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Cisco Population Genetic Structure in Minnesota

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Abstract – The Cisco *Coregonus artedi* is the most common coldwater stenotherm in Minnesota lakes but populations have been declining, likely due to eutrophication and climate change. Understanding the distribution of genetic diversity across Minnesota Cisco populations can help identify populations that would best maintain a portfolio of diversity, identify populations potentially at risk from low diversity and guide choice of donor populations if stocking is considered. This study examined genetic diversity within and among (i.e., spatial genetic structure) 40 samples from 37 Cisco populations using eight microsatellite DNA loci. Measures of genetic diversity, heterozygosity and allelic richness, were often low within populations from west-central Minnesota but also for some populations throughout the state. Multiple approaches (Fst and STRUCTURE analyses, principal coordinates analysis, and tree diagrams) indicated distinct populations persist across the state. Lake Superior and three populations introduced from Superior were clearly distinct from inland populations. Inland populations loosely grouped according to major drainages. Several individual populations were clearly distinct, but this may have been caused by small population size and rapid divergence through random genetic drift. Populations with low genetic diversity could be candidates for "genetic rescue" via translocations from other populations; however, a conservative approach would use local source populations for translocations to maintain regional, drainage-associated genetic structure among populations. Further studies with higher-resolution genomic markers are recommended to better resolve population relationships and assess possible impacts of past stocking.

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INTRODUCTION

The Cisco *Coregonus artedi* is widespread across northern North America and is the most common coldwater stenotherm in Minnesota lakes (Jacobson et al. 2012). Ciscoes are valued for human consumption and as important prey items for several other desired game species (Kaufman et al. 2009; Kennedy et al. 2018; VanderBloemen et al. 2020). Cisco populations have been declining in Minnesota, likely due to eutrophication and climate change (Jacobson et al. 2012), and are predicted to be extirpated from 30-70% of Wisconsin lakes by 2100 (Sharma et al 2011). Conservation efforts have focussed on maintaining coldwater habitat in refuge lakes deemed most resilient to system change (Fang et al. 2012; Jacobson et al. 2013). In some cases, introductory or supplemental stocking of Cisco has been proposed. Understanding the distribution of genetic diversity across Minnesota Cisco populations can help identify populations that would best maintain a portfolio of diversity, identify populations potentially at risk from low diversity and guide choice of donor populations if stocking is considered.

The study of North American Ciscoes is complicated by the high ecological and phenotypic diversity across their range (Turgeon et al. 2016; Eshenroder & Jacobson, 2020). Four extant species have been recognized in Lake Superior (Eshenroder et al., 2016). Most inland lakes in Minnesota have one morphotype *(C. artedi)* (Eddy and Underhill, 1974), while two others are occasionally found sympatrically with *C. artedi: C. zenithicus* and *C. nipigon* (Etnier et al., 2003). Turgeon et al. (2016) concluded that the Shortjaw Cisco *(C. zenithicus)* evolved independently from *C. artedi* in different lakes rather than represent a “Good Species,” i.e, *C. zenithicus* are more closely related to *C. artedi* within the same lake than they are to *C. zenithicus* in other lakes. Together, *C. artedi* displayed distinct western and eastern races (lineages), corresponding to Mississippi and Atlantic glacial refugia, with secondary mixing of lineages across much of north-central North America (Turgeon and Bernatchez 2001 a,b). As is common, finer-scale genetic population structure was observed among populations beyond that associated with the major races.

Populations will be similar based on shared ancestry, and conversely, differ as isolation (i.e., lack of gene flow) and time allow for genetic divergence. Spatial genetic structure of fish population is expected to relate to watersheds, as they are the pathway to population connectivity and gene flow. Contemporary watersheds may not reflect shared ancestry if post-glacial connectivity differed from the present; however, stocking is the most direct human impact on genetic structure of fish populations because it can immediately transcend historical boundaries. Stocking is often a confounding factor in assessing patterns of genetic diversity within and among fish populations (Miller et al. 2012, Turnquist et al. 2017).

This study examined genetic diversity within and among (i.e., spatial genetic structure) 37 Cisco populations in Minnesota. Populations with low genetic diversity, which may result from population bottlenecks driven by limited suitable aquatic habitat (Grow et al. In revision), may be susceptible to fitness-reducing inbreeding depression (Frankham 2015). Spatial patterns of genetic diversity were examined in relation to contemporary watersheds (mostly Hudson Bay and Mississippi drainages) and for evidence of stocking impacts. Genetic data can help guide management, especially in relation to stocking or translocations as a tool to maintain or enhance Cisco populations.

METHODS

Population sampling

Cisco used in this study were sampled as part of broader studies examing oxythermal habitat relationships of native (Jacobson et al. 2020; Grow et al. In revision) and introduced Cisco populations (Jacobson et al. 2018). This analysis includes 32 samples from Grow et al. and an additional eight samples not included in any of the earlier studies. The 40 samples came from 37 lakes; three lakes had separate samples from distinct basins (Basswood, Leech and Lake of the Woods – Traverse Bays). Samples were collected once per lake in July or August from 2013 to 2015. Cisco were collected in the field primarily with vertical gill nets used by the Minnesota Department of Natural Resources to sample pelagic fish in deep lakes (Jacobson *et al.*, 2018). The nets consisted of seven 61 m deep panels of monofilament webbing (bar-measure mesh size x panel width): 10 mm x 0.9 m, 13 mm x 0.9 m, 19 mm x 1.2 m, 25 mm x 1.8 m, 32 mm x 3.0 m, 38 mm x 3.0 m, 44 mm x 3.0 m). The 10 mm and 13 mm panels were sewn together vertically into one net, as were the 19 mm and 25 mm panels, while the 32 mm, 38 mm, and 44 mm panels were used individually for a total of five separate vertical nets. Each net was ganged together by a 2 m connecting rope and the gang (5 nets with 7 panels) was set as a unit in the deepest basins of a lake. One to three gangs per lake were set in deep basins depending on lake complexity. Each net was deployed to sample the entire water column and was fished for 24 hrs. Leech Lake and Lake of the Woods - Traverse Bay samples were collected during standard Minnesota DNR large lake survey gillnetting surveys and Lake Superior samples were collected during standard Minnesota DNR fall assessments with floating gill nets. Fin clips from sampled fish were collected and preserved in 100% ethanol for genetic analysis.

Genetic analysis

Cisco were genotyped at eight microsatellite DNA loci to measure population genetic diversity and assess population structure. All genotyping was conducted at the J. Cruise laboratory at the University of St. Thomas (St. Paul, MN). Genotyping methods were as described in Jacobson *et al.* (2018) except that two loci (ClaTet12 and ClaTet15) were removed because they had excessive deviations from Hardy-Weinburg equilibrium in larger sample collections. Briefly, DNA was extracted from 5 to 10 mg fin tissue using Qiagen Plant DNeasy Mini purification kits. Extracted DNA was amplified via polymerase chain reaction (PCR) using M13-tailed primers and fluorescent dye incorporation, as described by Shimizu et al. (2002). Primer sequences, reaction conditions, and microsatellite characteristics are summarized in Appendix 1. PCR products were submitted to the University of Minnesota Genomics Center for capillary electrophoresis on an Applied Biosystems 3730xl Genetic Analyzer. Internal size standards were used to determine PCR fragment lengths (allele sizes) of all fluorescent products, using PeakScanner 2™ software (Applied Biosystems).

