INTRALACUSTRINE SPECIATION OF Salvelinus namaycush

IN LAKE SUPERIOR : AN INVESTIGATION OF GENETIC AND

MORPHOLOGICAL VARIATION AND EVOLUTION OF LAKE TROUT
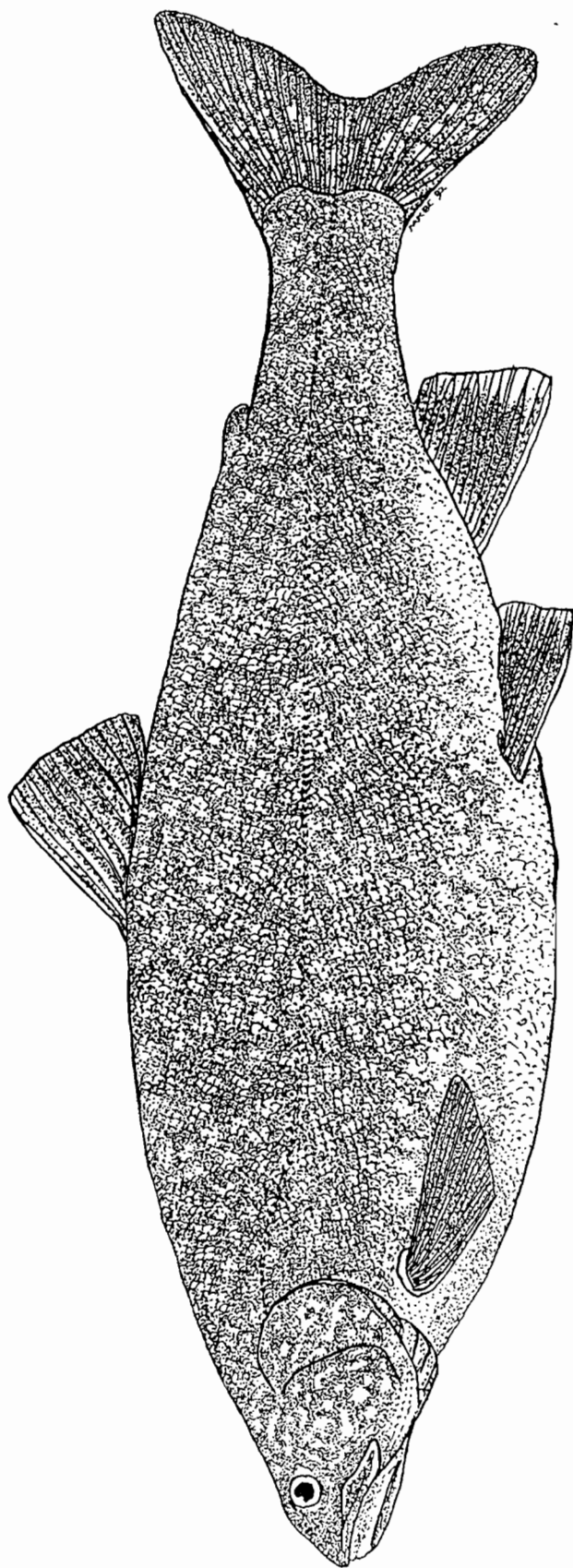
IN THE LAURENTIAN GREAT LAKES

by

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of the requirements for the degree of
Doctor of Philosophy
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" A lake is the earth's most beautiful and alluring
feature. It is the mind's eye, looking into which the
viewer can see into the depths of his reality."

Henry David Thoreau

"The most important thing is not to stop questioning."

Albert Einstein

For Those who love the lakes and
have accepted the responsibility of stewardship.

ACKNOWLEDGEMENTS

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CHAPTER I

LAKE TROUT (*Salvelinus namaycush*) IN LAKE SUPERIOR

Abstract

The lake trout (*Salvelinus namaycush*) is a ubiquitous top-level predator of deep freshwater lakes and rivers in the northern U.S. and Canada. In Lake Superior, morphological and possible genetic differentiation has resulted in at least three recognizable forms of lake trout. The three different morphological forms have been thought to be the result of environmental adaptation to different depths. The most widely recognized form is the "lean", which inhabits the inshore lacustrine areas of deep lakes less than 70 meters deep. A second form, the "siscowet," is a robust lake trout which inhabits the deep basins of Lake Superior, at depth of 50 to over 150 meters. A third form is called the "humper," or "paperbelly," and lives over isolated shoals about 50 meters deep surrounded by water greater than 100 meters deep. Previous investigations of physiological differences among leans, siscowets, and humpers showed that the phenotypic differences were heritable. Biogeographic changes since the Pleistocene glaciation had significant implications for divergence in life history adaptations among lake trout phenotypes. The possibility exists that these populations are undergoing speciation even though they are not separated by geographical barriers. The morphologically divergent populations of lake trout in Lake Superior offer a unique opportunity to investigate and test the intralacustrine speciation hypothesis in *Salvelinus namaycush*.

Introduction

This introduction will provide background on *Salvelinus namaycush* and its presence in the Laurentian Great Lakes system. I will describe the life history and biogeography of *S. namaycush*, and the legacy of investigations into morphological diversity of *S. namaycush* in Lake Superior. Finally, I will present the results of my investigations of morphological and genetic diversity among contemporary Lake Superior lake trout populations in Chapters 2 through 6 of this thesis.

The lake trout, *Salvelinus namaycush*, is an indigenous North American species whose native range is restricted to recently glaciated lakes in northern North America. Its broad distribution and lack of clear geographic differentiation support evidence of dispersal of lake trout throughout a network of lakes associated with the last glacial retreat (Lindsey 1964, Kahn and Qadri 1971, Behnke 1972). In Lake Superior, morphological and possible genetic differentiation has resulted in at least three recognizable forms of lake trout (Figure 1.1). The most widely distributed form is the "lean", which inhabits the inshore areas (less than 70 meters deep) of Lake Superior. The lake trout is a deep water inhabitant of many other deep lakes in North America whose depths are nota s extreme as Lake Superior. The lean is slender with a low body fat content, and has a straight, pointed snout. A second form, the "siscowet," inhabits the deep basins of Lake Superior from 50 to over 150 meters deep. The siscowet is distinguished by its robust body shape and high fat content, which are thought by some to be a result of environmental influence or differences in food habits (Jordan and Evermann 1911, Scott 1967). A third form is called the "humper," or "paperbelly," and lives over isolated shoals about 50 meters deep surrounded by water greater than 100 meters deep. The humper is distinguished by its large eye and thin abdominal wall. The body fat content of the humper is intermediate to the fat content of the lean and siscowet phenotypes. The lean lake trout is found in virtually every deep, cold lake in northern North America in

contrast to the siscowet and humper, which are endemic to Lake Superior. This distribution suggests that the origin of differentiation of siscowets and humpers occurred within the Lake Superior basin.

Interest in the differences between lean and siscowet lake trout in Lake Superior precedes the European settlement of the Laurentian Great Lakes. The word "*siscowet*" is derived from an Ojibwa word meaning "that which cooks itself" (Goode 1884), a reference to the extreme fatness of the deep-water form. Siscowet lake trout were prized for their rich taste when salted, and quickly became a delicacy in markets of the eastern United States. Louis Agassiz (1850) described the siscowet lake trout in his natural history of Lake Superior, and considered it a subspecies of lake trout, *Salmo siscowet*, based upon its morphological differences from *Salmo namaycush*. Ichthyologists have debated the validity of the subspecific designation throughout the twentieth century (Jordan and Evermann 1911, Eddy and Surber 1943, Lindsey 1964, Kahn and Qadri 1971, Behnke 1972, Becker 1983, Robins et al. 1991) because of unanswered questions about environmental versus genetic variation. An excerpt from the Lake Superior Journal of October 1852 attests to the distinctness of the siscowet in the eyes of the local population (Rakestraw 1967):

> "...The siskowit [*sic*] is without a doubt, the fattest fish that swims in either fresh or salt water. The fishermen say one of these fish, when hung up by the tail in the hot sun of a summer day, will melt and entirely disappear, except for the bones. In putting up about fifty barrels this season, one of the fishermen made two and a half barrels of oil from the heads and 'leaf lard' alone, without the least injury to the marketability of the fish. Besides this leaf fat, the fat or oil is disseminated throughout the fish. They are too fat to eat fresh, and are put up for market like whitefish and trout."

Interest in this particular project came from an evolutionary perspective as well as a management perspective. Morphological differences among lake trout populations occur today only in Lake Superior. Reports of "siscowet-like" lake trout were made in the late nineteenth century from northern Lake Michigan and northern Lake Huron (Strang 1854, Smith and Snell 1891, Brown et al. 1981).

The absence of a deepwater lake trout from these lakes since the early 1900 's leaves their similarity to the Lake Superior siscowet in question (Brown et al. 1981). Initial genetic investigations of the Lake Superior lake trout suggested that though morphological differences existed, genetic divergence was minor or absent (Eschmeyer and Phillips 1965, Dehring et al. 1981, Ihssen et al. 1988). The possibility remained that the Lake Superior populations were undergoing speciation even though they were not separated by geographical barriers (Behnke 1972, Utter et al. 1989). Hypotheses of intralacustrine speciation have been proposed for other species which inhabit recently glaciated lakes (Smith and Todd 1984). The morphologically divergent populations of lake trout in Lake Superior offer an opportunity to investigate and test the intralacustrine speciation hypothesis.

Management interest in the lean-siscowet problem is linked to lake trout recovery efforts in the Great Lakes. Strict controls were placed on exploitation of lean lake trout in Lake Superior after severe declines in population abundance in the 1960's and 1970's. Within the next decade, health experts discovered that certain fish, including siscowets, produced high levels of "Omega-3" oils which were said to be advantageous for the prevention of heart disease (Karahadian and Lindsay 1989). Exploitation pressure shifted to siscowet lake trout to address the newly developed market, and agencies were faced with the question of whether or not to manage siscowets as a separate species or to manage them under the same rules as they manage lean lake trout exploitation. They decided to treat leans and non-leans (siscowets) as different entities, and were then faced with the task of developing criteria with which to enforce new fishing regulations. This meant information on geographic and depth distribution, as well as criteria by which leans and siscowets could be identified in the field. Even though many managers and fishermen could tell most leans and siscowets apart, the question remained whether the differences being used to separate them were due to the environment or whether they were genetically based.

This project attempts to address both of the above issues and provide insight into processes of speciation in recently glaciated lakes. The observed morphological differentiation among lake trout populations appears to be associated with environmental adaptation, and the differences may have been leading to speciation. Principle differences between the three forms in addition to habitat depth include spawning depth and spawning time (Milner 1873, Royce 1951, Eschmeyer 1957, Thurston 1962, Rahrer 1965), fat content (Eschmeyer and Phillips 1965), buoyancy (Crawford 1966), and migratory behavior (Eschmeyer 1957, Thurston 1962, Lawrie and Rahrer 1973, Pycha and King 1975). The existence of endemic populations of lake trout in Lake Superior, and the presence of physical, ecological, and genetic differentiation between the lean and siscowet lake trout (Dehring et al. 1981) provide an opportunity to study the interaction of the processes of morphological and ecological differentiation and genomic evolution leading to intralacustrine speciation.

The ultimate goals of this study are: (1) to discover molecular-genetic and morphological correlates of the differences in fat content, habitat, and biochemical traits that characterize the several different forms of *Salvelinus namaycush* in Lake Superior; and (2) to determine, based on morphological, ecological, and behavioral differences, whether these features are homogeneous within spawning populations and differ between populations in ways consistent with hypothesized barriers to gene flow that could have arisen within the lake according to the intralacustrine speciation hypothesis. Phenotypic differences may be the consequence of original separation of daughter populations by different spawning times or places (Smith and Todd 1984). Persistence of significant differences in fat content and some morphological and osteological differences between leans and siscowets raised in controlled environments (Stauffer and Peck 1981) intimates that physiological variation is under at least some genetic control.

Life History of the Lake Trout, *Salvelinus namaycush*

Salvelinus comprises a monophyletic group in the family Salmonidae, distinguished by the presence of teeth on a vomerine crest. *Salvelinus namaycush* is distinguished from other *Salvelinus* by its grey-green coloration with pale, irregular spots, white leading edges on the paired and anal fins, absence of a kype or other breeding structures, and the absence of nest-building behavior.

