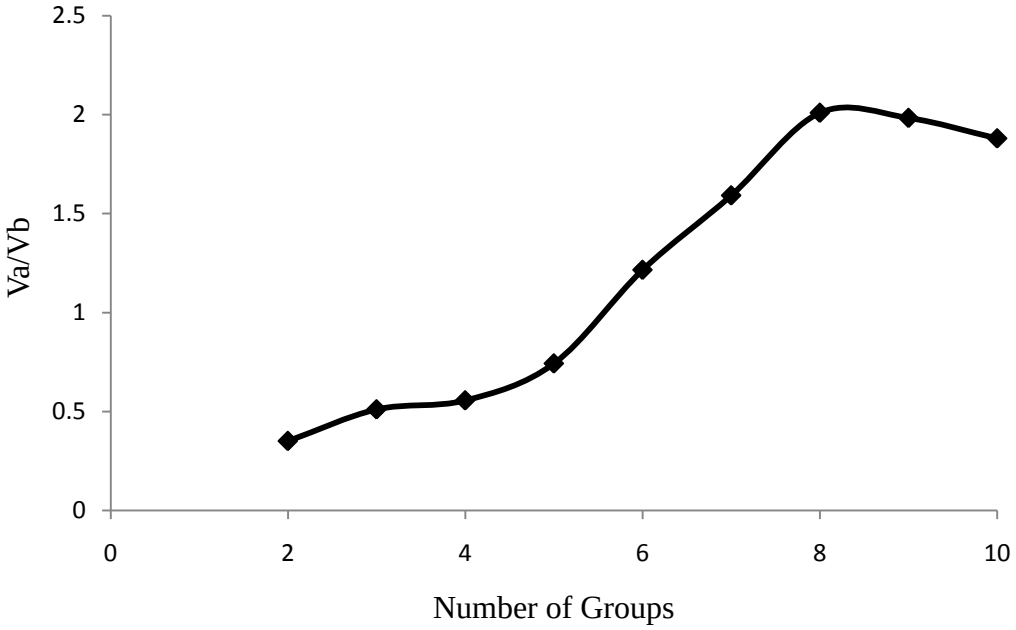

Figure 12. Graph of V_a/V_b vs. number of groups for all populations.

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	103.751	0.06755	1.72
Within Groups (V_b)	202.863	0.07263	1.85

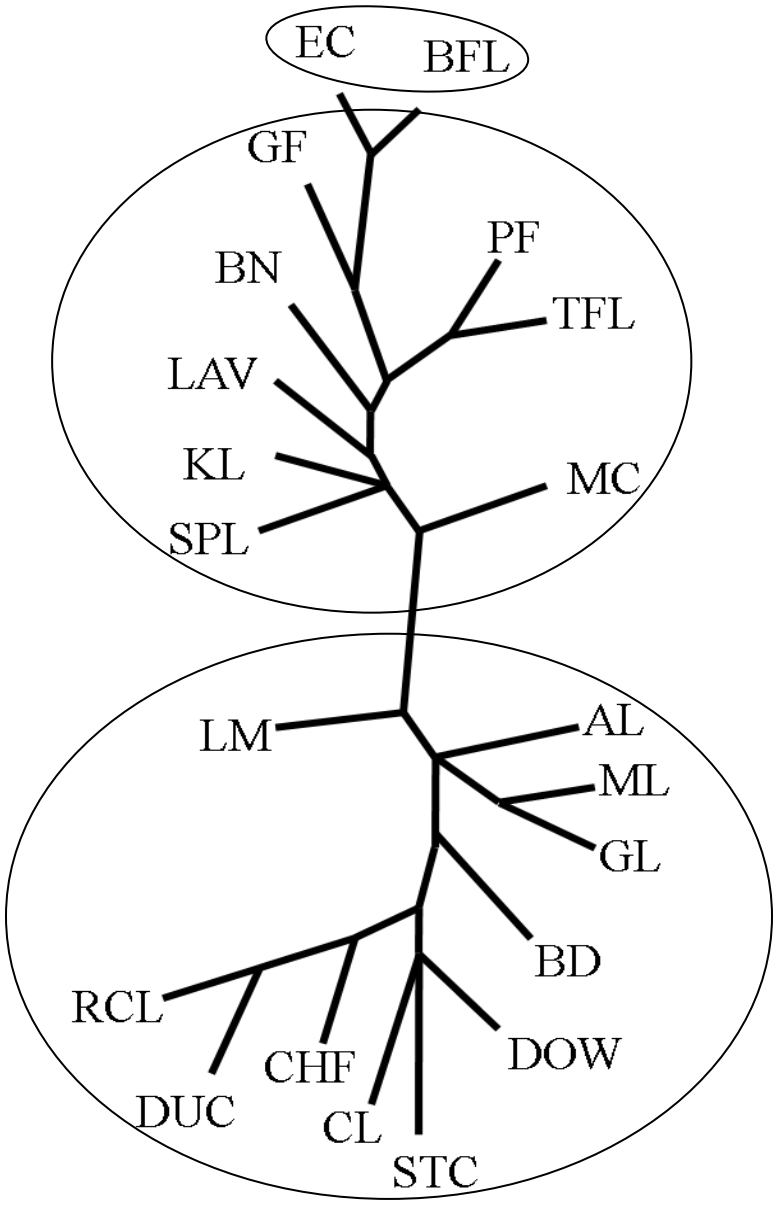

Figure 13. Hypothesis one of the 21 populations: a three group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	125.127	0.06535	1.67
Within Groups (V_b)	181.488	0.06749	1.72