For each sample, expected (H_E) and observed (H_O) heterozygosities were obtained using GENALEX 6.5 (Peakall and Smouse 2012, 2016). GENALEX was also used to test for deviations from Hardy-Weinberg equilibrium followed by a correction for multiple testing with a false discovery rate of 5% (Benjamini & Hochberg, 1995). Allelic richness (A_r) was calculated using HP-Rare (Kalinowski, 2005), with a sample size of 16 alleles (the minimum sample size included in this analysis; i.e., twice the number of fish genotyped from St. Mary's Lake). This rarefaction method standardizes allelic diversity to a common sample size to allow comparisons among unequally sampled populations.

Several approaches assessed population genetic structure (i.e., genetic relationships) among populations. Pairwise FST values, a measure of variance in allele frequencies, were calculated using GENALEX. The genetic composition of populations was further assessed in STRUCTURE (Pritchard et al. 2000). STRUCTURE uses a Bayesian clustering approach to identify the possible number of genetically-distinct population clusters (*K*) that, approximately, minimize Hardy-Weinberg and linkage disequilibrium in the data. Then, it estimates the proportion of alleles of each individual originating from each population. Ten replicate analyses for all numbers of distinct genetic clusters (K) from K = 1 to K = 20 were performed using an admixture model with correlated allele frequencies, a burn in length of 50,000 steps and a run length of 200,000 steps after burn in. The best supported K value(s) were determined using the method of Evanno et al. (2005). STRUCTURE analysis identifies distinct populations but does not indicate the degree of difference, whereas principal coordinate analysis (PCoA) reveals genetic relationships based on dissimilarity of allele frequencies. PCoA reduces the complexity of variation across multiple variables/ dimensions (here, allele frequencies) into axes that best resolve differences among samples. PCoA was first run in GENALEX for all the samples and then with the clearly resolved group of Lake Superior and introduced populations removed to examine inland populations with finer resolution. Finally, a neighbor-joining tree of genetic relationships among populations based on Cavalli-Svorsa chord distances (Cavalli-Sforsa and Edwards 1967) was constructed using the neighbor joining method of Saitou and Nei (1987). Tree construction was performed using FIGTREE (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Our final genetic data set consisted of 40 samples from 37 lakes (three lakes had separate samples from distinct basins: Basswood, Leech and Lake of the Woods - Traverse Bay) with an average of 25 individuals per sample (range 4 to 70; Clearwater-South with N=4 was removed from some of the subsequent analyses; Table 1, Figure 1). Significant deviations from Hardy-Weinberg equilibrium were indicated for 29 of 298 tests. Deviations were not associated with specific samples or loci, which would have indicated possible population or genotyping anomalies, so all data were deemed suitable for assessing diversity measures.

Within population genetic diversity

Measures of genetic diversity varied widely across populations (H_E: 0.39-0.80; H_O: 0.41-0.80; A_r: 3.1-8.1; Table 1). The relative ranking of populations was generally consistent for all measures (Figure 2). For example, Middle Spunk had the lowest diversity for all three measures and six populations were in the lowest quartile for all measures. Populations in the Mississippi drainage had lower diversity than those of the Hudson Bay drainage, but the differences were not statistically significant (Mann-Whitney U tests: A_R, U=94, p=0.08; H_E, U=99.5, p=0.11). Within the Mississippi drainage, however, disjointed populations to southwest had significantly lower genetic diversity than those to the north and east (Mann-Whitney U tests: A_R, U=17, H_E, U=16, both p=0.01).

TABLE 1. Genetic diversity of Minnesota Cisco populations based on eight microsatellite loci: observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}) and allelic richness standardized to a sample size of eight individuals (AR_8).

Pop ID	Population	Drainage	N	H_{obs}	H_{exp}	AR_8
1	Superior	Superior	20	0.71	0.72	7.8
2	Greenwood	Introduced	45	0.74	0.74	7.1
3	Loon	Introduced	50	0.76	0.76	7.4
4	Clearwater	Introduced	11	0.75	0.71	6.1
5	StMarys	Superior	8	0.80	0.74	5.8
6	Ojibwe	Hudson Bay	20	0.58	0.61	5.7
7	Moose	Hudson Bay	20	0.73	0.77	6.9
8	Newfound	Hudson Bay	15	0.73	0.74	6.3
9	Snowbank	Hudson Bay	20	0.74	0.78	8.1
10	Sucker	Hudson Bay	11	0.70	0.74	7.2
11	Basswood-E	Hudson Bay	25	0.73	0.78	7.4
12	Basswood-W	Hudson Bay	34	0.76	0.77	7.3
13	BearIsland	Hudson Bay	20	0.76	0.76	7.6
14	Farm	Hudson Bay	18	0.65	0.68	6.4
15	Fall	Hudson Bay	15	0.66	0.73	6.7
16	Shagawa	Hudson Bay	16	0.74	0.75	6.3
17	Trout	Hudson Bay	30	0.77	0.78	8.0
18	LOW-LTraverse	Hudson Bay	20	0.80	0.75	7.0
19	LOW-BTraverse	Hudson Bay	20	0.73	0.76	7.7
20	Elk	Mississippi-N	19	0.71	0.72	6.0
21	TenMile	Mississippi-N	20	0.73	0.77	7.6
22	Lasalle	Mississippi-N	20	0.66	0.77	6.7
23	Leech-West	Mississippi-N	20	0.65	0.76	7.0
24	Leech-Main	Mississippi-N	20	0.72	0.70	7.2
25	Kabekona	Mississippi-N	28	0.76	0.80	7.6
26	BigSand	Mississippi-N	18	0.73	0.77	6.8
27	Blue	Mississippi-N	20	0.67	0.68	6.9
28	Prairie	Mississippi-N	13	0.63	0.55	4.5
29	Long	Mississippi-N	49	0.75	0.75	6.9
30	Round	Mississippi-N	20	0.80	0.78	7.6
31	Cedar	Mississippi-N	47	0.74	0.76	7.5
32	Osakis	Mississippi-WC	21	0.64	0.70	6.4
33	Rachel	Mississippi-WC	17	0.65	0.63	5.4
34	Burgen	Mississippi-WC	23	0.72	0.70	6.3
35	Carlos	Mississippi-WC	29	0.72	0.75	7.0
36	MiddleSpunk	Mississippi-WC	20	0.41	0.39	3.1
37	Koronis	Mississippi-WC	42	0.52	0.55	4.7
38	Green	Mississippi-WC	70	0.73	0.73	6.8
39	WestSylvia	Mississippi-WC	17	0.69	0.67	5.0
40	ClearwaterS	Mississippi-WC	4	0.53	0.62	3.9