The lake trout is a ubiquitous top-level predator of deep freshwater lakes and rivers in Canada and the northern United States. Its historical range extends from the Labrador Peninsula, south to the headwaters of the Lake Ontario - St. Lawrence River - Hudson River system in New York, and west through the Great Lakes drainage basin, headwaters of the Columbia and Fraser Rivers, Vancouver Island, and north throughout Canada and into Alaska. Lean lake trout were widely introduced into many western lakes (Hubbs and Lagler 1941). Deep-bodied lake trout reportedly inhabited limited areas in Lake Michigan, northern Lake Huron (Goode 1884, Organ et al. 1978), and Rush Lake, Michigan (Hubbs and Lagler 1941), but their similarity to Lake Superior siscowets is put in question by their absence from the lower lakes after the turn of the twentieth century.

Lake trout spawning generally occurs over rocky or gravelly substrate in flowing or turbulent water usually associated with the autumn turnover (Van Oosten 1944). Evidence of spawning has been noted as early as June (Sweeney 1890, Eschmeyer 1954) and April (C. Bronte 1992, personal communication) in siscowets, and in August in humpers (Burnham-Curtis, unpublished data). Migratory behavior is present among leans, but is not suspected in siscowets or humpers (Dehring et al. 1981, Ihssen et al. 1988). Females do not always spawn every year (Van Oosten 1944).

Early life history of lean, siscowet, and humper lake trout is similar. Eggs remain in interstices of rocky substrate over winter, and hatching occurs in association with increasing water temperature from late March through early June in Lake Superior at 0.3 - 1.0 degrees Centigrade (°C) (Van Oosten 1944, Scott

and Crossman 1973, C. Bronte personal communication). Larval growth is rapid, and larvae move into nursery areas in deeper water after absorption of the yolk sac (Eschmeyer 1957). Sexual maturity is reached at about age 6 or 7. Lake trout are piscivorous, and an increase in food size accompanies increased growth. Major components of adult lake trout forage include ciscoes (*Coregonus* spp.), sculpins (*Cottus cognatus, C. ricei, Myoxocephalus thompsoni*), ninespine stickleback (*Pungitius pungitius*), burbot (*Lota lota*), occasional crustaceans including *Mysis relicta*, and terrestrial insects. Juvenile forage includes younger and smaller forms of some of the previous list as well as *Pontoporeia affinis*. Larval lake trout eat the most locally abundant zooplankton available (Rawson 1961, Scott and Crossman 1973). As carnivores, lake trout generally eat the most available forage items and demonstrate little prey preference (Dryer et al. 1965). Most differences in composition of diet between lean and siscowet lake trout can be attributed to habitat differences and differences in local forage availability (Eck and Wells 1986).

Commercial fishing for the lake trout commenced upon the settlement of the Great Lakes in the late 1700's (Agassiz 1850, Brown et al. 1981). The preference for fatty fish well-suited for salting created an instant market for the siscowet lake trout. The presence of a siscowet-like form in the Great Lakes in the mid-1800's is referred to in records of the Strang settlement on Beaver Island in Lake Michigan (Strang 1854), the Hudson Bay Company in Lake Superior (Brown et al. 1981), and commercial fishing records from Lake Huron (Goodier 1981). Extensive commercial exploitation of both forms of lake trout elicited a gradual decline in overall catches by the early 1900's (Pycha and King 1975, Baldwin et al. 1979, Brown et al. 1981, Hartman 1988). By the 1920's the siscowet-like form disappeared from commercial catches in Lake Michigan (Brown et al. 1981). With increased fishing effort, the efficiency of nylon nets, and the invasion of the parasitic sea lamprey (*Petromyzon marinus*) into the Great Lakes, a precipitous decline in lake trout abundance began in the early 1940's

from the lower Great Lakes (Lake Ontario and Lake Erie) and progressed into the upper Great Lakes (Lake Huron, Lake Michigan, and Lake Superior) (Lawrie and Rahrer 1972, Pycha and King 1975). By the late 1960's native populations of lake trout were absent from all of the Great Lakes except Lake Superior and Georgian Bay of Lake Huron, and extensive efforts began to protect the remaining stocks. In the 1960's extensive stocking of hatchery-reared lake trout was undertaken with some recovery and natural reproduction (Curtis 1990, Marsden et al. 1988). Pollution and physical degradation of spawning shoals have seriously impeded the goal of re-establishing natural stocks in the lakes (Eshenroder et al. 1984). Management interest in the species-level differences between lean and siscowet lake trout in Lake Superior have peaked in the face of continued decline in near-shore lake trout populations and increased exploitation pressure on offshore populations.

A Brief History of the Biogeography of the Laurentian Great Lakes Basin

A comprehensive analysis of the origin of the fish fauna of the Laurentian Great Lakes Basin can be found in Bailey and Smith (1981). A brief overview will be presented here. The Laurentian Great Lakes Basin is geologically young (14000 - 9000 ybp) relative to other lacustrine systems of similar magnitude such as the African rift lakes and Lake Baikal, which date back from hundreds of thousands to millions of years. Lake Superior is the largest, deepest, and most oligotrophic of the five Great Lakes. While Lake Superior is shallower than Lake Baikal or Lake Tanganyika, it covers the largest area of any lake on earth, with over 82,000 km^2 of surface area and over 210,000 km^2 of basin area. The average depth of Lake Superior is about 300 m above sea level and it has a maximum depth of about 450 m. The maximum surface temperature ranges from 11-16°C. The diversity of species found in the Great Lakes basin numbers 174 in 71 genera and 28 families (Bailey and Smith 1981).

The lake history is divided into several stages of glacial advance and retreat during Pleistocene glaciation (Prest 1970). Initial glaciation was followed by a stage allowing maximum isolation of inshore waters (10600 - 8100 ybp), succeeded by a period in which the lake basins were widely connected (8100 - 6000 ybp) leading to the modern configuration (Prest 1970). At the fluctuating boundaries of the ice lobes were large periglacial lakes within which many species of coldwater fish existed. Glacial refugia for warmwater fish were provided in numerous tributary waters, most notably the Mississippi, Maumee, Fox, and Mohawk drainages. Within these refugia many species survived the glaciation and subsequently colonized the Great Lakes. Lake trout colonized from the Bering, Atlantic, and Mississippi refugia as indicated by fossil and extant forms found throughout North America (McPhail and Lindsey 1970, Scott and Crossman 1973).

Species patterns within the Great Lakes suggests that differentiation at the species level occurred within the lake basin, the most extensive divergence being within the polymorphic coregonid species complex (Todd and Smith 1980, Smith and Todd 1984). While some believed that the level of differentiation within *S. namaycush* was merely stock or population differentiation and not species-level differentiation (Jordan and Evermann 1911, Scott and Crossman 1973, Robins et al. 1991), there were others who supported discrimination at least at the sub-specific level (Agassiz 1850, Eddy and Surber 1943, Eschmeyer 1957, Hubbs and Lagler 1949). Opponents to the separation of leans and non-leans into separate taxonomic classes used the presence of phenotypic intergrades and the likelihood that hybridization occurs freely as evidence of locally adapted races rather than reproductively segregated populations (Jordan and Evermann 1911, Scott and Crossman 1973). Advocates of lean or non-lean separation consistently supported arguments with ecological evidence of population differentiation--by differences in spawning time and place--maintaining that while interbreeding may occur, it is insignificant (Eddy and Surber 1943, Eddy and Underhill 1974, Brown et al. 1981).

Several stages of lake formation during the latter part of the Wisconsonin glacial retreat could have provided the potential for reproductive isolation among colonizing lake trout populations in the Laurentian Basin. Glacial Lake Keweenaw formed around 12,500 years before present (ybp), but was again filled in by glacial advance about 11,800 ybp (Figure 1.3). Deep, cold water-adapted forms would probably not have survived in the glacial refugia, and the development of glacial Lake Duluth about 11,600 ybp would be the earliest that lake trout could have begun to diverge. Tributary connections between glacial Lake Duluth and glacial Lake Algonquin could have allowed shallow water populations to migrate, but probably were not deep enough for a deepwater adapted form of lake trout to survive and migrate (Leverett 1928). The eastern basin of glacial Lake Duluth opened around 10,600 ybp, followed by lowering water levels in glacial Lake Chippewa and Lake Stanley. The lower basins achieved maximum isolation around 8200 ybp. However, glacial Lake Minong and glacial Lake Houghton-Nipissing at this time retained an apparently larger volume of water in a part of the basin with high topographic diversity. Rising water levels (7800 ybp) would eventually bring isolated populations into secondary contact if they were occupying similar habitats (deeper inshore waters) and lake trout would be widely distributed in the current Laurentian Great Lakes.

Phenotypic Divergence in Lake Superior Lake Trout

Three forms of adult lake trout are currently recognized primarily according to amount of body fat and depth of capture (Goodier 1981). Initial identification of the siscowet form was a direct result of the recognition of differentiation of body depth and snout shape (Agassiz 1850). Thomson (1883) and Goode (1884) listed nine forms of lake trout, most recognized by flesh color and average weight. These characteristics are highly correlated with diet and the interaction of the environment with physiology, and as a result are difficult to use as indicators of species level differentiation. Commercial fishermen recognized up

to twelve "races" of trout during the early twentieth century, most of which were identified in the Isle Royale area (Rakestraw 1967, Cochrane 1982). Traditional morphological characters considered to be more reliable indicators of divergence do not show significant differences between the lean and siscowet forms recently (Khan and Qadri 1970). Such characters as fin ray counts, body length, gill raker number, dentition, jaw shape, and breeding morphology are considered good characters to distinguish most species of fishes (Hubbs and Lagler 1941). Measurements of these revealed no major differences between the siscowet and lean forms (Eschmeyer 1957, Qadri 1964, Khan and Qadri 1970).

Prior to 1953 abundance of lake trout in Lake Superior was high, as reflected in commercial fishing records (Lawrie and Rahrer 1973) and different populations were considered to be differentiated stocks. The presence of diverse populations of lake trout sustained the pressure of "fishing-up," or targeting different stocks of lake trout, by commercial fishermen long before the severe depletion of lake trout was aggravated by sea lamprey predation. The severe decline in lake trout abundance was not lake-wide, but basin-wide as well. Lake trout abundance declined so drastically in the lower four Great Lakes (Lake Michigan, Lake Huron, Lake Erie, and Lake Ontario) that they became extinct. Recovery efforts began early enough in Lake Superior that some wild populations survived, but the historical morphological diversity may have been lost. After 1960, the only morphological differences evident among Lake Superior lake trout defined the extremes: lean, siscowet, and humper. Fishing pressures and sea lamprey predation would have had the greatest deleterious effects on inshore lake trout populations--lean populations would have been the most adversely affected. There is no documented evidence that siscowet populations suffered the same severe declines in abundance. Historical morphological diversity may not be reflected in the character distribution among extant lake trout populations if reproductive isolation among discrete stocks was incomplete. Introgression between formerly segregated lake trout populations will have significant implications for contemporary character distribution. However, if behavioral and

ecological differences are maintained, morphologically differentiated populations, or stocks, may still survive in Lake Superior.

The most convincing study of morphological divergence between leans and siscowets was an analysis of body fat content by Eschmeyer and Phillips (1965). Wild lean and siscowet lake trout and hatchery-raised controls were analyzed for body fat content as percent of dry weight. Results showed non-overlapping, length-specific differences in fat content between leans and siscowets for both wild and hatchery groups (Figure 2 and Figure 5 of Eschmeyer and Phillips 1965, see Figure 1.2 and Figure 1.3). Siscowets consistently had a higher fat content at specific lengths than leans (Eschmeyer and Phillips 1965). This study complemented a previous study by Thurston (1962) of the chemical composition of lean and siscowet lake trout which showed differences in the protein and oil composition of the two forms, and a study by Crawford (1966) which demonstrated buoyancy differences. A 1989 study of oil composition by Karahadian and Lindsey demonstrated significant differences in the oleic acid composition of lipids from lean and siscowet lake trout. A summary of distinguishing characteristics of lake Superior lake trout is presented in Table 1.1.

Tributary waters and fluvial systems which support lake trout have populations that differ slightly in allozyme and DNA patterns (Phillips and Ihssen 1986, Grewe and Hebert 1988). Differences occur in the frequency of polymorphic alleles and not in chromosomal structure, or chromosome presence or absence (Phillips and Ihssen 1986, Phillips et al. 1989). There is little or no morphological divergence among lake trout populations in lakes outside the Great Lakes basin. All of the non-Great Lakes lake trout resemble the lean phenotype. Historical evidence for the presence of siscowets in areas other than Lake Superior is convincing but scant (Strang 1854, Brown et al. 1981, Dehring et al. 1981). The presence of lean lake trout in the other Great Lakes, in Canadian lakes, and their tributaries suggests that the lean form was the initial colonist in the post-glacial Great Lakes.