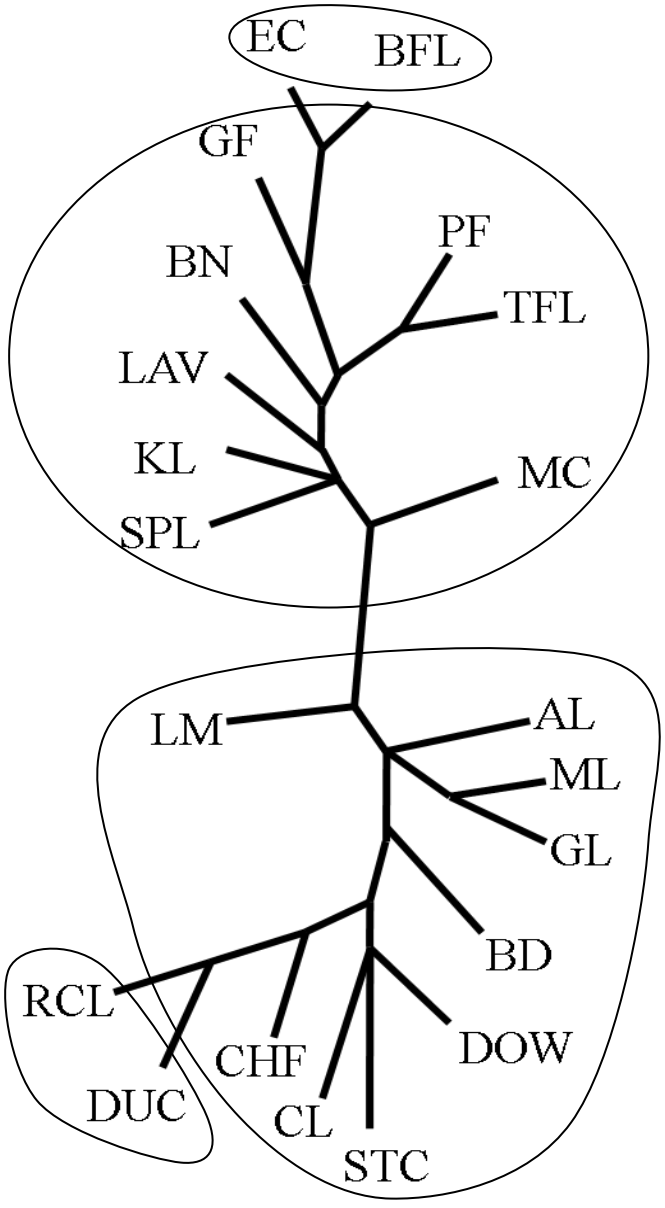

Figure 14. Hypothesis two of the 21 populations: a four group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	189.664	0.08563	2.19
Within Groups (V_b)	116.950	0.04355	1.11

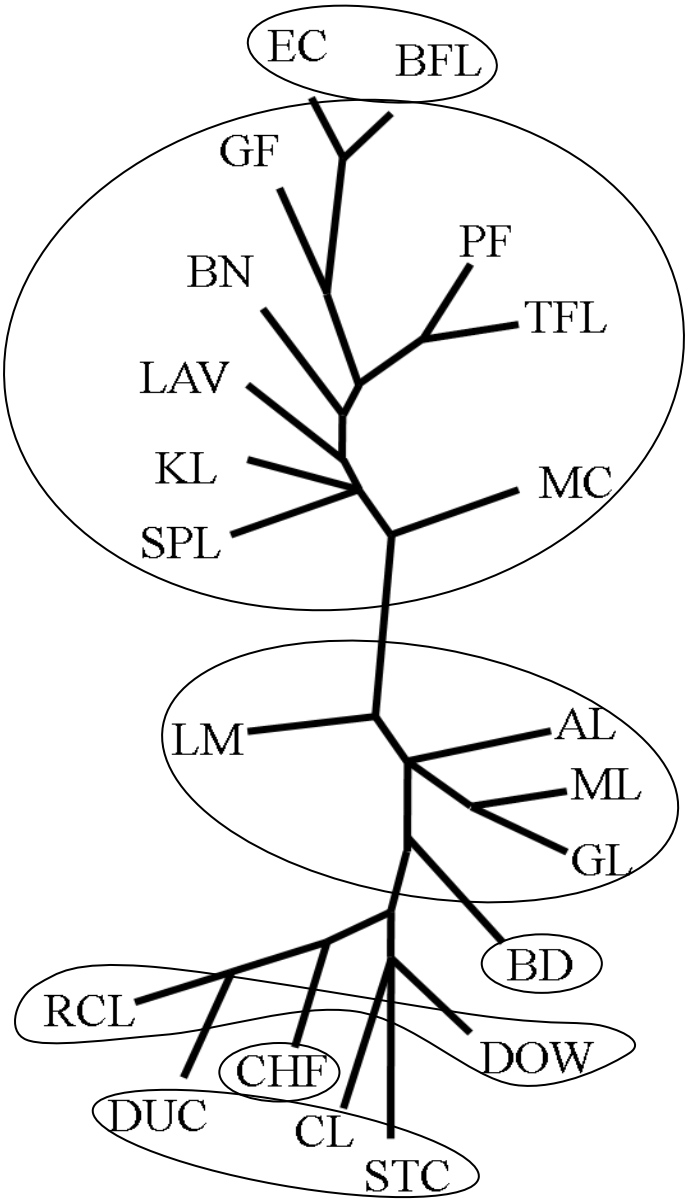

Figure 15. Hypothesis three of the 21 populations: a seven group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	199.825	0.08155	2.09
Within Groups (V_b)	106.789	0.04218	1.08