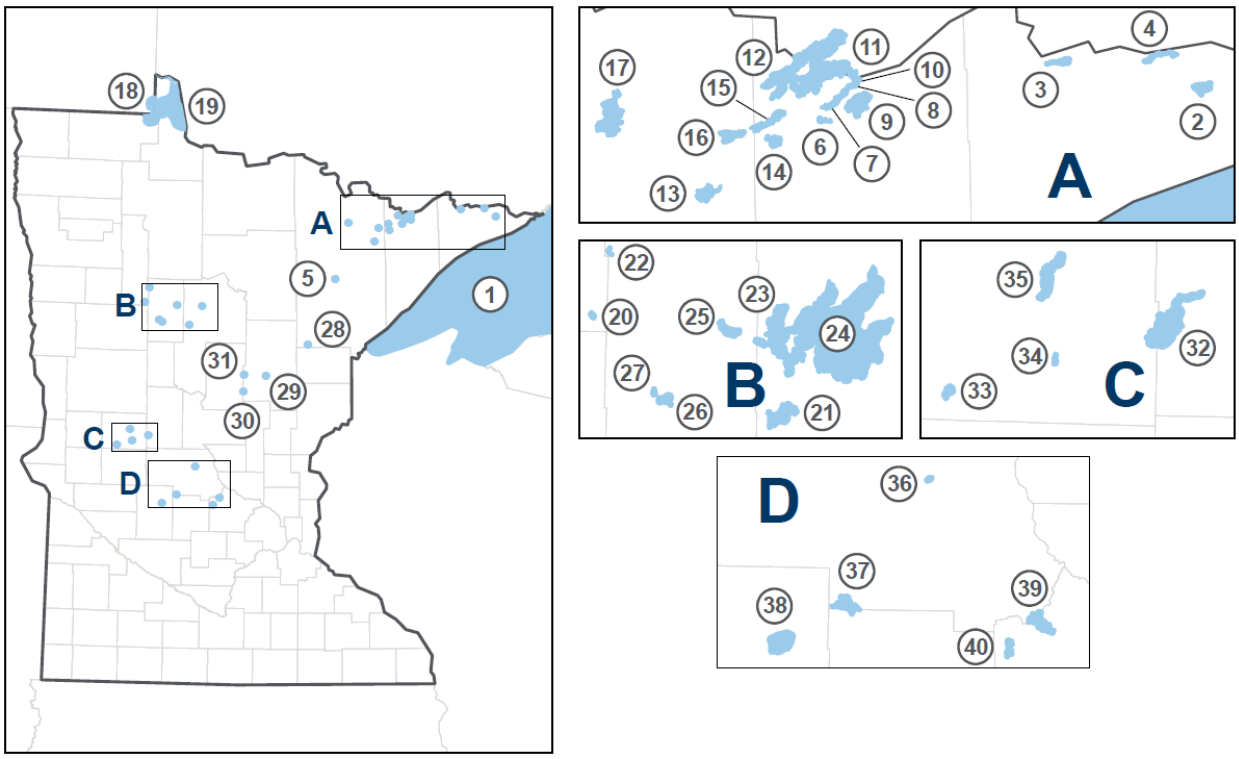

FIGURE 1. Map of Minnesota Cisco populations assessed for genetic diversity. Lake names associated with Populations IDs are listed in Table 1.

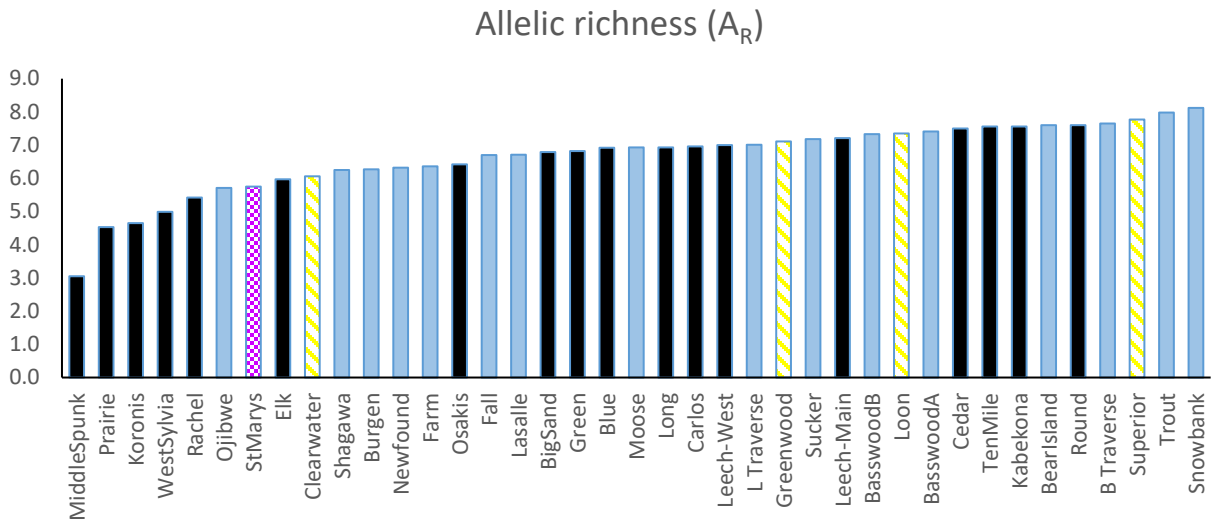

FIGURE 2. Minnesota Cisco populations displayed in increasing order of allelic richness. Colors on bars indicated drainages: solid blue – Hudson Bay drainage, black – Mississippi drainage, stippled purple – Lake Superior drainage, cross-hatched yellow – Lake Superior and inland populations founded from Lake Superior sources.

Population genetic structure

Significant genetic structure was evident between most samples, indicating that limited gene flow and resulting genetic drift is common among populations. All but 10 of 741 pairwise comparisons indicated significant variance in allele frequencies between populations as measured by F_{ST} (Appendix 2). Six of the non-significant comparisons were between basin samples within lakes or lakes in the same drainage, so a lack of strong isolation and subsequent drift is unsurprising. Four non-significant comparisons involved lakes from the Hudson Bay and Mississippi drainages, which likely reflected low power and sampling error.

Bayesian clustering analysis in STRUCTURE provided the highest support for just two ancestral groups (K=2) that were not informative about overall population differences. Low values of K produced by the Evanno method are common when multiple levels of hierarchical structure are present (Evanno at el. 2005). The next best support was for nine distinct ancestral groups (K=9) with various associations with individual samples, geographic clusters or stocking histories. First, Lake Superior and three populations founded from Lake Superior sources (Clearwater, Greenwood and Loon) showed a strong similarity (i.e., mostly the same color – yellow; Figure 3) and were distinct from all other inland populations, as was shown previously with fewer inland samples (Jacobson et al. 2018). Next, in several cases, single populations or pairs of populations formed distinct clusters (Ojibwe-Prairie, Elk, Middle Spunk, Koronis, and Green-West Sylvia). At the next-best supported K=12, the pairs Ojibwe-Prairie and Green-West Sylvia further resolved into their own groups (Figure 3, lower). Finally, two groups of geographically nearby populations were predominantly composed of one ancestral group (Farm-Fall-Shagaw, brown in Figure 3, upper; Osakis-Rachel-Bergen-Carlos, dark blue in Figure 3, upper). The remaining populations had poorly-resolved mixtures of multiple ancestral groups.