Genetic and Morphological Tests of Speciation Hypotheses
of *Salvelinus namaycush*

Hypotheses of divergence and speciation in the absence of geographic isolation were tested using morphometric and mitochondrial DNA analyses. In the second chapter I describe a breeding experiment which was originally conducted to uncover morphological differences between lean and siscowet offspring which could be useful in field identification of juveniles. Upon closer inspection, and reanalysis of the data using principal component analysis, the data not only show that leans and siscowets are different, but that differences in fat content and body shape are transmitted from parent to offspring. Chapter Three provides additional support for the hypothesis that leans and siscowets are partially reproductively isolated. Differences in the shape of dermal cranial bones were discovered which correspond to lean and siscowet phenotypes. The humper phenotype appears to be intermediate for all of these characters.

In Chapter Four I report on tests of hypotheses of reproductive isolation and intralacustrine speciation using restriction enzyme length polymorphism analysis of mitochondrial DNA (mtDNA). This tests the presence of subtle genetic differentiation in a rapidly evolving molecule which may be indicative of reproductive isolation. If leans and siscowets diverged within Lake Superior, it might have been geologically recent (since the last glacial retreat). Divergence in more obvious genetic characters may not have had enough time to occur, but divergence in mtDNA resulting from assortative mating could be detected. Chapter Five presents the results of the test of the hypothesis that reproductive isolation can be detected in morphological divergence among lean, siscowet, and humper lake trout. A principal component analysis, parallel to the one performed on hatchery specimens described in Chapter Two, was used as an application of traditional multivariate analyses to investigate correlated size and shape changes which reflect divergence in characters that discriminate lean, siscowet, and humper lake trout. Finally, in Chapter Six, hypotheses of speciation are discussed

in relation to the data presented in the preceding chapters. The purpose of this study was to determine whether the morphological characters among lean, siscowet, and humper lake trout had a genetic basis, and whether phenotypic expression in the wild was modified by environmental factors affecting growth and metabolism. Anthropogenic disturbances in Lake Superior in the last century may have perturbed the system, perhaps compromising the analysis of whether morphological divergence is leading to speciation in *Salvelinus namaycush* in Lake Superior.
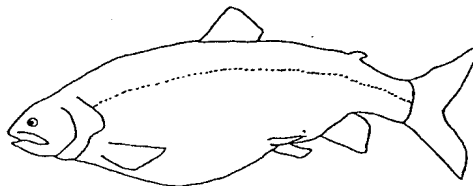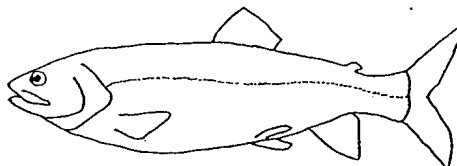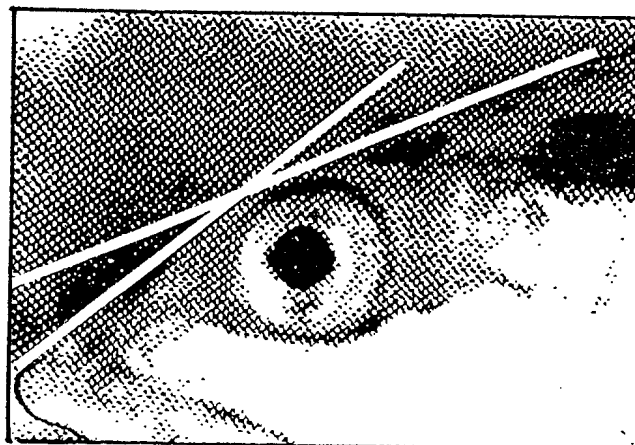
Figure 1.1. Body shape profiles of lean (top), siscowet (middle), and humper (bottom) lake trout with angle of snout outlined.
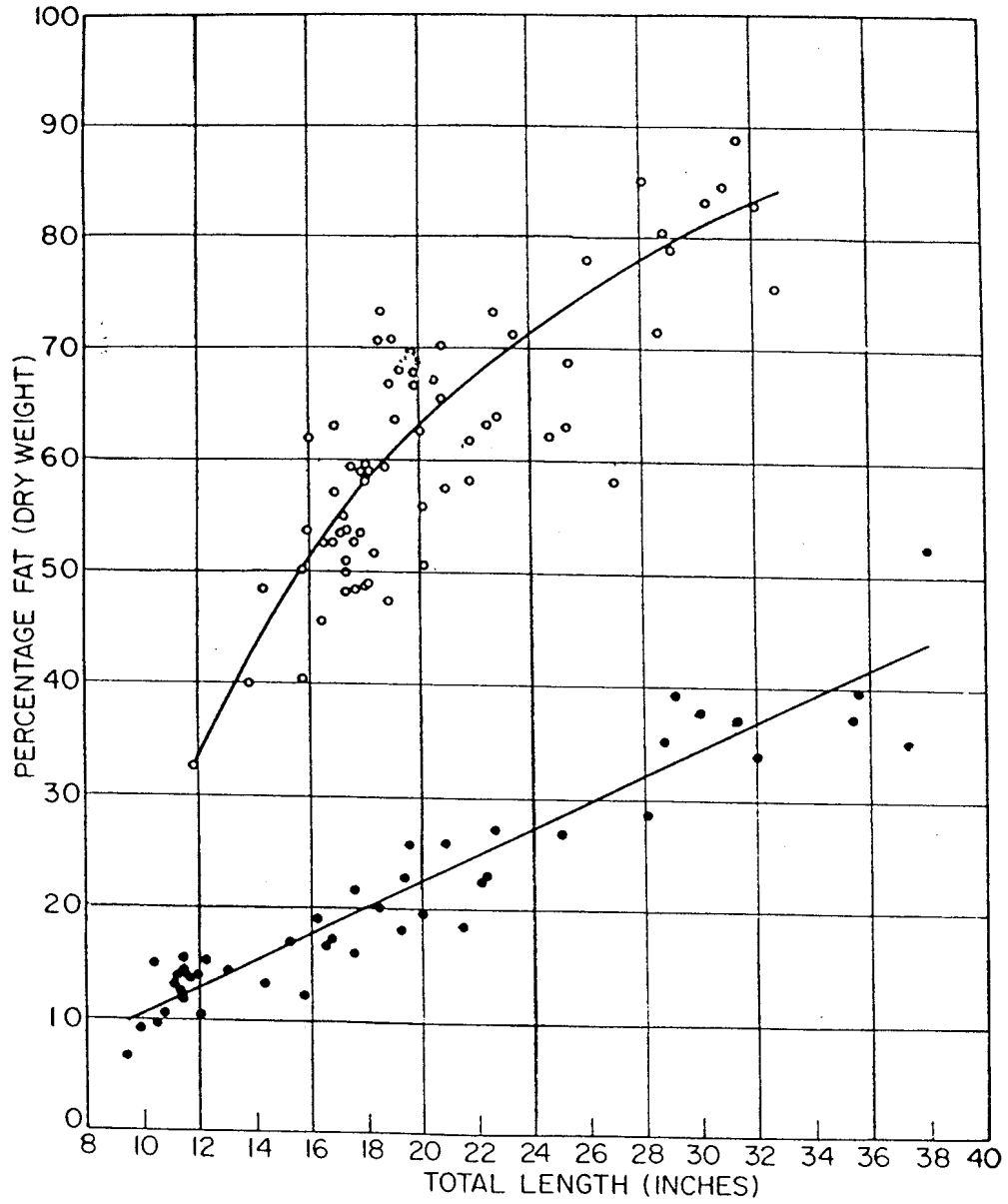
Figure 1.2. Percentage of fat in the flesh of Lake Superior siscowets (open circles) and leans (closed circles) of different lengths. Each circle represents one fish. The curves were fitted by inspection. Reprinted from Figure 2, Eschmeyer and Phillips 1965.
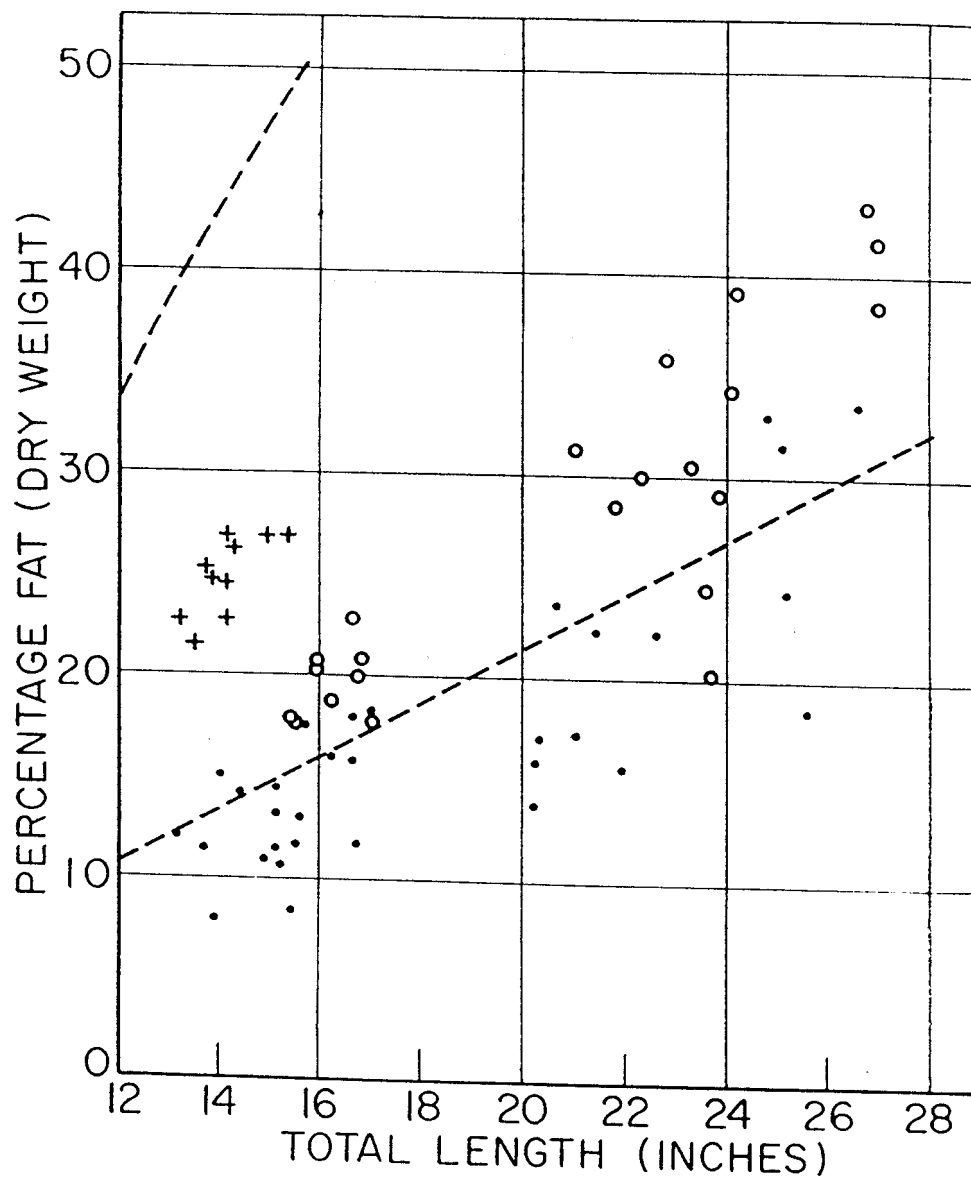
Figure 1.3. Percentage of fat in the flesh of hatchery-reared lake trout (dots), lean x siscowet hybrids (open circles), and siscowets (crosses). The broken lines are for native Lake Superior leans (lower) and siscowets (upper) from Figure 2 of Eschmeyer and Phillips 1965. Reprinted from Figure 5, Eschmeyer and Phillips 1965.
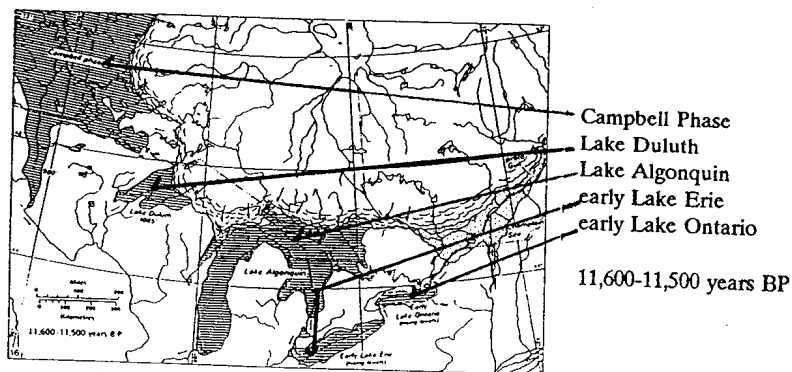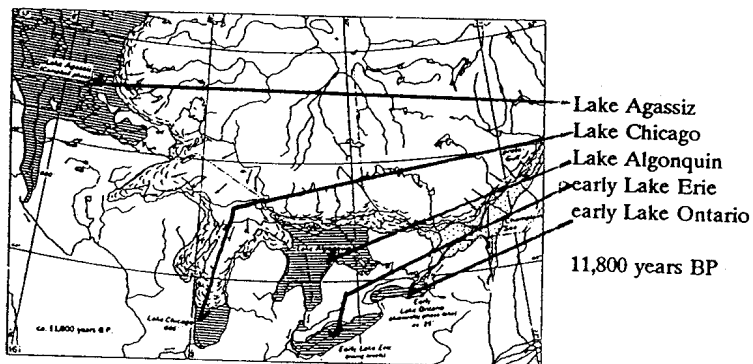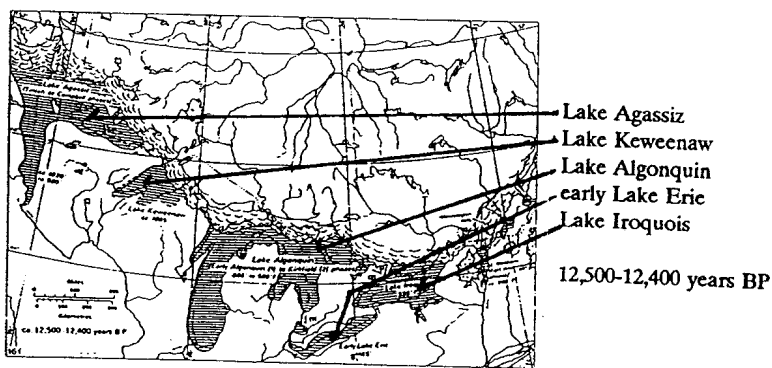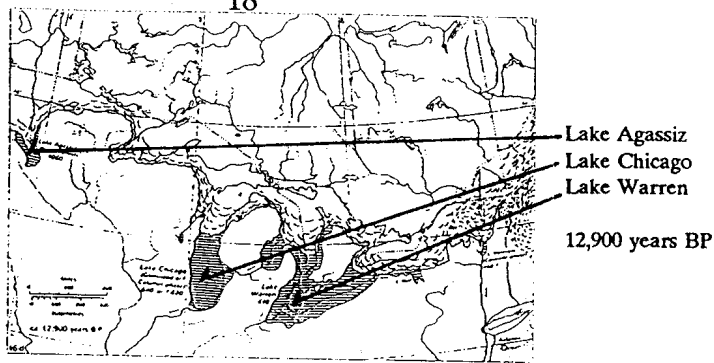
Figure 1.4. Pattern of Wisconsonin glacial retreat leading to the current Laurentian Great Lakes configuration (from Prest 1970, Bailey and Smith 1981). Origin of the siscowet lake trout could have occurred only since the glacial Lake Duluth stage, about 11,600 years before present.