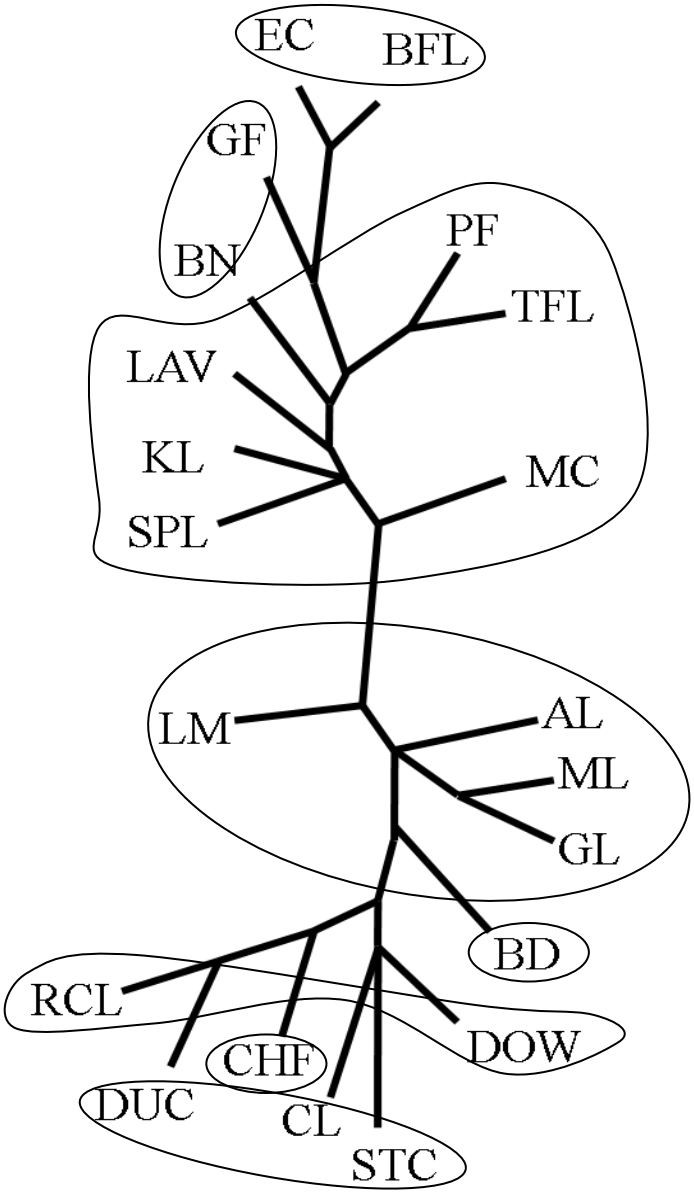

Figure 16. Hypothesis four of the 21 populations: an eight group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	209.898	0.07980	2.05
Within Groups (V_b)	96.717	0.04066	1.04

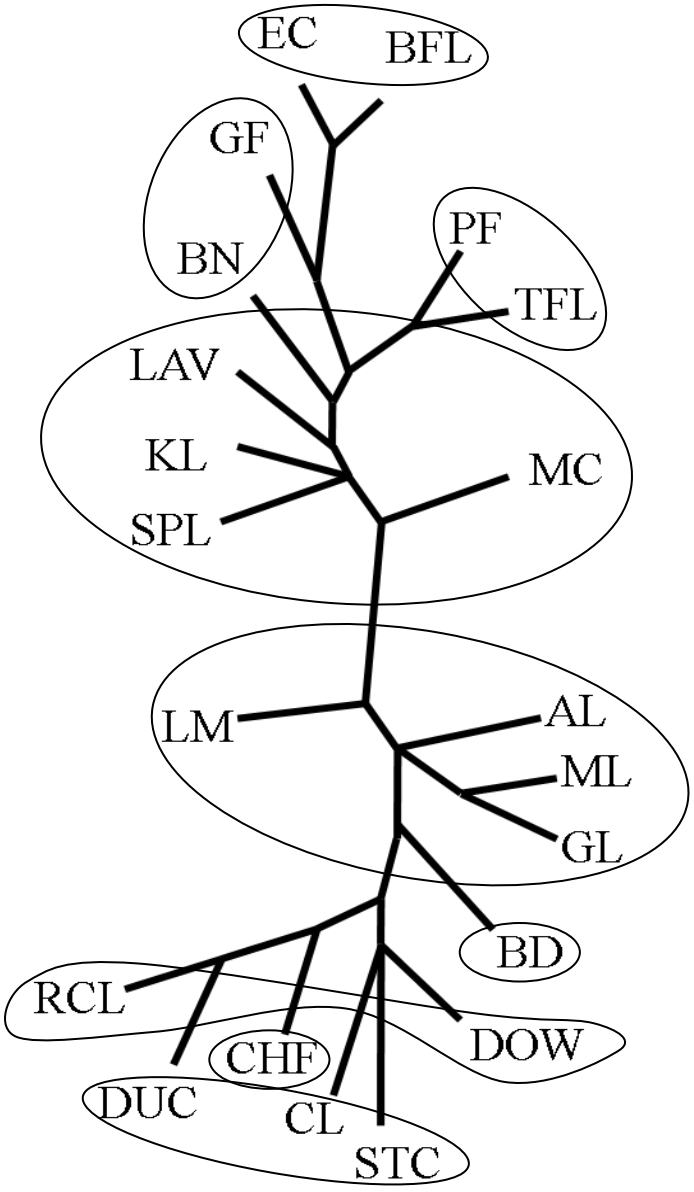

Figure 17. Hypothesis five of the 21 populations: a nine group scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c).