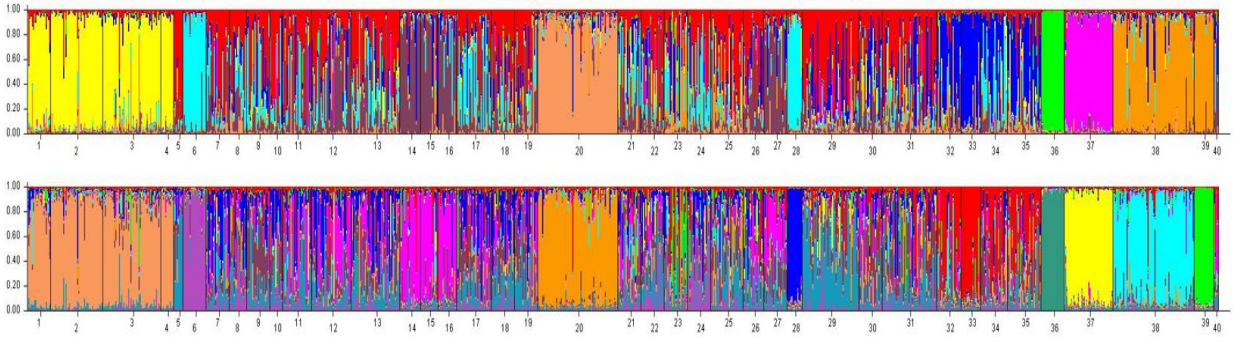

FIGURE 3. Genetic structure of Minnesota Cisco populations revealed by Bayesian clustering analysis in the program STRUCTURE (Pritchard et al. 2000). Colors indicate (upper) the 9 distinct clusters, i.e., ancestral groups, best supported by the analysis (after the uninformative K=2) and (lower) the 12 distinct clusters that had moderate support in the analysis. Note that colors show contrasts within graph and are not related across graphs. Vertical lines represent individuals with colors representing proportions of ancestry estimated to derive from each ancestral group. Multiple colors for an individual may indicate ancestry derived from multiples sources (e.g., resulting from stocking) or result from incomplete resolution of ancestral groups (low assignment power). Sample locations are indicated by numbers in Table 1.

The PCoA analysis is a way to show how populations related to each other, not just which ones differ as in STRUCTURE. The PCoA of all samples clearly grouped Superior and introduced populations with each other and separate from all other inland samples (Figure 4). Among inland populations, Hudson Bay and Mississippi drainage populations tended to separate left-to-right along the first axis, but with considerable overlap. Because PCoA considers all data simultaneously, removing samples can change the relative position of the remaining samples.

The analysis without Superior populations showed some of this effect, but most patterns were similar (Figure 5). A trend of Hudson Bay and Mississippi drainage populations separating along axis 1 remained. Some groupings of geographically close populations were evident, including the interconnected lakes Moose, Newfound, Sucker and Basswood in the Hudson Bay drainage and Green, Burgen, Osakis and Rachel in west-central Minnesota. Middle Spunk and Ojibwe were consistent outliers in both analyses.

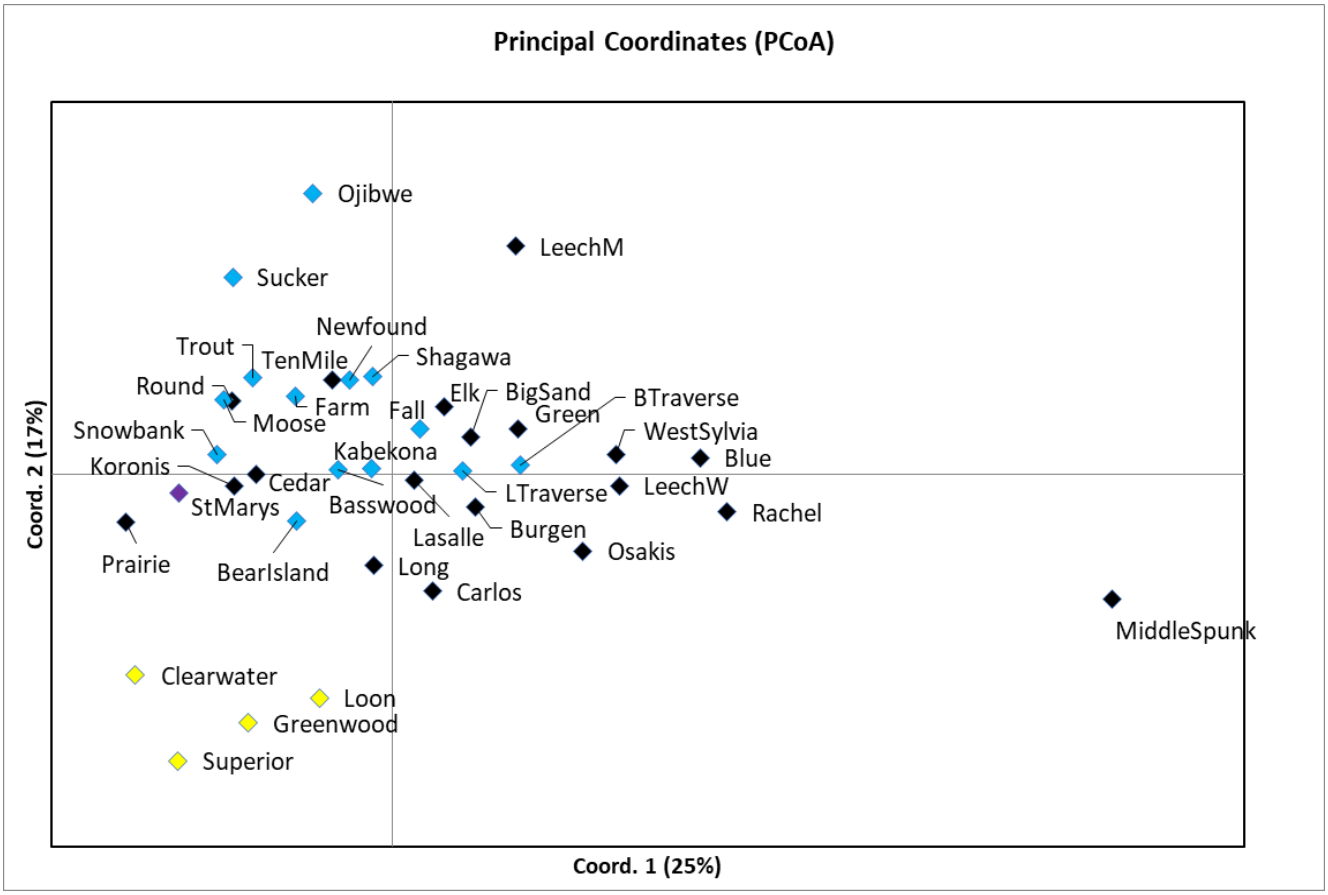

FIGURE 4. Population structure of Minnesota Cisco populations assessed by a principal coordinate analysis (PCoA). Individual scores on PCoA axes 1 and 2 (percentage of genetic variance explained in parentheses) are presented as points and colored by region: blue – Hudson Bay drainage, black – Mississippi drainage, purple – Lake Superior drainage, yellow – Lake Superior and inland populations founded from Lake Superior sources. Basswood east and west basin samples were combined.