Lake Agassiz
Post-Duluth Lake
Lake Chippewa
Lake Stanley
early Lake Erie
early Lake Ontario

10,600 years BP

Lake Agassiz
Minong-Houghton transition
Lake Chippewa
Lake Stanley
Lake Hough
early Lake Erie
early Lake Ontario

9,000 years BP

Lake Agassiz
Lake Houghton-Nipissing
Lake Chippewa
Lake Stanley-Nipissing
Lake Erie
Lake Ontario

8,200 years BP

The Nipissing Great Lakes
three outlet phase

Lake Erie
Lake Ontario

6,000 years BP

Figure 1.4 continued. Later stages of glacial retreat included water level fluctuations that could have isolated lake trout populations in separate basins within the Laurentian Great Lakes basin.

	LEAN	SISCOWET	HUMPER
BODY SHAPE	Slender; Anal fin angled ventrally	Deep-bodied; Anal fin angled posteriorly	Deep-bodied; thin abdominal wall; Anal fin angled ventrally
HEAD SHAPE	Straight line over snout	Blunted (convex) over snout	Slightly blunted over snout; large eye
OPERCLE[3]	STRAIGHT, angled dorsally	NOTCHED, angled anteriorly	NOTCHED, angled anteriorly
SUPRAETHMOID[3]	Usually no radii at anterior; covered by thin skin	Radii present at anterior; covered by thick skin	Radii usually present at anterior; covered by thin skin
BODY FAT[1]	Low; mostly found in visceral cavity	High; found in visceral cavity, high concentration in muscle	Intermediate; found mostly in visceral cavity, some in muscle
STOMACH CONTENTS	Smelt, bloater chub, herring, sculpins, mysis	Bloater chub, herring, sculpins, mysis; some smelt and terrestrial insects	Mostly herring, sculpins, and mysis
MATURITY[1,2,3]	6-10 yr, > 500 mm total length	6-10 yr, > 400 mm total length	6-10 yr, << 500 mm total length

Table 1.1. Summary of characteristics distinguishing lean, siscowet, and humper lake trout in Lake Superior.

1. Eschmeyer and Phillips, 1965
2. Rahrer, 1975
3. Burnham-Curtis, unpublished data

CHAPTER II
ENVIRONMENTAL VS GENETIC CONTROL OF PHENOTYPE:
THE THOMPSON HATCHERY EXPERIMENT

Abstract

Offspring of known lean and siscowet lake trout (*Salvelinus namaycush*) from Lake Superior reared in a hatchery were examined for discriminating characters among juvenile lake trout of different phenotypes. The progeny of homotypic crosses of lean and siscowet phenotypes were raised under controlled environmental conditions for three years and sacrificed for fat and morphometric analysis. Fat analysis of eight parent lake trout confirmed their identification as leans or siscowets. Differences in fat content that defined lean and siscowet parents were also found to be different in their hatchery-raised progeny. Differences between leans and fats were found in univariate morphological characters, but the differences did not correspond to parental phenotypes. I measured additional morphometric and meristic characters on the progeny and performed a principal component analysis to look for correlated differences in size and shape. The pattern of correlation among morphometric and meristic characters in principal component analysis supported the presence of heritable genetic differences between lean and siscowet lake trout.

Introduction

Fishermen and those familiar with Lake Superior have long been aware of the morphological diversity among the native salmonid populations. The

identification of adult "lean," "siscowet," and "humper" lake trout in Lake Superior is not particularly difficult for seasoned fishermen or experienced fishery managers. The morphological and physiological differences of these adult lake trout are well-documented and not widely disputed (Thurston 1962, Eschmeyer and Phillips 1965, Rahrer 1965, Khan and Qadri 1970, Crawford 1966), but the relative contributions of environment and genotype remain unresolved. Lean lake trout inhabit inshore waters at depths of 15-80 meters. They have a slender fusiform body with a pointed nose and straight snout. The body fat content of lean lake trout ranges from 12% to 42% of ash-free dry weight. The siscowet lake trout inhabits offshore areas at depths of 50 to over 150 meters. The siscowet is a deep-bodied, robust fish with a rounded and convex snout. Its body fat content ranges from 32% to 85% of its ash-free dry weight, and most of the fat is found in the visceral cavity and interlaced throughout the muscle (Thurston 1962). The humper lake trout inhabits offshore waters at about 50 meters depth over isolated deepwater shoals surrounded by depths greater than 100 meters. Humpers are characterized by a thin abdominal wall and a large eye, but their body shape and fat content are intermediate to those of the lean and siscowet. Humper populations are now only found around Isle Royale and Caribou Island where they are sympatric with lean and siscowet populations.

Eschmeyer and Phillips (1965) demonstrated conclusively that leans and siscowets have different growth characteristics which are reflected in non-overlapping measures of fat content, but they could not dismiss the possibility that the differences in the wild were due to environmental influence (Jordan and Evermann 1911, Eddy and Surber 1943, Scott and Crossman 1973). Eschmeyer and Phillips (1965) also demonstrated that hybrid offspring of leans and siscowets had intermediate fat content, an indication that differences between the parent forms were genetic. The ability of commercial fishermen to target lake trout populations with specific phenotypes year after year enhanced support for the hypothesis that lean, siscowet, and humper lake trout were genetically distinct and reproductively segregated. As a result, breeding experiments were undertaken by

the Michigan Department of Natural Resources at the Thompson Hatchery to test the hypothesis that the phenotypic differences between lean and siscowet lake trout are heritable and can be discerned in offspring raised under controlled environmental conditions.

If lean and siscowet lake trout are not genetically segregated and offspring were raised under controlled environmental conditions, then they should develop similar (convergent) characteristics. If the phenotypic differences have a genetic basis, then offspring raised under controlled conditions should express their parental phenotype. The Thompson Hatchery experiment was originally designed to document morphological characters that would be useful in discrimination of wild juvenile lean and siscowet lake trout (Stauffer and Peck 1981). Inspection of the data, and re-analysis with principal components, revealed evidence supporting the hypothesis that morphological differences among lean and siscowet lake trout have a genetic basis.

Methods

The Original Thompson Hatchery Study: Stauffer and Peck 1981

Evaluation of lake trout rehabilitation efforts in Lake Superior depend partly upon identification of the different lake trout phenotypes to determine if planted lake trout are reproducing successfully. In 1973, lake trout breeding studies were conducted by the Michigan Department of Natural Resources (MI DNR) to determine whether detectable differences existed that would enable fishery managers to distinguish between juvenile lean and siscowet lake trout whose spatial distributions overlap (Stauffer and Peck 1981). Identification of younger lake trout was difficult because the most useful distinguishing characters (body fat content, body depth, facial characteristics) were less pronounced in juveniles and virtually absent or undetectable in young of the year and yearlings.

Ripe siscowet lake trout were collected from the Apostle Islands in western Lake Superior. Lean lake trout offspring were obtained from homotypic crosses

of hatchery stock which originated from populations in Marquette Harbor, on the south shore of Lake Superior. The identity of all parents was confirmed by analysis of fat content and size at maturity (Stauffer and Peck 1981). Two lean males and two lean females provided gametes for the lean crosses and two siscowet males and two siscowet females provided the gametes for the siscowet crosses. The offspring were reared separately in Thompson Hatchery for one year, then in common tanks for a period of two years under identical conditions, at temperatures of 7-10°C. Siscowet progeny were identified by a left pelvic fin clip, and lean progeny had no fin clip. Offspring were fed to promote maximum growth, and uneaten food was removed from the tanks. At 3 years of age the young were sacrificed, morphological measurements were taken, and comparisons were made between the lean and siscowet phenotypes.

Morphometrics

I re-analyzed the morphological data from the offspring raised at Thompson Hatchery using principal components analysis. The advantage of using this method over univariate comparisons is that principal components represent combinations of all available characters and emphasized the variation in a data set. Principal component analysis provided one way to statistically sample and apply the variation found in multiple characters to the identification problem (Bookstein et al. 1985). Graphical representation of the individual component scores in two dimensions illustrated differences corresponding to external grouping criteria that would otherwise have been masked in univariate analyses. The goal was to detect intraspecific differences attributed to genetic control. Differences that persist in offspring of the progeny of homotypic crosses of the phenotypic extremes under identical environmental conditions indicate a genetic basis to observed diversity.