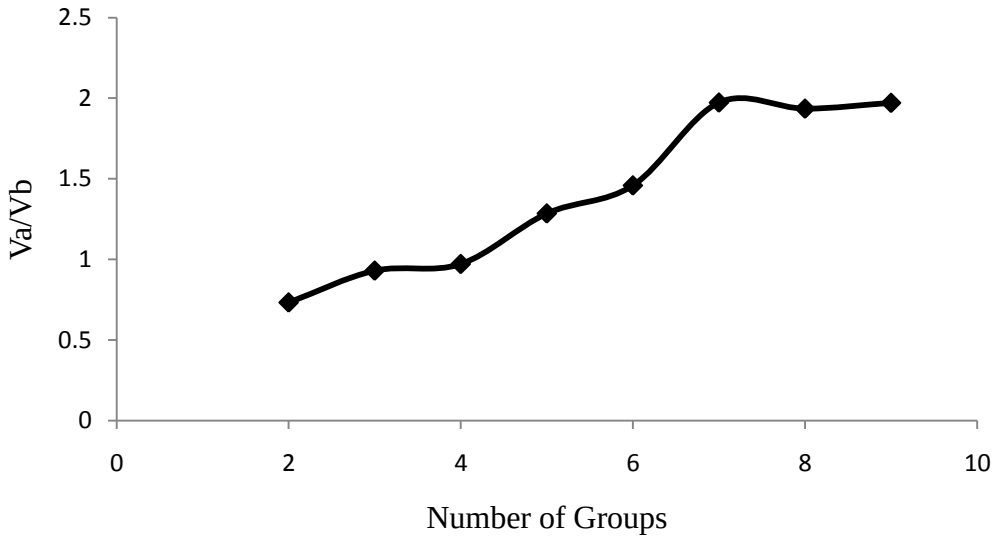

Figure 18. Graph of V_a/V_b vs. number of groups for 21 populations.

	Sum of Squares	Variance of Component	% of Variance
Among Groups (V_a)	121.986	0.02504	0.64
Within Groups (V_b)	280.173	0.10200	2.62

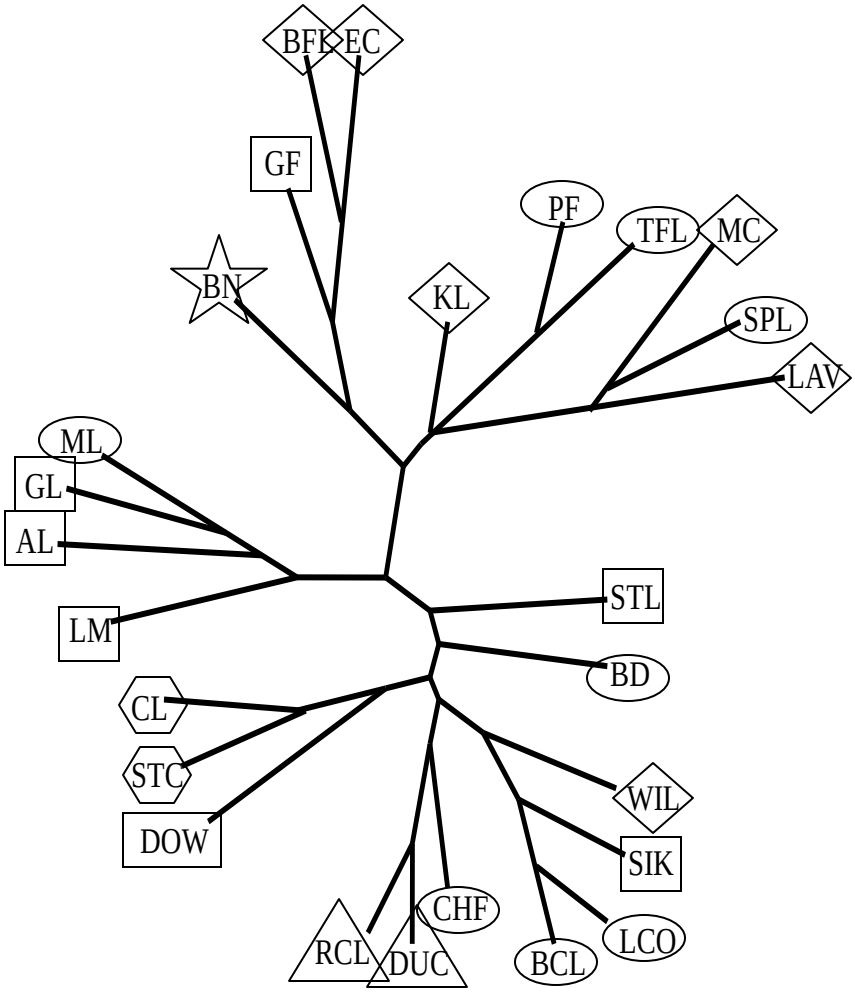

Figure 19. Contemporary genetic units scenario. AMOVA analysis: sum of squares, variance components, and percent of variance in table. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_c). Black triangles represent lower Chippewa management unit, black circles represent upper Chippewa management unit, black star represents Green Bay management unit, black squares represents Superior management unit, black octagon represents St. Croix management unit, and black diamonds squares represent upper Wisconsin River management unit.