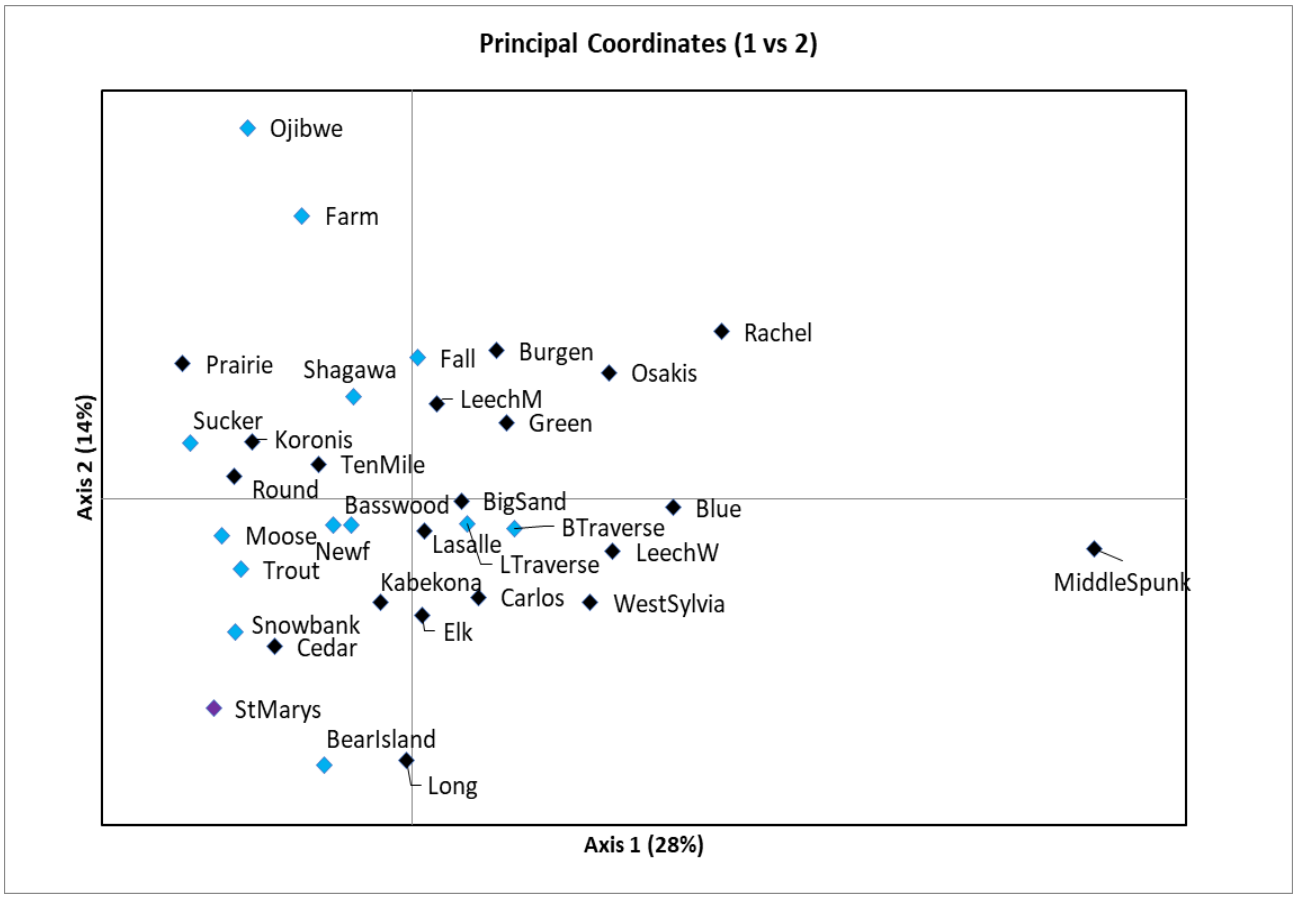

FIGURE 5. Population structure of Minnesota Cisco populations assessed by a principal coordinate analysis (PCoA) with Lake Superior and introduced populations removed. Individual scores on PCoA axes 1 and 2 (percentage of genetic variance explained in parentheses) are presented as points and colored by region: blue – Hudson Bay drainage, black – Mississippi drainage, purple – Lake Superior drainage. Basswood east and west basin samples were combined.

The genetic tree revealed patterns of relationships among populations similar to those of PCoA and STRUCTURE (Figure 6). Superior and introduced populations were on a distinct branch. Other main branches tended to group Hudson Bay or Mississippi drainage populations but some populations were interspersed across drainage groups. Branch lengths are proportional to genetic distances and long branches typically occurred for populations that were outliers in PCoA or distinctive in STRUCTURE.

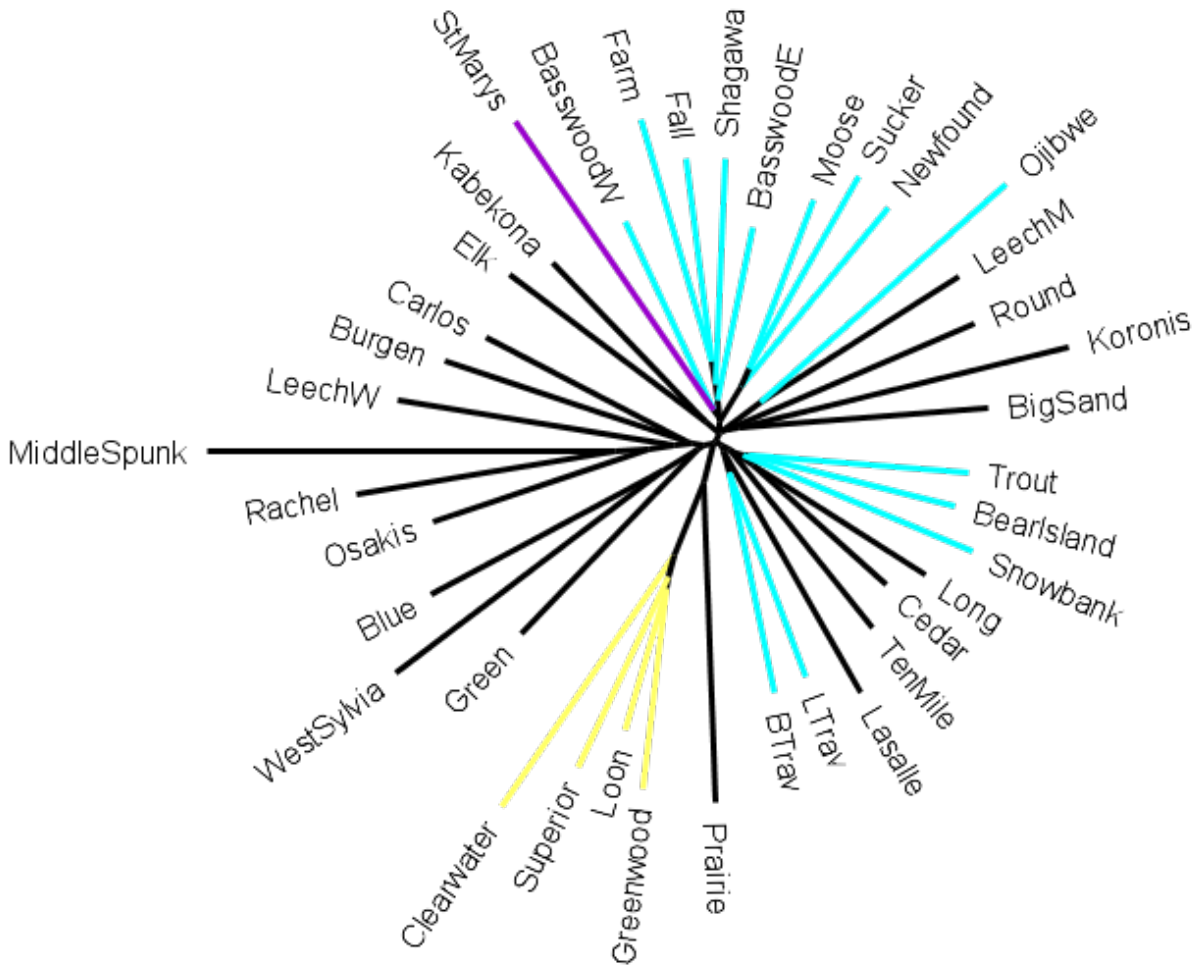

FIGURE 6. Neighbor-joining tree diagram of genetic relationships among Minnesota Cisco populations based on chord distances using eight microsatellite loci. Colored lines indicate geographic groupings of populations: blue – Hudson Bay drainage, black – Mississippi drainage, purple – Lake Superior drainage, yellow – Lake Superior and inland populations.