I measured the following morphometric characters for ten offspring from each of the lean and siscowet crosses reared at Thompson Hatchery: total length, head length, head depth, body depth, pre-dorsal length, pre-orbital length, post-

orbital length, sub-orbital length, post-maxillary length, premaxillary height, premaxillary width, dentary length, dentary tooth row length, and mandible length (Figure 2.1). The following meristic counts were recorded: ventral pores, number of gill rakers on first basibranchial arch, branchiostegal rays, dorsal rays, pectoral rays, pelvic rays, anal rays, lateral line pores, scales in diagonal rows, scales in vertical rows (dorsal insertion to pelvic insertion), and scales around the caudal peduncle (Figure 2.1). Emphasis was placed on measuring characters that represented girth or body depth because the results of the original Thompson Hatchery study suggested that significant differences were present in girth measurements for the hatchery offspring. Body depth measurements were most likely to be positively correlated with body fat content, which was shown to differ significantly between lean and siscowet lake trout (Eschmeyer and Phillips 1965).

Univariate statistics were calculated for these data using the STATS module of the SYSTAT statistical package (Wilkinson 1988). Principal component analysis was performed using the FACTOR module of SYSTAT (Wilkinson 1988). Correlation matrices were used as input for initial principal component analyses because all of the characters did not have the same units of measure. Principal component scores were plotted for the first two principal components in two-dimensional plots to illustrate variation among the morphotypes. Principal component analysis was then performed separately on the morphometric and meristic data. Morphometric measurements were analyzed in a covariance matrix and meristics were analyzed in a correlation matrix. The first principal component of morphometrics was plotted against the first principal component of meristics.

Results

The progeny of siscowets appeared to grow faster in the hatchery than the progeny of the lean lake trout, even though length at age data from wild populations indicated that length at age for leans was greater than length at age

for siscowets (USFWS Ashland Biological Station, unpublished data). Siscowet progeny also had lower specific gravity than leans, meaning they were more buoyant.

Stauffer and Peck's analysis of morphological characters of the progeny indicated siscowets had greater girth than leans at the same age. Graphs of girth and length measurements are shown in Figure 2.2. Meristic measurements showed little difference in the number of rays in the pectoral and pelvic fins, but dorsal ray counts for siscowets averaged higher than leans. The ranges for most of the measurements overlapped widely and the differences among means were not biologically significant, as they varied within less than one discrete unit.

In my morphometric analysis, significant differences were present between lean and siscowet phenotypes for all measurements except head length (Table 2.1). Significant differences were present for gill raker number, number of pyloric caeca, dorsal rays, pectoral rays, scales in diagonal rows, scales in vertical rows, and caudal scales. Siscowets raised under controlled environmental conditions were longer and more robust than their lean counterparts. Statistically significant differences between lean and siscowet offspring ($p < 0.05$) occurred for all measurements except ventral pores, anal rays, and lateral line pores.

Lean lake trout progeny were separated from siscowet progeny by principal component 1 (PC 1), with no overlap, from the analysis of all data in a correlation matrix. PC 1 accounted for 64.4% of the variance in the data set. PC 2 accounted for 8.7% of the variance. Because all of the loadings of size estimates on PC 1 are high (0.86-0.99), PC 1 is clearly a size component. PC 2 does not discriminate leans and siscowets. Though the major loadings on PC 1 were mostly measures of body size, six meristic measurements (diagonal scale rows, vertical scale rows, caudal scales, gill rakers, pectoral rays, and pelvic rays) had loadings greater than 0.50 (Table 2.2). Two meristics, vertical scale rows and diagonal scale rows, had loadings of 0.96 and 0.83. PC1 plotted against PC 2 formed two distinct and non-overlapping groups as seen in Figure 2.3a. Morphometric variables and some meristic variables were highly correlated.

In the second principal component analysis, morphometric and meristic variables were dissociated and analyzed separately. When PC 1 for morphometrics is plotted against PC 1 for meristics, lean and siscowets show two distinct clusters which have no overlap in either morphometrics or meristics (Figure 2.3b).

Discussion

Under controlled conditions, offspring of extreme lean and extreme siscowet parents express their parental traits, and provide evidence that morphological differences have a genetic basis. Because the comparative analysis of the fat content of parents and their offspring were similar, growth characteristics of lean and siscowet phenotypes bred true. In addition, the pattern of morphological differences between parental leans and siscowets prevailed in their hatchery progeny. Siscowets grew faster than lean offspring under controlled environmental conditions, a characteristic observed in other studies[1]. The observation that the fat content of the hatchery progeny was lower than wild leans and wild siscowets of the same age could be attributed to hatchery diet composition (Phillips et al. 1957). I think it is unlikely that diet would account for corresponding differences in growth, body shape, or meristics between lean and siscowet progeny.

One of the hypotheses of this experiment was that offspring of homotypic crosses of known parents representing extremes of each phenotype would express the parental phenotype when raised under controlled environmental conditions. The original goal was to produce criteria by which wild juvenile lake trout could be separated into lean and siscowet phenotypes. An implicit assumption was that

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[1]Foster, N.R., W.H. Berlin. 1983. Performance and physiological characteristics of young of different strains of lake trout. Research Completion Report. USFWS Great Lakes Fishery Laboratory.

the observable morphological differences were heritable. Controlling environmental variation, phenotypic differences that were expressed in the offspring of homotypic lean and siscowet crosses were assumed to be due to genetic determination. The results of morphometric analyses clearly show that the morphology of leans and siscowets has a genetic basis. The hypothesis that differentiation is due only to environmental variation must be rejected.

Persistent non-overlapping differences in fat content of hatchery-raised lean and siscowet progeny are not purely ecophenotypic. The fat content for wild lean and siscowet lake trout measured by Stauffer and Peck (1981) corresponded to values published by Eschmeyer and Phillips (1965). Eschmeyer and Phillips also measured the fat content of hatchery raised leans and siscowets as well as lean x siscowet hybrids. They found that hatchery leans and siscowets still had non-overlapping fat content, but the fat content was lower than for wild fish. More importantly, Eschmeyer and Phillips found that the fat contents of hybrid progeny were intermediate to those of the parents. There can be no other explanation than genetic control for persistent differences in the fat content and morphological characters of hatchery-raised lean and siscowet progeny.

While it is common for size characters to be influenced by environmental variation and season (LeCren 1951, Paloheimo and Dickie 1966), meristic measurements may vary according to temperature or developmental rate (Barlow 1961). These progeny were raised under conditions which limited the environmental influence on phenotypic expression, yet the gross morphological differences and fat content differences persisted. The phenotypic differences observed among lean and siscowet lake trout cannot be the result of either random events or convergence. The similarity of hatchery lean progeny to their wild parents and the similarity of hatchery siscowet progeny to their wild parents is evidence that some differentiated heritable genetic control is involved in the expression of the morphological characteristics of lean and siscowet lake trout.

Leans and siscowets, while closely related, have unique evolutionary histories. Some form of genetic control directs the transmission of traits

associated with fat deposition and growth. Separate analyses of the morphometric and meristic variables clarified the contributions of length-dependent and length-independent characters to observed patterns of diversity. The pattern of variation for meristic characters when morphometric characters are excluded demonstrated that differences among length-independent characters of lean and siscowet lake trout have a genetic basis. Even without a quantitative measure of heritability, it is clear that there are heritable differences in fat deposition, morphology, and meristic characters between lean and siscowet lake trout from Lake Superior.

If some of the morphological differences are heritable, then selection on phenotype in highly divergent habitats, combined with the tendency of lake trout to return to natal spawning grounds (Martin 1960) would be expected to enhance assortative mating and lead to the evolution of genetic differences (Dickinson and Antonovics 1973). The re-analysis of the Thompson Hatchery data now provides a baseline for the analysis of molecular genetic and morphological variation of wild populations of lean and siscowet lake trout. Given initial evidence that the morphological differences between lean and siscowet lake trout are genetically based, it would be valuable to examine aspects of genetic variability in wild populations.

Conclusion

The observation that heritable genetic differences existed between lean and siscowet lake trout progeny demonstrated that observable phenotypic differences in the wild were not due solely to environmental factors. Principal component analysis showed that lean and siscowet progeny could be discriminated in two distinct and non-overlapping clusters. Characters that contributed to the variance in the major components were not restricted to morphometric measurements, but also included a significant contribution from meristic measurements. The ability to discriminate lean and siscowet progeny and the inability to discriminate wild lean and siscowet progeny suggests that phenotypic expression is influenced by