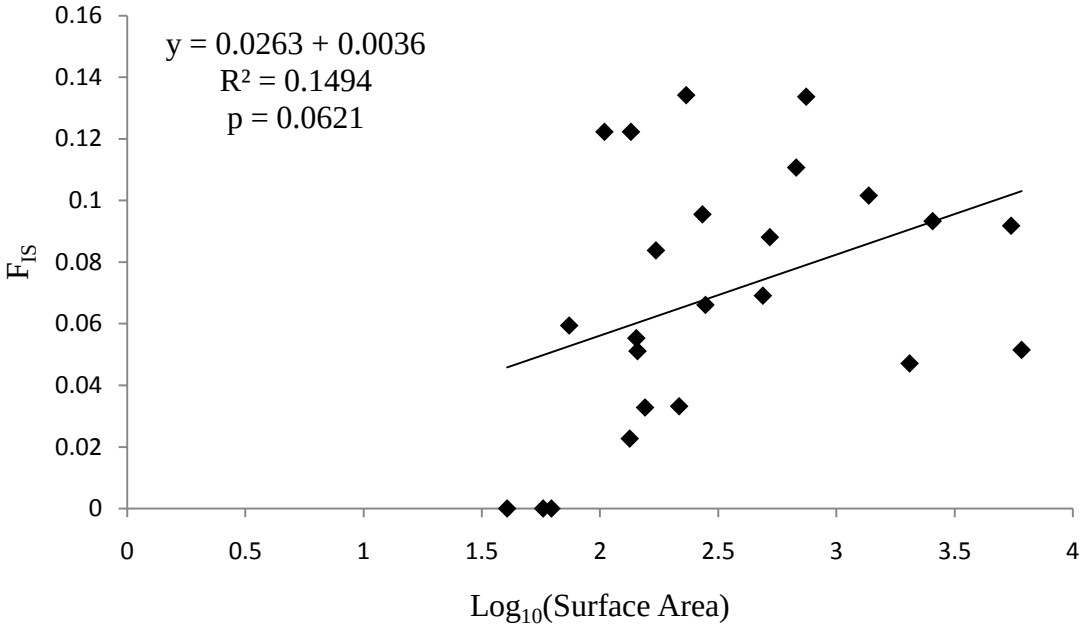

Figure 20. Relation of inbreeding coefficient (F_{IS}) to surface area of sampled populations. Slope, R^2, and p-value shown in top left corner. Diamonds represent sampled populations.

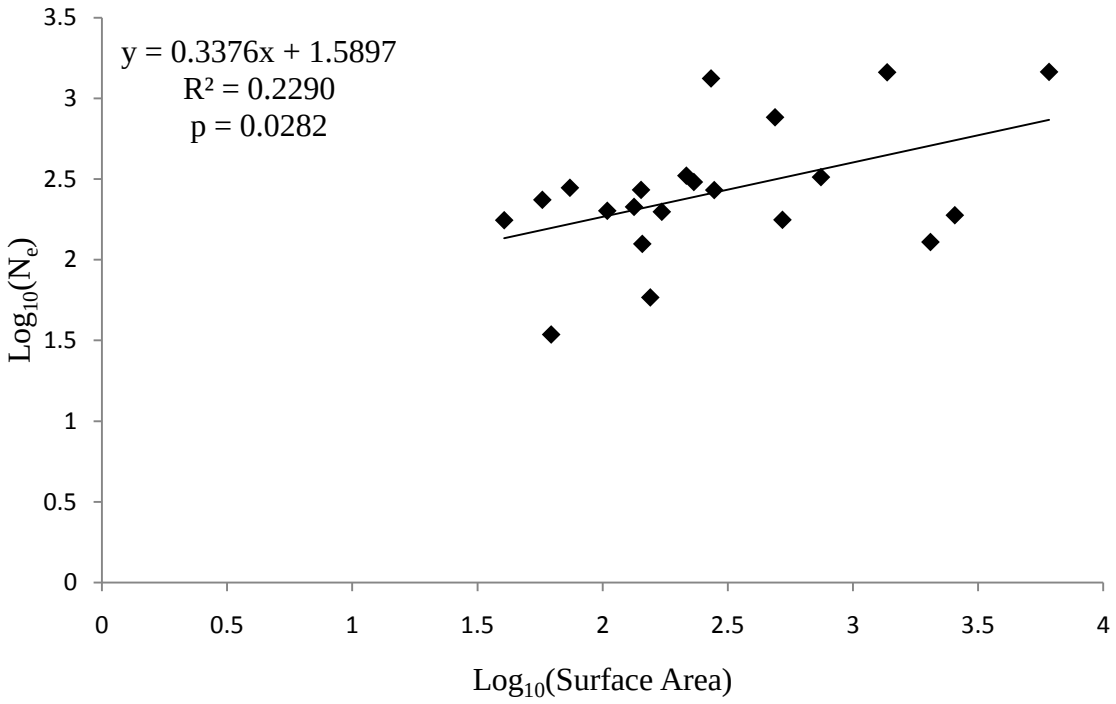

Figure 21. Relation of surface area to effective population size (N_e) of sampled populations. Slope, R^2, and p-value shown in top left corner. Diamonds represent sampled populations.

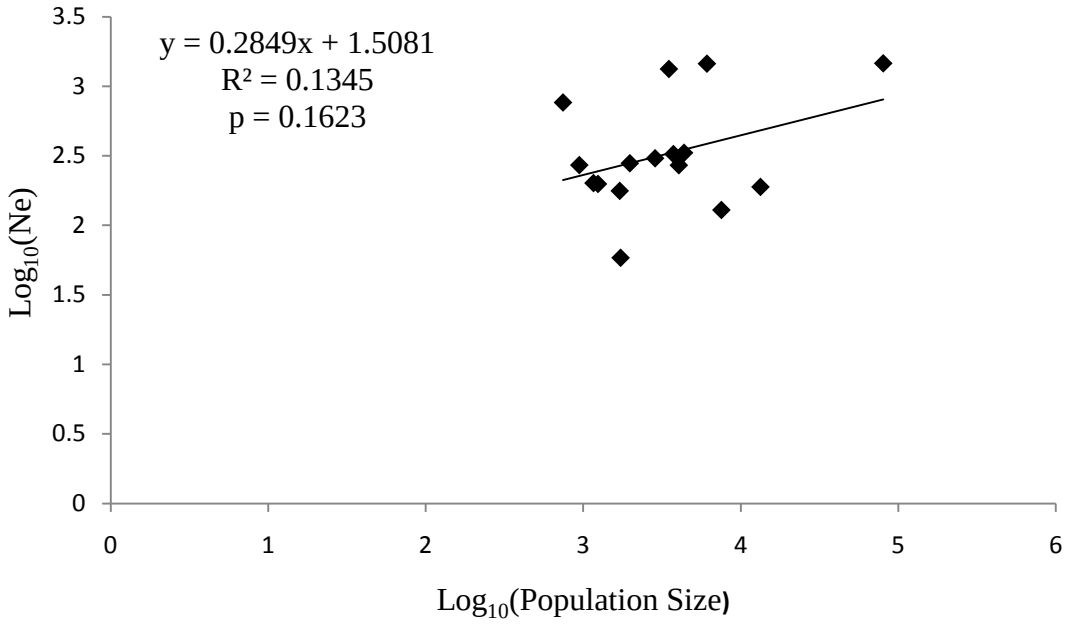

Figure 22. Relation of population size to effective population size (N_e) of sampled populations. Slope, R^2, and p-value shown in top left corner. Diamonds represent sampled populations.