DISCUSSION

Groups of genetically similar populations suggest connectivity and shared history. There is a slight separation of Hudson and Mississippi populations in the PCoAs, suggesting that structure is at least loosely associated with contemporary major drainages, as for walleye *Sander vitreus* (Bootsma et al. 2021) and Muskellunge *Esox masquinongy* (Turnquist et al. 2017). In addition, several branches of the tree diagram group geographic clusters of populations within drainages: Carlos-Burgen-Middle Spunk-Rachel-Osakis, Shagawa-Farm-Fall-Basswood, and Moose-Sucker-NewFound. The latter two are connected lake groups within the Boundary Waters. The measure of differentiation F_{ST} is low, and sometimes non-significant, between populations in these groups, indicating that contemporary gene flow and/or maintenance of high population sizes has limited divergence among these populations.

In contrast to population groups, the distinctiveness of many individual populations in STRUCTURE or PCoA analyses (Ojibwe, Prairie, Elk, Middle Spunk, Koronis, Green, and West Sylvia) likely reflects isolation and bottlenecks leading to relatively strong divergence due to rapid genetic drift. With the exception of Green, these populations all fall in the bottom quartile for one or more of the genetic diversity measures. Importantly, divergence due to rapid drift in small populations does not necessarily imply long isolation and may obscure historical population connectivity and shared colonization history. A good example is Middle Spunk, which has by far the lowest genetic diversity. It is farther from the main inland group than are Superior populations in the PCoA (Fig. 4), but branches along with other west-central populations in the genetic tree. This suggests shared ancestry with other populations in the region, rather than an introduction from a highly divergent source population. In contrast, Koronis does not group with other populations in west-central Minnesota, but it also has low diversity. Rapid divergence of this apparently bottlenecked population may be obscuring a relationship to other Cisco populations in the region. The grouping of Ojibwe and Prairie at K=9 may be an example of random drift resulting in similarity that does not reflect shared history. These populations are from different drainages and it seems unlikely that one would have been stocked into the other. The populations separate at K=12 and PCoA and the tree diagram do not support their pairing.

Most published studies of Cisco genetic structure have focused on large-scale patterns across the range (Turgeon 2001a; b; 2003) and there is less comparable information on genetic structure across the smaller scale of this study. Prior studies identified two major groups associated with distinct glacial refugia that have mixed in the Great Lakes and inland into Canada. Native inland populations in Minnesota likely fall into the western refugial lineage (Turgeon and Bernatchez 2001a). Although not the focus of their study, Turgeon et al. (2016) indicated that STRUCTURE identified distinct individual populations or groups of nearby populations over approximately 500 hundred kilometers in Ontario, which is similar to our findings. Another study using microsatellite data found low diversity and high divergence among inland populations in Michigan, with little impact of historical stocking from Great Lakes sources (J. Homola, Michigan State University, unpublished data; 2021 Midwest Fish and Wildlife Conference abstract). A study using thousands of genomic markers and widespread sampling across mostly Wisconsin populations surveyed genomic diversity, differentiation, and effective population sizes and their relationships to environmental factors (A. Ackiss, UW-Steven's Point, unpublished data; 2021 Midwest Fish and Wildlife Conference abstract). They also found a mix of geographic groupings of populations and highly distinct, likely bottlenecked, individual populations. Their genomic markers should provide much higher resolution of genetic structure, within population diversity and potential stocking impacts. Future efforts should focus on using the same markers for Minnesota populations. Combining the data would then increase the understanding of fine-scale genetic relationships in Minnesota and place them in the context of broader structure across the region.

Possible influences of historical stocking on genetic structure of Cisco populations are plausible yet not clearly evident, with the exception of three northeastern populations introduced from Lake Superior. Although Cisco stocking has been uncommon in recent decades, historical records indicate that coregonids were stocked in the late 1800s from federal agencies and other northern states (Eddy and Underhill 1974). These authors describe stocking from Lake Superior into central Minnesota lakes and specifically name Green Lake, which is in this study. However, detailed records of sources, Cisco forms, or even species (i.e., possibly Lake Whitefish *C. clupeaformis*) were often lacking (Eddy and Underhill 1974). The three northeastern populations known to have been introduced from Lake Superior over 90 years ago were genetically similar to each other, yet largely distinct from all other populations (Jacobson et al. 2018 and this study). It follows that other introduced populations from Lake Superior should likely also fall within this group. In contrast, other inland population, including several from west-central Minnesota, show little Lake Superior ancestry in STRUCTURE analysis and they all form one large grouping in PCoA, with the exception of a few outliers that are best explained as bottlenecked populations rapidly diverged via genetic drift. The Detroit River/Lake Erie area was a known major source for widespread coregonid stocking from the late 1800s to early 1900s (Roseman et al. 2007), but a review of stocking records indicated that Minnesota likely received more whitefish than Cisco (L. Pashnik, USGS Great Lakes Science Center, unpublished data). If Lake Erie or other Great Lakes Cisco had been successfully introduced, they would likely be from the eastern lineage and highly divergent from Minnesota populations (Turgeon and Bernatchez 2001a); at least of the magnitude of differences with Lake Superior populations. Although chance similarity is possible, the PCoA suggests that any possible historical stocking of non-local sources has not substantially contributed to Cisco populations in this study, including those in west-central Minnesota. The data are less able to discern whether stock transfers within Minnesota, if they occurred, affected current genetic structure. Population grouping are not entirely consistent with present-day drainages. The exceptions could be due to stocking across genetic boundaries (Turnquist et al. 2017), differences between current and post-glacial connectivity pathways (Eshenroder and Jacobson 2020), bottlenecks resulting in rapid genetic drift that obscures relationships, or simply poor resolution because of moderate sample sizes and numbers of genetic markers. Combining Minnesota samples with those from across the region that could have been historical sources should clarify possible stocking impacts, especially by using more extensive genomic markers (A. Ackiss, UW-Steven's Point, unpublished data).

Ciscoes are characterized by multiple phenotypic forms (Eshenroder & Jacobson, 2020) but the degree of genetic divergence is population specific (Turgeon et al. 2016). Two lakes in this study have possible multiple forms, Basswood and Trout. Basswood Lake appears to have dwarf artedi and larger Nipigon Ciscoes. The east basin has the largest divergence between the two forms whereas the western basin has primarily Nipigon. Trout Lake appears to have dwarf artedi and large zenithicus. However, neither sample from these lakes provided evidence for multiple genetic groups. The two samples from distinct basins of Basswood Lake showed no differentiation in F_{ST} tests. STRUCTURE analyses for Trout Lake did not indicate distinct ancestries within the sample. Further testing of possible genetic differences among forms would benefit from larger samples of each form within lakes assessed with greater numbers of genomic markers to enhance resolution.