environment. When the environmental influences are removed or controlled, the differences attributable to genomic control become evident. Most morphological characters in the parent lake trout show a wide range of overlap. Among hatchery-raised progeny, variation in morphological and meristic characters was great enough to enable group discrimination. Lean and siscowet lake trout in Lake Superior represent locally adapted populations that are segregated enough to allow differential growth and development, but not enough to allow intrinsic reproductive isolation. Further examination of genetic and morphological variability in the wild are necessary to answer questions of reproductive isolation, evolution, and speciation.
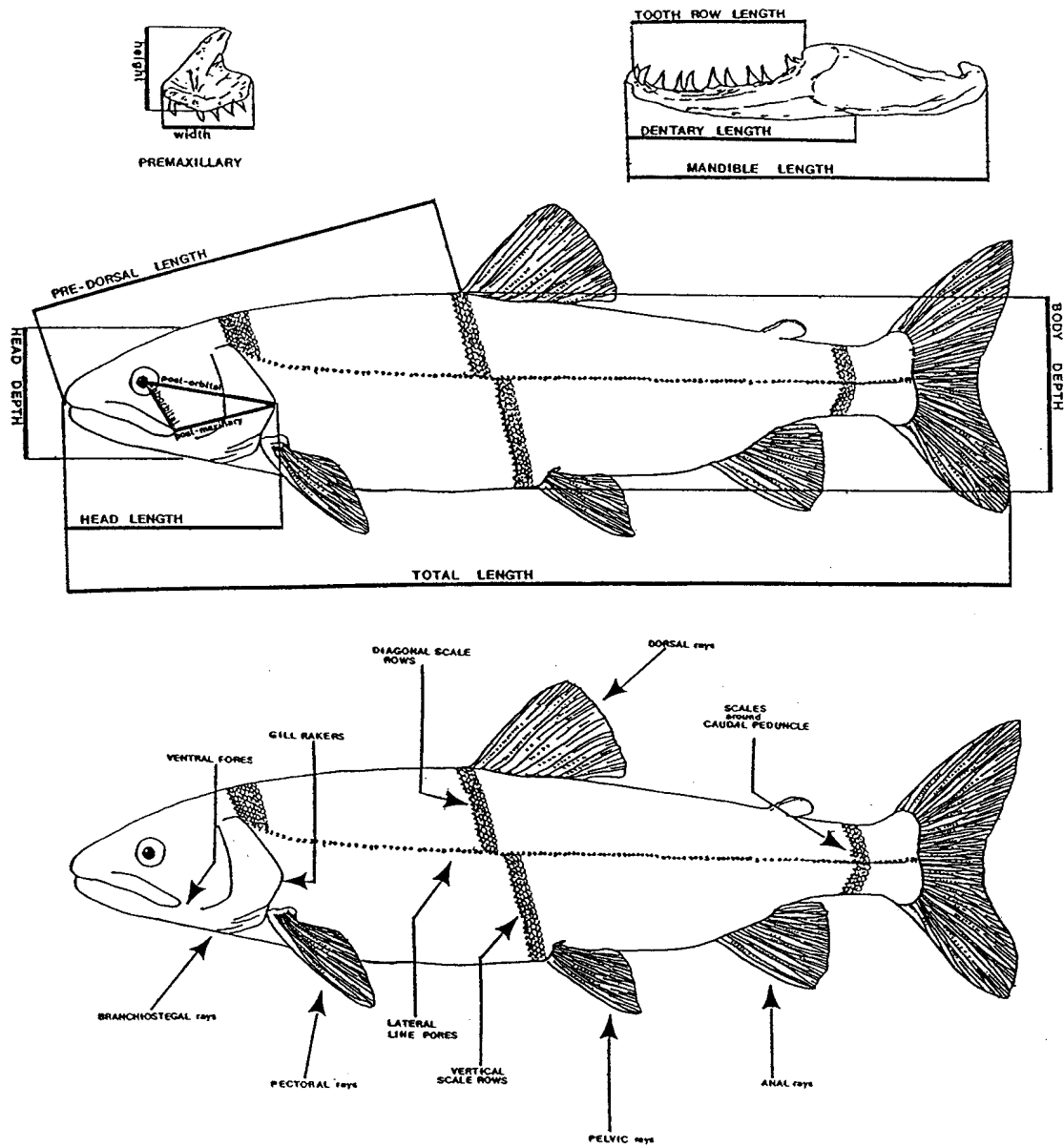
Figure 2.1. Morphometric and meristic measurements collected from Lake Superior *Salvelinus namaycush*.
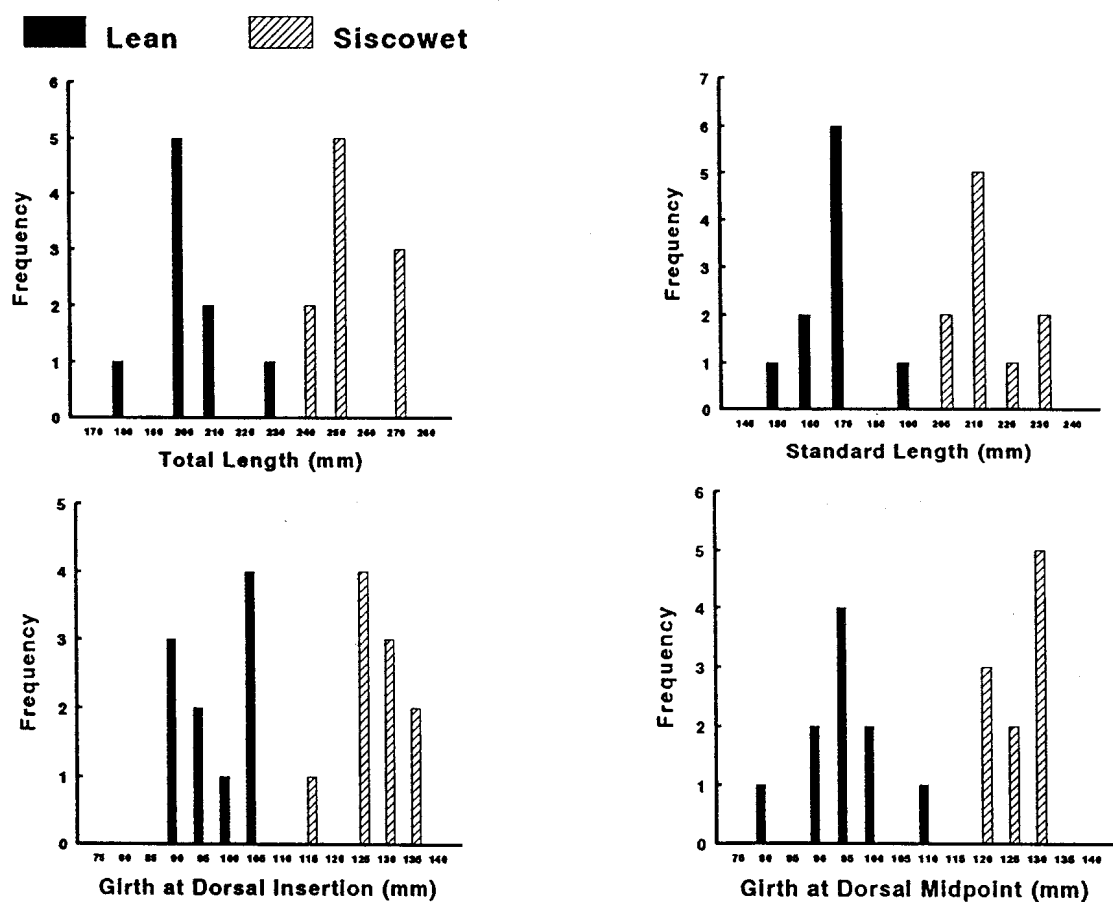
Figure 2.2. Distribution of morphometric measurements of 3-year-old progeny of lean and siscowet lake trout raised in Thompson Hatchery. Girth was measured as the distance around the body at the dorsal insertion and the midpoint of the dorsal fin. 3-year-old progeny of lean and siscowet lake trout raised under identical conditions showed non-overlapping differences in length and body depth measurements.
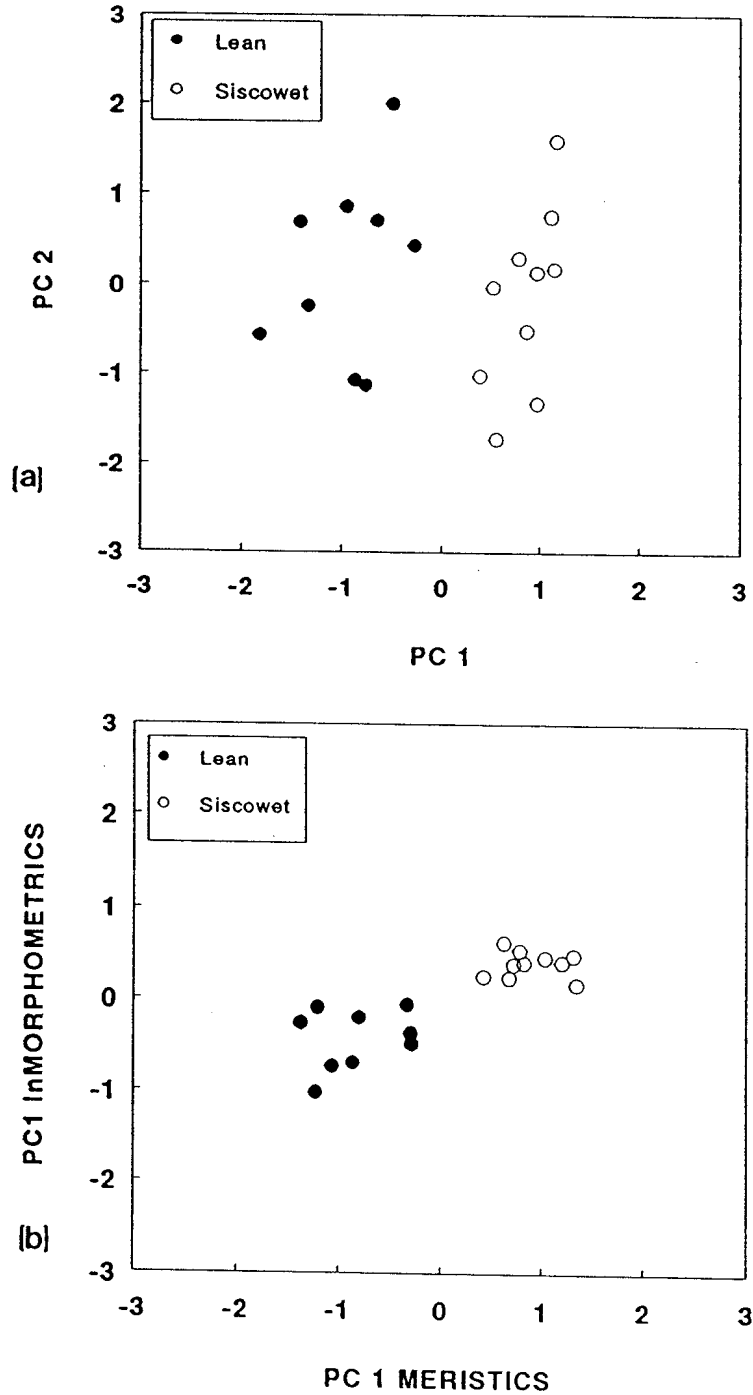
Figure 2.3. Principal components plotted for morphometric and meristic characters from Thompson Hatchery lake trout progeny. (a) PC 1 vs PC 2 from a correlation matrix of morphometric and meristic variables combined. (b) PC 1 for morphometrics from a covariance matrix plotted against PC 1 for meristics from a correlation matrix. In (a), non-overlapping differences are present in PC 1 as a size component. When analyzed separately as in (b), both morphometrics and meristics contribute to non-overlapping differences between leans and siscowets.

	LEAN (N = 10)			SISCOWET (N = 10)		
	MEAN	VARIANCE	SEM	MEAN	VARIANCE	SEM
Total Length	245.7	405.1	6.4	316.6	82.0	2.9
Head Length	53.6	17.3	1.3	66.0	8.7	0.9
Head Depth	25.5	10.6	1.0	35.4	6.1	0.8
Body Depth	47.7	34.2	1.8	67.4	18.2	1.3
Pre-dorsal Length	108.8	110.7	3.3	138.7	16.9	1.3
Pre-orbital Length	20.3	2.6	0.5	24.9	1.6	0.4
Post-orbital Length	35.4	8.4	0.9	43.0	4.7	0.7
Sub-orbital Length	13.8	3.2	0.6	16.7	1.9	0.4
Post-maxillary Length	28.2	4.8	0.7	33.4	2.5	0.5
Dentary Length	25.7	11.7	1.1	32.2	7.0	0.8
Dentary Tooth Row	19.8	3.7	0.6	26.2	9.1	0.9
Mandible Length	34.4	10.7	1.1	42.1	6.6	0.8
Premaxillary Width	6.7	0.9	0.3	8.3	1.1	0.3
Premaxillary Height	5.4	0.4	0.2	6.5	0.5	0.2
Dorsal-caudal Length	102.9	97.2	3.1	131.7	19.6	1.4
Adipose-Caudal Length	34.4	20.8	1.4	44.1	7.5	0.9
Ventral Pores	9.7	0.4	0.2	9.3	0.4	0.2
Gill Rakers	16.4	0.3	0.2	17.7	0.4	0.2
Pyloric Caeca	110.2	115.2	3.4	93.9	20.9	1.4
Branchiostegal Rays	13.2	0.4	0.2	12.1	0.3	0.2
Dorsal Rays	8.9	0.1	0.1	10.0	0.0	0.0
Pectoral Rays	13.8	0.4	0.2	14.6	0.5	0.2
Pelvic Rays	9.1	0.3	0.2	9.7	0.2	0.2
Anal Rays	9.3	0.2	0.1	9.4	0.3	0.2
Lateral Line Pores	126.6	2.7	0.5	127.5	4.3	0.6
Scales in Diagonal Rows	175.0	18.2	1.3	201.1	10.1	1.0
Scales in Vertical Rows	73.9	44.3	2.1	83.5	11.8	1.1
Caudal Scales	69.6	4.9	0.7	77.9	14.3	1.2

Table 2.1. Univariate statistics for Thompson Hatchery lean and siscowet lake trout progeny. All individuals are age 3.

	CORRELATION MATRIX		MORPHOMETRIC (ln) COVARIANCE MATRIX		MERISTIC CORRELATION MATRIX	
	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Eigen Values	15.13	1.92	0.24	0.02	5.40	1.32
Percent Variance	63.03	7.99	84.34	7.51	49.10	11.97
Total Length	0.99	0.02	0.13	0.01		
Body Depth	0.95	-0.00	0.19	0.01		
Head Length	0.98	0.12	0.12	0.01		
Head Depth	0.95	0.03	0.18	0.00		
PreDorsal Length	0.97	0.02	0.13	-0.00		
PreOrbital Length	0.96	0.08	0.12	0.01		
PostOrbital Length	0.97	0.18	0.11	0.01		
SubOrbital Length	0.85	0.42	0.12	0.03		
PostMaxillary Length	0.93	0.11	0.10	-0.00		
Dentary Length	0.87	0.14	0.14	0.05		
Mandible Length	0.94	0.14	0.13	0.02		
Premaxillary Width	0.69	0.04	0.13	-0.11		
Premaxillary Height	0.77	-0.45	0.11	-0.07		
Ventral Pores	-0.24	0.42			-0.47	0.01
Gill Rakers	0.65	-0.20			0.71	0.31
Branchiostegal Rays	-0.48	0.38			-0.66	-0.19
Dorsal Rays	0.86	-0.236			0.90	0.12
Pectoral Rays	0.61	0.09			0.66	0.26
Pelvic Rays	0.50	-0.63			0.65	-0.58
Anal Rays	0.13	-0.57			0.31	-0.76
Lateral Line Pores	0.31	0.39			0.34	0.34
Diagonal Scale Rows	0.96	-0.11			0.93	0.17
Vertical Scale Rows	0.80	-0.17			0.84	-0.19
Caudal Scales	0.77	-0.45			0.89	-0.14

Table 2.2. Principal component loadings for hatchery raised lean and siscowet progeny. PC 1 in a correlation matrix is a size component, but when meristics and morphometrics are run separately, it is evident that some meristic characters are highly correlated with morphometric characters.