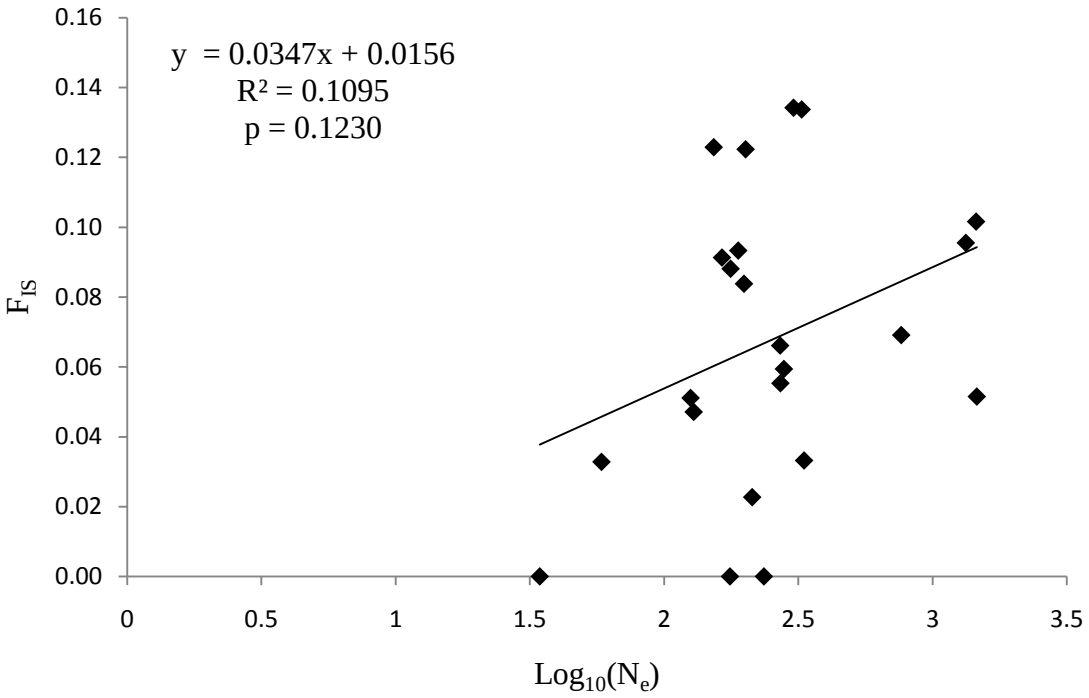

Figure 23. Relation of effective population size (N_e) to inbreeding coefficient (F_{IS}) of sampled populations. Slope, R^2, and p-value shown in top left corner. Diamonds represent sampled populations.

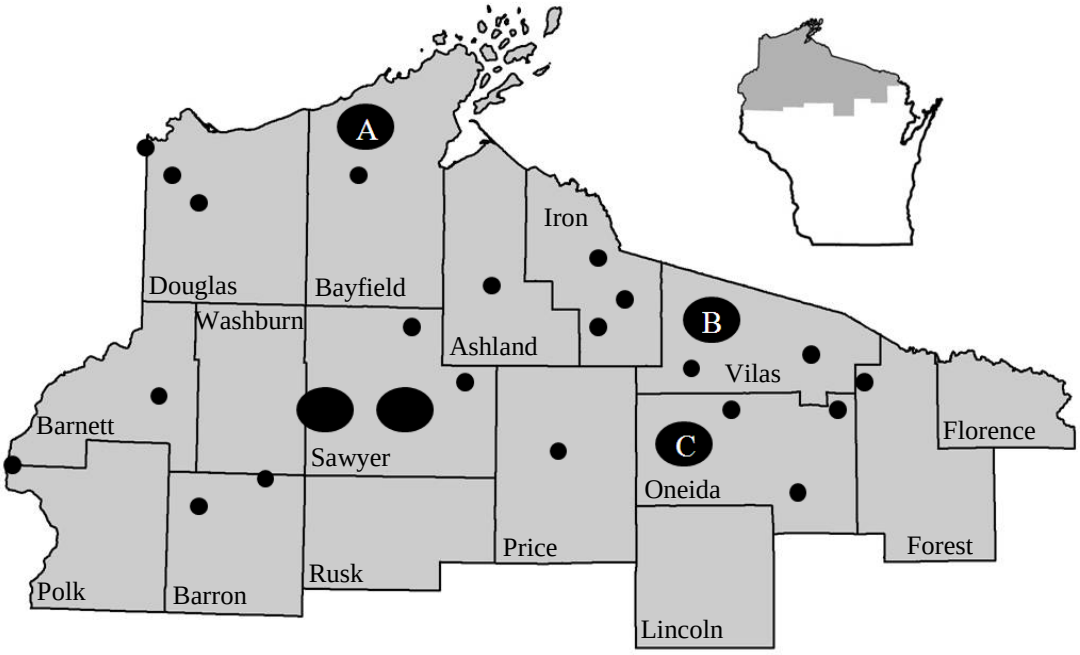

Figure 24. Map of the Ceded Territory of Wisconsin with all sampled populations and key points of disagreement between genetic resolution and geographic proximity. Black dots represent sampled populations. Large dark circles represent populations that genetically grouped together but were inconsistent with geographic location. Siskiwit Lake corresponds to circle A, Big Crooked Lake corresponds to circle B, and Willow Flowage corresponds to circle C.