Management implications

Maintaining genetic diversity within populations may be essential to avoid inbreeding depression and threats to long-term sustainability (Frankham 2015; Ralls et al. 2017). Although low genetic diversity may result from bottlenecks and small population size due mostly to non-genetic factors, the loss of diversity may then contribute to further decline through an extinction vortex (Gilpin & Soule 1986). This has led some conservation scientists to recommend "genetic rescue" - the introduction of new genetic diversity through translocations

(Whiteley et al. 2015; Ralls et al. 2020). Grow et al. (In revision) showed that genetic diversity of 32 of the populations in this present study was inversely related to a measure of habitat quality based on temperature and oxygen. Poor Cisco oxythermal habitat may lead to summer kills and population bottlenecks. Populations with relatively low genetic diversity occurred throughout the state, but many of these were west-central populations on the southern edge of the species range and among the most vulnerable to changing climate (Fang et al. 2012).

Although it is challenging to define a criterion based on these data for how much genetic diversity is too little, the relatively low-diversity populations are potential candidates for genetic rescue. The use of translocations (stocking), however, is controversial (Tallmon et al. 2005; Mable 2018) because of the countering concern of outbreeding depression, or the introduction of maladaptive genes or disruption of beneficial gene complexes. To reduce the risk of outbreeding depression, Miller and Kapuscinski (2003) recommended using sources that are similar by genetic, life-history and ecological/environmental criteria. The MNDNR currently applies these principals to Genetic Management Units (GMU) based on major watersheds in northern Minnesota, although this is only formally applied to Walleye in relation to stocking (Fields et al. 1997, MNDNR 1996). For Cisco, the northern populations, primarily in the Hudson Bay drainage, could be managed as a separate GMU from the Mississippi drainage populations. In practice, this calls for translocations within GMUs if attempts are made to enhance genetic diversity of populations. More conservatively, the populations of west-central Minnesota might be treated as a separate unit. Although their genetic distinctiveness may be attributed to isolation and bottlenecks, they are also on the edge of the Cisco range in the warmest climate zone. Although likely at risk (Fang et al. 2012), they have also persisted thus far, possibly due to adaptation to warmer environments and other habitat characteristics. Translocation among populations within this region may have a better chance at success than using northern sources. Separate from the question of GMUs, larger, more genetically diverse, populations are preferred sources (Frankham 2015). The data in this study provide a rank of possible source populations based on this criterion. The only other way to maintain (although not enhance) genetic diversity of isolated populations is to increase abundance and avoid population bottlenecks. This emphasizes the critical need to maintain habitat and water quality (e.g., oxythermal conditions) for Cisco to avoid pushing more populations to the point where lack of genetic diversity may further contribute to an extinction vortex.

The extent of genetic diversity related to possible multiple forms of Cisco in Minnesota is another important question not fully answered. The existence of multiple forms has primarily been documented in North American lakes of the Canadian Shield (Turgeon et al. 2016; Eshenroder and Jacobson 2020). That certainly holds true for Minnesota where Schmidt (2016) noted a number of lakes with multiple forms of Cisco in northeast Minnesota. Many of these forms exist in large, complex lakes with excellent water quality and warrant special conservation efforts. Indeed, Shortjaw and Nipigon Cisco are designated as Species of Special Concern in Minnesota (Minnesota DNR 2016), and Shortjaw Cisco have special status in Canada (Todd 2003). Further collections of ciscoes in northeastern Minnesota lakes and subsequent analyses in collaboration with Great Lakes and Canadian geneticists would be useful for developing meaningful conservation plans for these important fish.

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APPENDIX 1. Microsatellite locus characteristics, including allele characteristics, genetic diversity, primers and PCR reaction conditions. H_O, observed heterozygosity; H_E, expected heterozygosity; T, annealing temperature. Loci Clatet12 and Clatet15 were removed because numerous samples had significant heterozygote deficits (i.e., not in Hardy-Weinberg equilibrium). These loci had a large size range and deficits may have been caused by large allele drop-out.

Locus	Total alleles	Size range (bp)	Mean alleles per lake (range)	Mean H_O per lake (range)	Mean H_E per lake (range)	Primers	Reference	T(˚C)
Cisco90	6	114-130	4.1 (2-5)	0.49 (0.10-0.75)	0.60 (0.10-0.71)	CAGACATGCTCAGGAACTAG CTCAAGTATTGTAATTGGGTAC	A	55
Cisco126	6	187-225	2.6 (1-6)	0.32 (0.00-0.55)	0.34 (0.00-0.61)	GCCAGAGGGGTACTAGGAGTATG GCAGAGAAAGAGCCTGATTGAAC	A	60
BWF2	7	168-180	4.0 (3-6)	0.48 (0.15-0.73)	0.50 (0.23-0.64)	CGGATACATCGGCAACCTCTG AGACAGTCCCCAATGAGAAAA	B	55
Cisco157	13	157-181	7.6 (5-10)	0.83 (0.70-1.00)	0.80 (0.72-0.84)	CTTAGATGATGGCTTGGCTCC GGTGCAATCACTCTTACAACACC	A	60
Cisco181	64	183-403	20.5 (12-27)	0.87 (0.73 – 0.95)	0.95 (0.85-0.98)	GGTCTGAATACTTTCCAAATGCAC CCATCCCTTTGCTCTGCC	A	65
Cisco200	29	214-282	14.0 (9-19)	0.80 (0.56-1.00)	0.91 (0.87-0.94)	GGTTAGGAGTTAGGGAAAATATG GTTGTGAGGTAGGCCTGG	A	60
Clatet1	33	180-304	14.1 (6-20)	0.85 (0.60-1.00)	0.88 (0.69 – 0.96)	GAGCCCATCATCACTGAGAAAGA CTGCTACCCACAAACCCCTG	C	60
Clatet6	79	232-496	23.0 (17-28)	0.95 (0.90-1.00)	0.97 (0.92-0.98)	GAATCGGCATCTCCTGAGTCA GCTTGGGGCATAATAACCACC	C	60
Clatet12*	63	150-488	22.5 (13-28)	0.89 (0.81-1.00)	0.97 (0.95-0.98)	TCTTTGGGTTCTTAGGCTGG GGGAAACTGTATTTTGGAGC	C	57
Clatet15*	44	232-390	16.9 (11-23)	0.83 (0.70-0.95)	0.94 (0.91-0.96)	CCGAAATGGTCATAACTGAA GTGGTCCTCTGTAGCCCA	C	57

A. Turgeon, et al. (1999); B. Patton et al. (1997), as cited in A; C. Winkler and Weiss (2008).

APPENDIX 2. Pairwise Fst values between 39 samples of Cisco populations. Non-significant values at the P <0.05 criterion are indicated in bold italics. Populations IDs are listed in Table 1.