CHAPTER III
OSTEOLOGICAL DIFFERENCES BETWEEN LEAN AND SISCOWET
LAKE TROUT PHENOTYPES

Abstract

Two osteological characters found among *Salvelinus namaycush* lean, siscowet, and humper phenotypes in Lake Superior provide additional evidence that morphological divergence is not due solely to ecophenotypic differentiation. The presence of radii on the anterior of the supraethmoid bone is found in siscowets and humpers and is absent in a majority of leans. A notch in the opercle bone near the articulation with the hyomandibular is consistently present in siscowets and humpers and absent in leans. The opercle notch was referred to in observations by Agassiz (1850), though it has been little used in investigations of lake trout morphological divergence. The consistency of the opercle notch and the dermethmoid radii provide evidence of the unique shared ancestry of siscowet lake trout and the possible hybrid origin of humpers. The opercle notch provides an additional character by which lean and siscowet lake trout can be distinguished in the field.

Introduction

Resistance to classifying the siscowet and humper phenotypes of Lake Superior lake trout as separate subspecies is apparently due to the general tendency of salmonids to rapidly evolve morphologically divergent sympatric forms in recently glaciated lakes (Behnke 1972). These forms are often characterized by

ecological adaptations which contribute to assortative mating in the absence of complete reproductive isolation. The family Salmonidae are soft-rayed teleost fishes in the order Clupeiformes (suborder Salmonoidei). The Salmonidae are grouped into three subfamilies, the Salmoninae, Thymallinae, and Coregoninae, based on morphological and behavioral characteristics (Norden 1961). The subfamily Salmoninae includes five genera of trout, char, and salmon of which *Salvelinus* Richardson is one. Osteological features characteristic of the subfamily Salmoninae include the presence of an orbitosphenoid bone, a suprapreopercle bone, a basibranchial plate, teeth on the maxilla, no dermosphenotic bone, and parietals separate at the midline (Regan 1914, Norden 1961, Behnke 1972). *Salvelinus* are distinguished from other Salmoninae by the presence and pattern of teeth on the vomer and palatine, as well as by pigmentation pattern (light spots on a dark background) (Morton and Miller 1954), and the size and shape of the scales (Stokell 1951). *Salvelinus namaycush* (Walbaum) is distinguished from other *Salvelinus* species by the presence of a toothed vomerine crest, resulting in an argument by some for its classification as a separate genus, *Cristivomer* (Vladykov 1963, Qadri 1964).

A genetic basis for morphological differentiation among Lake Superior lake trout populations has been demonstrated in fat analyses (Eschmeyer and Phillips 1965), and in breeding studies (Stauffer and Peck 1981; Chapter 2). If leans, siscowets, and humpers are genetically segregated then differences should exist in physical characters not normally influenced by environmental variation. To test the hypothesis that genetic divergence among lean, siscowet, and humper lake trout is reflected in physical characters, the cranial bones were examined for structural differences that were consistent with phenotypic boundaries. The bones of the cranium are of great taxonomic significance for salmonines. At the generic

and specific level, the variations in the shapes of the supraethmoid (dermethmoid), premaxilla, and circumorbitals are distinct. The presence of a shape difference in the opercle bone between lean and siscowet lake trout (*Salvelinus namaycush*) was noted by Louis Agassiz (1850) but has not been used in subsequent investigations of morphological divergence (Eschmeyer 1957, Thurston 1962, Qadri 1964, Eschmeyer and Phillips 1965, Khan and Qadri 1971, Stauffer and Peck 1981). The implication of consistent differences in osteology is that morphological differences have a genetic basis. Consistent differences in osteology corresponding to lean, siscowet, and humper phenotypes will support the presence of genetic segregation.

Methods

The shape of the supraethmoid is characteristic at the generic level among the Salmoninae (Smith and Stearley 1989, and Figure 3.1). Its overall shape is triangular, and it is posteriorly fimbriate, with posterior projections overlapping the frontals. It borders but does not articulate laterally with the nasals. Its anterior portion is rounded or triangular and fits between the upper extensions of the premaxilla. In *S. namaycush* the supraethmoid is longer than it is wide, distinguishing it from most other *Salvelinus*. The supraethmoid is referred to as the dermethmoid by Smith and Stearley (1989) and Stearley (1992) in their analyses of salmonid phylogeny. This bone will herein be referred to as the supraethmoid (*sensu* Norden 1961, and Cavender 1980) because of its position directly on top of the ethmoid cartilage below the dermis.

The opercle series consists of four lateral dermal bones of the cranium (Figure 3.2). The shape of the preopercle is taxonomically significant at the generic level, but does not vary within *S. namaycush*. It lies just behind the suspensorium of the lower jaw and partially covers the 3 opercular bones. The opercle is the topmost of three flat intramembranous bones which cover the gills.

The subopercle, located below the opercle, overlaps the branchiostegal rays. The interopercle is a triangular bone which lies below the preopercle and separates the preopercle from the subopercle.

Dermal cranial bones were removed from fresh or frozen specimens according to the method of Ridewood (1904). Skin and excess muscle were removed from dried preparations using dermestid beetle larvae. Supraethmoid bones were removed from the dorsal ethmoid cartilage after the removal of dermal cranial bones, and were cleaned of dermis by immersion in hot water.

Observations of opercle bone shape were made on prepared specimens. Observations of supraethmoid structure were made using a stereomicroscope. Illustrations were made from photographs or from drawings using a compound microscope. Comparisons of supraethmoid structure and opercle shape were made with skeletal specimens in the collections of the University of Michigan Museum of Zoology (UMMZ) Division of Fishes.

Specimens examined

Salvelinus namaycush:

Lean phenotype.74 wild fish collected from locations in Lake Superior. 6 fin-clipped hatchery-raised
adults collected from Keweenaw Bay, Lake Superior; 10 hatchery-raised progeny of wild
lean phenotype;
UMMZ 172464 (1956), Marquette, MI (2); UMMZ 173951 (1955), Blue Lake, MI (1);
UMMZ 177542 (1960), Green Lake, WI (1); UMMZ 66300 (1930), Lake Michigan, MI (1);
UMMZ 98596 (1931), Torch Lake, MI (1); UMMZ 79343 (1927), Paris Hatchery, MI (1);
UMMZ 53662 (1921), Lake Superior, Ontanogon, MI (1);

Siscowet phenotype. 180 wild fish collected from locations in Lake Superior; 10   hatchery-raised
progeny of wild siscowet phenotype;
UMMZ 55640 (1921), Lake Superior, Marquette, MI (2);
UMMZ 115947 (1937), Stannard Rock reef, Lake Superior (8).

Humper phenotype. 47 wild fish collected from Isle Royale, Lake Superior.

Rush Lake phenotype. UMMZ 80508 (1924), Rush Lake, MI (9).

Salvelinus fontinalis:

UMMZ 171011, Silver River, MI (1); UMMZ 183701, Montreal River, MI (1); UMMZ 186231, Marquette, MI (1).

Salvelinus confluentus:

UMMZ 188852, Long Creek, Quebec, Ontario, Canada (1).

Salvelinus alpinus:

UMMZ 157351, Northwest Territories, Canada (1); UMMZ 183685, Northwest Territories, Canada (1).

Salvelinus leucomaenis:

UMMZ 187614, Hokkaido, Japan (1).

Hucho perryi:

UMMZ 187612, Hokkaido, Japan (1).

Oncorhynchus mykiss:

UMMZ 201666, Lake Michigan, Berrien County, MI (1).

Identification criteria

Lake trout specimens were assigned to "lean," "siscowet," or "humper" phenotype categories on the basis of a combination of several external morphological characteristics used by fisheries managers and commercial fishermen. Lake trout were considered to be "leans" if they had a straight, pointed snout and slender body. Lake trout were considered to be "siscowets" if they had a convex snout (bent over the eye) and a deep body. Lake trout were considered to be "humpers" if they had a disproportionately large eye and a thin abdominal wall. Humpers had facial characteristics similar to the lean phenotype, and they lacked the excessive visceral fat of the siscowet phenotype. In some cases, identification of leans and siscowets was difficult and gross observation of the amount of visceral and intramuscular body fat was utilized as an additional

criteria for identification. Siscowets had a much greater amount of visceral body fat (lining the dorsal wall of the visceral cavity) than leans. In addition, the excessive fat in the muscle tissue of siscowets was easily observed by squeezing the flesh between one's fingers. Sampling locations, depths, and numbers of lean, siscowet, and humper lake trout identified are listed in Table 3.1. Fisheries managers and commercial fishermen often targeted siscowet populations based on depth of capture. Management regulations restricted state-licensed gill net fishing to depths greater than 60 fathoms (110 meters). All fish collected at depths greater than 110 meters by commercial fishermen in this study (Copper Harbor, Port Wing, Duluth) consistently possessed "siscowet" characteristics. Around Isle Royale, leans, siscowets, and humpers were taken in the same nets, set across-contour, but with only slight overlap in depths. I observed that if leans and siscowets were both taken from the deeper water, siscowets did not bloat as severely as leans when brought to the surface.

Results

Among Lake Superior *S. namaycush*, the supraethmoid bone showed variation in the presence of bony ridges or radii in the anterior portion of the bone (Figure 3.1). The radii extended from the center where the supraethmoid was in contact with an indentation in the ethmoid cartilage and continued anteriorly to the edge of the supraethmoid. In larger specimens, distinct ridges were seen at the anterior edge of the bone. The presence of radii on the supraethmoid bone varied considerably among wild leans and siscowets from Lake Superior. The radii were present in 26.1% (± 0.2%) of the lean phenotype, 80.4% (± 0.1%) of the siscowet phenotype, and 66.7% (± 0.5%) of the humper phenotype. Among other species (*S. alpinus, S. leucomaenis, S. fontinalis, Hucho perryi,* and *Oncorhynchus mykiss*) bony ridges on the supraethmoid resembling the radii seen among Lake Superior *S. namaycush* were only found in *S. alpinus*

(Figure 3.1). The supraethmoid radii were not found in any *S. namaycush* of hatchery origin with the lean phenotype. The supraethmoid radii were also absent in wild *S. namaycush* from lakes in arctic Canada. Among the progeny from the Thompson Hatchery study, all siscowet progeny possessed the supraethmoid radii, and the lean progeny lacked them (Figure 3.2).

The opercle bone of siscowets had a noticeable notch on the dorso-anterior corner just behind the hyomandibular (Figure 3.3). This opercular notch could be readily seen when the dermis was removed from the opercle. The presence of the opercular notch was less variable than the supraethmoid radii among the lean, siscowet, and humper lake trout in Lake Superior. The opercular notch was present in 18.5% (± 0.1%) of the lean phenotype, 92.8% (± 0.05%) of the siscowet phenotype, and in 100% of the humper phenotype. The opercular notch was absent from all other *Salvelinus* species examined (Figure 3.3). The opercular notch was also absent from all *S. namaycush* of hatchery origin, and from all wild *S. namaycush* examined from lakes in arctic Canada. Among the Thompson Hatchery lean and siscowet progeny, the opercular notch was absent in all lean progeny and present in all siscowet progeny. A slight differences could be seen in the angle of the opercle bone among specimens possessing the opercular notch. An angle formed by a line drawn in the lateral plane at the top of the preopercle and a line connecting the top of the opercle bone with the top of the preopercle was more acute among specimens which lacked the opercular notch (Figure 3.4).

The presence of the supraethmoid radii and the opercular notch was lowest among leans and greatest among siscowets (Table 3.2, Figure 3.5). Leans are distinguished by the near absence of both the supraethmoid radii and the opercular notch and siscowets are distinguished by the general presence of both the supraethmoid radii and the opercular notch. Humpers showed the highest frequency of individuals with the opercular notch present and the supraethmoid radii absent, while the frequency of supraethmoid radii absence and opercular notch presence among leans and siscowets was comparable.

Discussion

The general presence of the opercular notch in siscowets and its near absence in leans is evidence that the siscowets share a recent common ancestor. The absence of the notch is a primitive condition, and its presence is unique to a Lake Superior lineage. The identification of the opercular notch as a siscowet characteristic is supported by the historical association of the opercular notch with the siscowet phenotype (Agassiz 1850). The supraethmoid bone, however, seems particularly susceptible to the effects of interbreeding. The supraethmoid bone of splake (*Salvelinus namaycush x S. fontinalis*) shows a blend of *S. namaycush* characteristics (anterior is large and triangular) and *S. fontinalis* characteristics (indentation at anterior, corners of anterior triangle are rounded) (personal observation). Individuals of lean and siscowet phenotypes that possessed supraethmoid and opercular characters that conflicted with *a priori* identification as lean or siscowet may be offspring of lean x siscowet interbreeding. The potential for interbreeding among lean and siscowet populations is thought to be great in some areas of the lake where habitats show significant overlap into the spawning season (James Peck, MI DNR, personal communication).