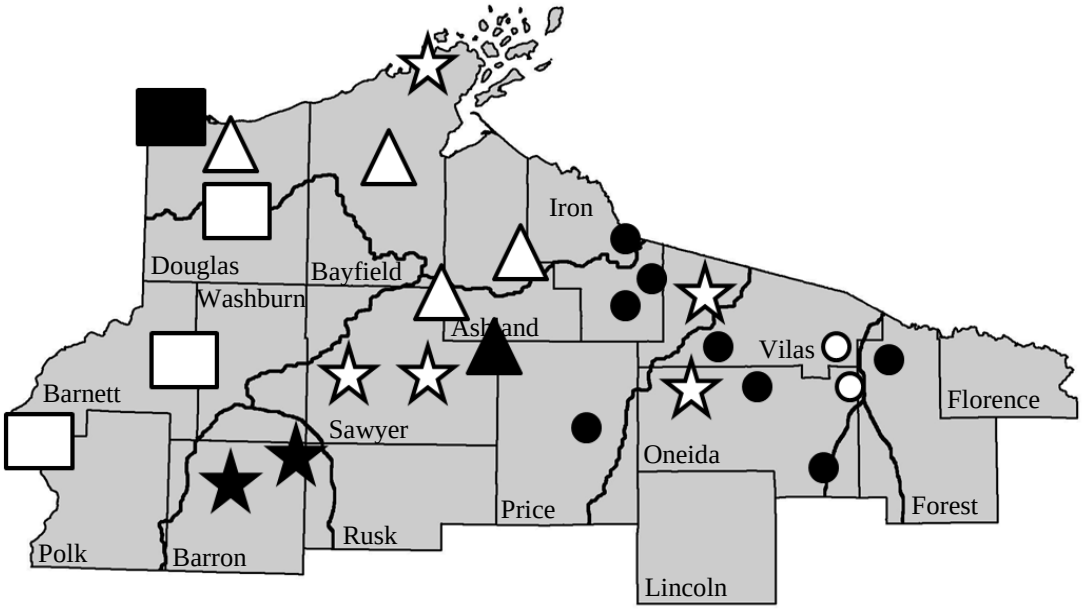

Figure 25. Eight genetic units identified through GSI compared to contemporary genetic units (black lines) in the ceded territory Black squares represents the St. Louis River, white squares represent a St. Croix River genetic unit, black stars represent a lower Chippewa River genetic unit, white stars represent a 'central ceded territory' genetic unit, black triangles represent Blaisdell Lake, white triangles represent a Lake Superior genetic unit, black circles represent an upper Wisconsin genetic unit, and white circles represent a Eagle River genetic unit.

Appendix 1.

Tissue Collection: Field Methods Standard Operating Procedure (SOP)
Molecular Conservation Genetics Laboratory

Items Needed for this Procedure:

Screw Cap Tubes (pre-labeled)	Clipboard	Squeeze Bottle
Tissue Scissors	Data Entry Sheet	95% Ethanol
Forceps	Pencil/Pen	
Tube rack		

Procedure:

1) Organize work space to maximize fish handling efficiency.

2) Collect morphological data (i.e., length, weight, etc…).

3) Before releasing fish collect tissue sample.

4) Cut a "nickel" size piece of fin tissue usually from the caudal or pelvic fin using scissors.

5) Using forceps, place tissue in the labeled screw cap tube (Note: place tissue in tubes in consecutive order beginning with the smallest number to minimize confusion).

6) Fill tubes with ethanol and screw on cap securely to prevent the ethanol from evaporating (Note: if handling a large number of fish, you can wait for a pause in sampling to add ethanol; just make sure the lids are put back on the tubes to prevent mixing tubes and lids).

7) Place tubes in tube rack in sequential order.

8) Record tube number on the data sheet so tissue samples can be matched up with morphological data collected for each individual.

9) Rinse scissors and forceps in water between samples to minimize contamination risk (Note: lake/river water is sufficient; no visible blood, 'slime', or tissue should be present between samples).

10) Label tube box with site specific information (location, date, range of sample numbers, and name of individuals collecting sample).

11) Rinse scissors and forceps in water between samples to minimize contamination risk (Note: lake/river water is sufficient; no visible blood, 'slime', or tissue should be present between samples).

12) Label tube box with site specific information (location, date, range of sample numbers, and name of individuals collecting sample).

Appendix 2.

Polymerase chain reaction (PCR) standard operating procedure

1. Aliquot DNA template into a 96-well PCR tray. Cover DNA template aliquots with sealant strips. Place DNA aliquots on ice or in the freezer until use. Minimizing the number of freeze-thaw cycles will maximize the longevity of your samples
2. Fill Styrofoam cooler with ice from ice-maker located on the fourth floor of the College of Natural Resources (CNR)
3. Turn on thermocycler to allow top heat-plate to reach 105° C before use.
4. Organize bench space (i.e., pipettes, tips, tubes, etc…) to facilitate rapid progression through the following procedure. Good organization limits the potential for human error, one of the primary causes of reaction failure.
5. Label tubes and 96-well PCR trays.
6. Thaw PCR reagents then place on ice until use. Placing reagents on ice maintains their integrity and insures their longevity.
7. Add molecular grade water to 1.5 ml tube. Note: water should always be added first, no exceptions.
8. Add 10x reaction buffer
9. Add dNTPs
10. Add $MgCl_2$
11. Add primer pairs.
12. Add *Taq* polymerase. *Taq* polymerase should be handled with extreme care, minimizing the length of time it is out of the freezer. Before adding *Taq* polymerase make sure to have the necessary materials to complete the following steps quickly. Minimizing the length of time between adding *Taq* polymerase and placing the reaction in the thermocycler will insure consistent results.
13. Vortex PCR cocktail briefly before aliquoting into PCR tray
14. Aliquot PCR reaction cocktail into 96-well PCR tray using a multi-channel pipette. Place 96-well PCR tray on ice before aliquoting reaction cocktail.
15. Add DNA template to the 96-well PCR tray containing the PCR cocktail aliquots. Remember to centrifuge 96-well PCR tray containing the DNA template before removing sealant strips
16. Cover 96-well plate this sealant strips
17. Label the plate with: project code, loci, your name, date, and book/page number
18. Centrifuge 96-well PCR tray containing the PCR reaction cocktail/DNA template mixture
19. Place PCR reaction cocktail/DNA template into the thermocycler. Thermocycler should ALWAYS be allowed to reach denaturation temperature (~ 94°) before placing PCR reaction into thermocylcer (HOT start)
20. Upon completion of amplification PCR reaction should be placed in the refrigerator or freezer until use. PCR reactions may be held in the freezer in excess of 7 days and still produce good gel image

Appendix 3. Stocking records from 1972-2006 for all populations sampled including the year and hatchery source.