Pop	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.005	-								
3	0.004	0.004	-							
4	0.017	0.012	0.019	-						
5	0.051	0.055	0.047	0.055	-					
6	0.132	0.111	0.104	0.108	0.104	-				
7	0.047	0.048	0.038	0.045	0.025	0.060	-			
8	0.061	0.056	0.043	0.058	0.040	0.060	0.010	-		
9	0.039	0.039	0.028	0.046	0.034	0.069	0.012	0.019	-	
10	0.075	0.070	0.059	0.065	0.035	0.040	***0.000***	***0.005***	0.013	-
11	0.039	0.037	0.024	0.041	0.024	0.060	0.007	0.010	0.009	0.014
12	0.037	0.035	0.028	0.035	0.030	0.069	0.008	0.019	0.015	0.016
13	0.038	0.043	0.028	0.056	0.031	0.098	0.023	0.030	0.011	0.036
14	0.077	0.061	0.062	0.052	0.067	0.062	0.036	0.046	0.049	0.035
15	0.058	0.048	0.042	0.048	0.053	0.055	0.023	0.027	0.034	0.025
16	0.065	0.057	0.048	0.057	0.050	0.057	0.016	0.020	0.027	0.017
17	0.054	0.051	0.039	0.054	0.036	0.056	0.010	0.017	***0.003***	0.007
18	0.049	0.043	0.031	0.047	0.042	0.075	0.017	0.017	0.024	0.022
19	0.057	0.048	0.033	0.053	0.046	0.072	0.020	0.022	0.029	0.030
20	0.076	0.071	0.053	0.078	0.053	0.087	0.027	0.021	0.038	0.034
21	0.054	0.046	0.038	0.050	0.040	0.040	0.012	0.019	0.009	0.009
22	0.045	0.039	0.028	0.045	0.044	0.060	0.019	0.025	0.020	0.027
23	0.066	0.055	0.040	0.069	0.059	0.080	0.038	0.031	0.045	0.048
24	0.110	0.091	0.077	0.095	0.068	0.044	0.033	0.023	0.043	0.019
25	0.039	0.036	0.023	0.042	0.025	0.067	0.006	0.011	0.016	0.017
26	0.058	0.049	0.039	0.052	0.043	0.071	0.019	0.021	0.031	0.023
27	0.094	0.081	0.065	0.102	0.083	0.111	0.055	0.050	0.065	0.065
28	0.088	0.090	0.087	0.078	0.113	0.136	0.067	0.092	0.078	0.085
29	0.039	0.045	0.029	0.058	0.037	0.108	0.024	0.035	0.021	0.045
30	0.049	0.047	0.039	0.041	0.035	0.054	***0.005***	0.024	0.014	0.008
31	0.042	0.042	0.032	0.047	0.037	0.076	0.016	0.026	0.004	0.023
32	0.060	0.044	0.036	0.053	0.073	0.087	0.046	0.042	0.053	0.059
33	0.097	0.080	0.065	0.100	0.107	0.109	0.072	0.062	0.082	0.081
34	0.052	0.040	0.035	0.051	0.074	0.066	0.036	0.044	0.042	0.046
35	0.030	0.030	0.020	0.044	0.046	0.089	0.029	0.035	0.026	0.044
36	0.209	0.188	0.160	0.225	0.220	0.250	0.190	0.172	0.188	0.208
37	0.127	0.125	0.121	0.128	0.120	0.152	0.075	0.127	0.115	0.109
38	0.074	0.057	0.049	0.070	0.062	0.065	0.040	0.038	0.041	0.041
39	0.102	0.093	0.076	0.112	0.089	0.122	0.065	0.073	0.062	0.078

APPENDIX 2. Pairwise Fst value…continued.

Pop	11	12	13	14	15	16	17	18	19	20
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	-									
12	***0.002***	-								
13	0.011	0.022	-							
14	0.032	0.023	0.071	-						
15	0.012	0.008	0.042	0.011	-					
16	0.008	0.009	0.033	0.020	0.009	-				
17	0.008	0.015	0.014	0.041	0.026	0.017	-			
18	***0.005***	0.011	0.025	0.042	0.014	0.019	0.022	-		
19	0.006	0.011	0.029	0.048	0.014	0.020	0.026	***0.003***	-	
20	0.026	0.038	0.035	0.078	0.038	0.035	0.029	0.031	0.029	-
21	0.008	0.012	0.025	0.031	0.015	0.014	0.004	0.017	0.016	0.031
22	***0.006***	0.011	0.023	0.045	0.019	0.022	0.021	0.015	0.010	0.038
23	0.020	0.033	0.037	0.068	0.030	0.037	0.041	0.018	0.014	0.030
24	0.028	0.039	0.050	0.060	0.033	0.030	0.027	0.027	0.028	0.031
25	***0.004***	0.011	0.014	0.046	0.017	0.019	0.014	0.008	0.010	0.014
26	0.012	0.012	0.034	0.040	0.015	0.019	0.026	***0.006***	0.009	0.029
27	0.034	0.040	0.059	0.077	0.040	0.038	0.061	0.028	0.024	0.059
28	0.069	0.062	0.096	0.085	0.080	0.070	0.076	0.077	0.089	0.100
29	0.015	0.020	0.014	0.073	0.040	0.040	0.029	0.022	0.020	0.038
30	0.012	0.011	0.030	0.031	0.021	0.017	0.011	0.023	0.026	0.038
31	0.009	0.012	0.012	0.052	0.030	0.024	0.008	0.024	0.024	0.036
32	0.028	0.028	0.056	0.044	0.021	0.034	0.055	0.022	0.021	0.052
33	0.051	0.050	0.083	0.070	0.038	0.053	0.081	0.041	0.034	0.071
34	0.024	0.020	0.050	0.039	0.018	0.025	0.040	0.022	0.016	0.053
35	0.015	0.017	0.026	0.054	0.026	0.033	0.030	0.023	0.022	0.038
36	0.146	0.159	0.174	0.208	0.153	0.166	0.180	0.135	0.125	0.154
37	0.097	0.085	0.122	0.123	0.102	0.100	0.115	0.110	0.108	0.122
38	0.026	0.033	0.054	0.050	0.026	0.036	0.039	0.031	0.029	0.043
39	0.042	0.049	0.066	0.094	0.055	0.058	0.063	0.049	0.037	0.066

APPENDIX 2. Pairwise Fst value…continued.

Pop	21	22	23	24	25	26	27	28	29	30
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21	-									
22	0.012	-								
23	0.032	0.022	-							
24	0.023	0.031	0.031	-						
25	0.014	0.013	0.017	0.025	-					
26	0.019	0.013	0.022	0.022	0.010	-				
27	0.047	0.039	0.027	0.051	0.040	0.028	-			
28	0.068	0.080	0.118	0.125	0.079	0.090	0.145	-		
29	0.029	0.016	0.033	0.057	0.016	0.025	0.052	0.096	-	
30	0.009	0.020	0.045	0.034	0.013	0.019	0.066	0.068	0.034	-
31	0.013	0.014	0.042	0.044	0.014	0.027	0.063	0.072	0.014	0.015
32	0.040	0.025	0.025	0.048	0.029	0.025	0.042	0.094	0.045	0.044
33	0.064	0.048	0.038	0.064	0.050	0.041	0.040	0.138	0.067	0.075
34	0.026	0.023	0.034	0.048	0.029	0.027	0.040	0.083	0.043	0.036
35	0.028	0.018	0.023	0.057	0.018	0.027	0.046	0.092	0.017	0.029
36	0.162	0.141	0.108	0.163	0.146	0.138	0.125	0.276	0.156	0.183
37	0.108	0.088	0.127	0.136	0.091	0.089	0.143	0.150	0.090	0.086
38	0.023	0.030	0.030	0.035	0.027	0.031	0.045	0.105	0.054	0.036
39	0.050	0.048	0.049	0.066	0.047	0.041	0.052	0.142	0.054	0.064

APPENDIX 2. Pairwise Fst value…continued.

Pop	31	32	33	34	35	36	37	38	39
1									
2									
3									
4									
5									
6									
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31	-								
32	0.048	-							
33	0.079	0.015	-						
34	0.039	0.018	0.037	-					
35	0.020	0.026	0.043	0.024	-				
36	0.176	0.118	0.125	0.150	0.132	-			
37	0.097	0.115	0.138	0.108	0.098	0.283	-		
38	0.044	0.032	0.049	0.034	0.036	0.138	0.134	-	
39	0.059	0.062	0.071	0.059	0.058	0.160	0.145	0.048	-