Population	Year	Hatchery Source
GL	No Recorded Stocking Events between 1972-2006	
RCL	1973	Maple Plains
	1973	Maple Plains Pond
	1992	NWD Outlying Ponds
	1992	Waterbody Ponds
	1994	NWD Outlying Ponds
	1996	NWD Outlying Ponds
	1996	St.Croix Tribal Hatchery
	1997	Unknown
	1997	Gov. Thompson
	1998	GT-Farlow Pond
	1998	Gt. Leisch Pond
	1999	Gov. Thompson
	2000	Gov. Thompson
	2000	St. Croix Tribal Hatchery
	2002	Gov. Thompson
	2004	Gov. Thompson
	2004	St. Croix Tribal Hatchery
DUC	No Recorded Stocking Events between 1972-2006	
LM	No Recorded Stocking Events between 1972-2006	
STC	No Recorded Stocking Events between 1972-2006	
CL	No Recorded Stocking Events between 1972-2006	
STL	1989	Lund
	1989	Spooner
	1990	Johnecheck#1
	1990	Johnecheck32
	1991	NWD Outlying Ponds
	1991	Spooner Hatchery
	1991	Waterbody name
	1992	NWD Outlying Ponds
	1993	NWD Outlying Ponds

Appendix 3. Continued

Population	Year	Hatchery Source
AL	2002	Gov. Thompson
	2003	Gov. Thompson
	2005	Gov. Thompson
	2006	Gov. Thompson
DOW	No Recorded Stocking Events between 1972-2006	
BN	1988	Private Purchase
	2002	Art Oehmcke
	2003	Art Oehmcke
	2004	Art Oehmcke
	2005	Art Oehmcke
	2006	Art Oehmcke
GF	No Recorded Stocking Events between 1972-2006	
SPL	No Recorded Stocking Events between 1972-2006	
TFL	1991	Spooner Hatchery
	1992	Spooner Hatchery
	1994	Woodruff Hatchery
MC	No Recorded Stocking Events between 1972-2006	
BFL	1973	Ross Lake
	1975	Winding Creek
KL	1974	Unspecified
	1976	Winding Creek
	1987	Woodruff Hatchery
	1987	Woodruff Hatchery
	1988	Woodruff Hatchery
	1992	Woodruff Hatchery
WIL	1975	Unspecified
	1976	Unspecified
	1994	Unspecified
PF	1990	Unspecified
BD	No Recorded Stocking Events between 1972-2006	

Appendix 3. Continued

Population	Year	Hatchery Source
ML	No Recorded Stocking Events between 1972-2006	
CHF	1976	Woodruff
	1977	Spooner
	1978	Spooner
	1979	Spooner
	1980	Spooner
	1981	Spooner
	1982	Spooner
	1984	Spooner
	1985	Spooner
	1986	Spooner
	1987	Spooner
	1988	Spooner
	1989	Spooner
	1990	Spooner
	1991	Spooner
	1992	Spooner
	1995	Art Oehmcke
	2000	Gov. Thompson
EC	1974	Unspecified
	1976	Winding Creek
	1979	Presque isle
LAV	1972	Unspecified
	1973	Unspecified
	1974	Unspecified
	1975	Unspecified
	1976	Unspecified
	1988	Unspecified
	1990	Unspecified
	1997	Unspecified
BCL	1972	Unspecified
	1973	Presque Isle
	1973	Lac Du Flambeau

Appendix 3. Continued.

Population	Year	Hatchery Source
LCO	1972	Unspecified
	1972	Unspecified
	1972	Spooner
	1977	Christenson
	1977	Dahlberg
	1977	Kurtz
	1977	Prose
	1977	Sorenson
	1977	Spooner
	1978	Sand Lake
	1979	Spooner
	1981	Dahlberg
	1981	Ellingson
	1981	Jeschke
	1981	Maple Plains
	1981	P. Martin
	1983	Fisk
	1985	Ellingson
	1985	Torgerson
	1987	Hermstad
	1989	Freeman, South
	1989	Freeman, West
	1989	Morrow
	1989	Radke (Radtke)
	1989	Spooner
	1990	Spooner
	1990	Spooner
	1991	NWD Outlying Ponds
	1991	Spooner Hatchery
	1992	Spooner Hatchery
	1993	NWD Outlying Ponds
	1993	Spooner Hatchery
	1994	Wild Rose
	1995	Art Oehmcke
	1996	Gov. Thompson
	1997	Gov. Thompson
	1997	Gov. Thompson
	1998	Gov. Thompson
	1999	Gov. Thompson
	1999	Gov. Thompson

Appendix 3. Continued.

Population	Year	Hatchery Source
LCO	2000	Gov. Thompson
	2001	Gov. Thompson
	2001	Gov. Thompson
	2002	Gov. Thompson
	2002	St. Croix Tribal Hatchery
	2003	Gov. Thompson
	2003	Gov. Thompson
	2003	AO-Presque isle
	2003	GT-Farlow
	2004	Gov. Thompson
SIK	No Recorded Stocking Events between 1972-2006	