The consistent presence of the opercular notch is evidence that the humper was derived from the Lake Superior lineage, while the high frequency of absence of the supraethmoid radii supports predictions of the hypothesis that humpers originated from introgression among lean and siscowet populations. The distribution of humper populations today is more restricted than the lean or siscowet populations within Lake Superior, and no lake trout populations with the humper phenotype are found outside of this lake. The humper phenotype has unique features as will as a mosaic of characteristics typical of lean and siscowet phenotypes. A thin abdominal wall and large eyes are unique to the humper phenotype. However, humpers are deep-bodied and have fat content intermediate to the lean and siscowet. The presence of radii on the supraethmoid bone is

highly variable among humpers, but the opercular notch is consistently present and indicates post-hybrid segregation. Humper spawning is in August, and its habitat is limited to offshore deepwater shoals. The humpers do not overlap the spawning times or habitats of leans or siscowets, and no leans or siscowets in spawning condition have been found in the presence of spawning humpers, both of which reduce the potential for recent backcrossing events. The pattern of morphology, osteology, and life history differences of the humper phenotype supports the hypothesis of historical hybrid origin from Lake Superior lean and siscowet ancestors.

The similar osteological characteristics of different siscowet populations and humper populations in Lake Superior could not have occurred as a result of convergent evolution. The presence of the supraethmoid radii and the opercular notch provides unambiguous evidence supporting the unique ancestry of the siscowet and humper lake trout phenotypes in the Laurentian Great Lakes. Low population densities which accompanied the sea lamprey invasion and increased fishing pressures are the most likely causes of interbreeding among lean and siscowet phenotypes in the last half century. Contemporary backcrossing probably resulted in the occasional presence of conflicting osteological character states in leans and siscowets. Evidence for the historical hybrid origin of the humper phenotype from lean and siscowet ancestors is provided by the variation in the presence of the supraethmoid radii and the intermediacy of other morphological characters in concert with stable differences in spawning time and place.

The presence of the opercular notch is useful in field identification of siscowets from Lake Superior. Because of the incidence of conflicting character states among lean lake trout, it is advisable to use the opercular notch in combination with external morphological features such as body depth, snout shape, fatness, and depth of capture when making field identifications.

Conclusion

The supraethmoid bone and the opercular bone of *S. namaycush* from Lake Superior were examined for differences consistent with external morphological features defining the lean, siscowet, and humper phenotypes. The supraethmoid bone of siscowets possessed bony ridges (radii) extending from a point at the center of the anterior of the bone. These radii were present in 80% of the siscowets and 67% of the humpers examined but absent in 74% of the leans. The supraethmoid radii were also absent in all leans of hatchery origin and all *S. namaycush* from outside of the Lake Superior basin. The absence of supraethmoid radii is a primitive condition in salmonids.

The opercle bone of siscowets had a notch present at the dorso-anterior corner (Agassiz 1850). This notch was present in 93% of the siscowets and 100% of the humpers but was absent in 82% of the wild leans examined. The opercular notch was absent in all leans of hatchery origin and all *S. namaycush* from outside of the Lake Superior basin. The opercular notch was also absent among all sister taxa to *S. namaycush*. The combination of the opercular notch and supraethmoid radii is characteristic of the *S. namaycush* siscowet phenotype. The frequency of opercular notch presence and supraethmoid ray absence among individuals of the humper phenotype in combination with mosaic patterns of morphology and life history are evidence supporting the hybrid origin of the humper phenotype in Lake Superior. Conflicting character states for the opercle bone and the supraethmoid bone in some lean and siscowet phenotypes in Lake Superior supported a hypothesis that hybridization among extant populations of *S. namaycush* was, or is occurring.

The use of the opercular notch in combination with morphological characters including fat content, body depth, snout shape, and depth of capture is advocated as a useful method of field identification of siscowet lake trout in Lake Superior.
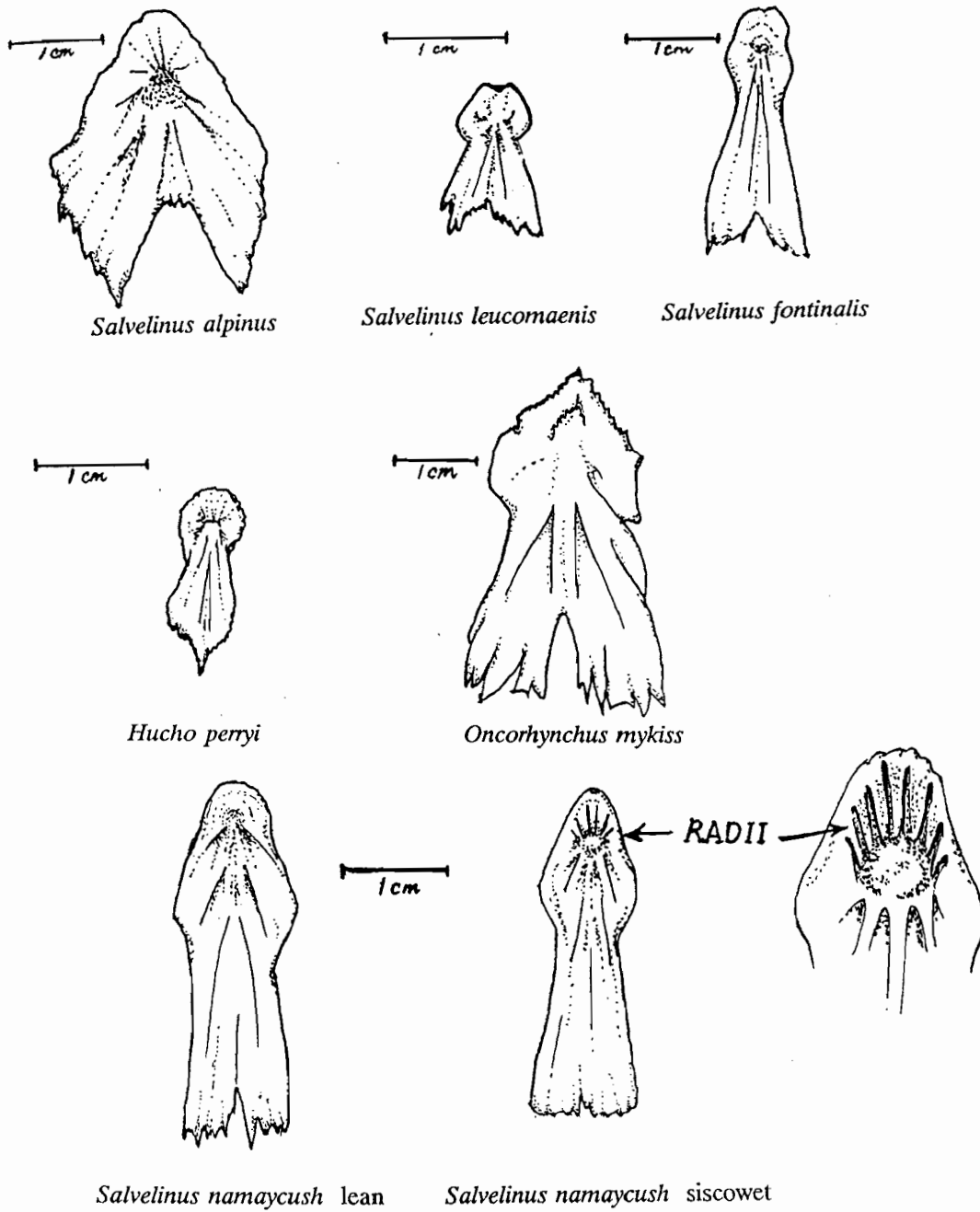
Figure 3.1. Supraethmoid bone of representative salmonid species.

Isle Royale Lean

Isle Royale Siscowet

Isle Royale Humper

Lean   Siscowet
Thompson Hatchery

UMMZ 172463
Lean x Siscowet hybrid
Marquette Hatchery

Lean - L. Superior
Apostle Islands

Siscowet - L. Superior
Copper Harbor

UMMZ 172464
L. Superior - Marquette

UMMZ 173951
Blue Lake,
Kalkaska Co., MI

UMMZ 177542
Green Lake, WI
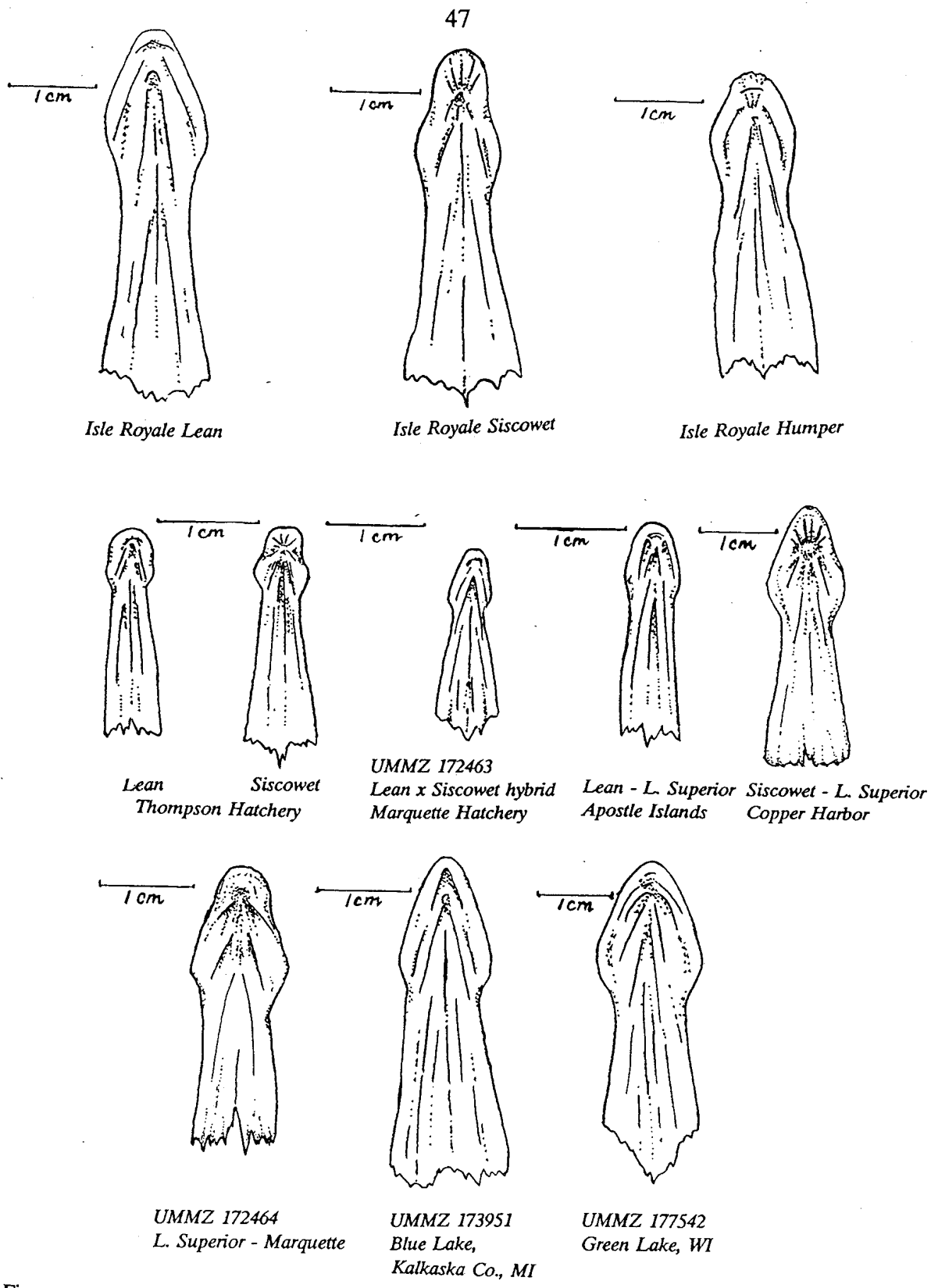
Figure 3.2. Supraethmoid bones of *Salvelinus namaycush* from Lake Superior, Thompson Hatchery progeny, and two inland lakes.

Salvelinus alpinus   *Salvelinus leucomaenis*

5 cm

Salvelinus confluentis   *Salvelinus fontinalis*

NOTCH

Salvelinus namaycush lean   *Salvelinus namaycush* siscowet

5 cm
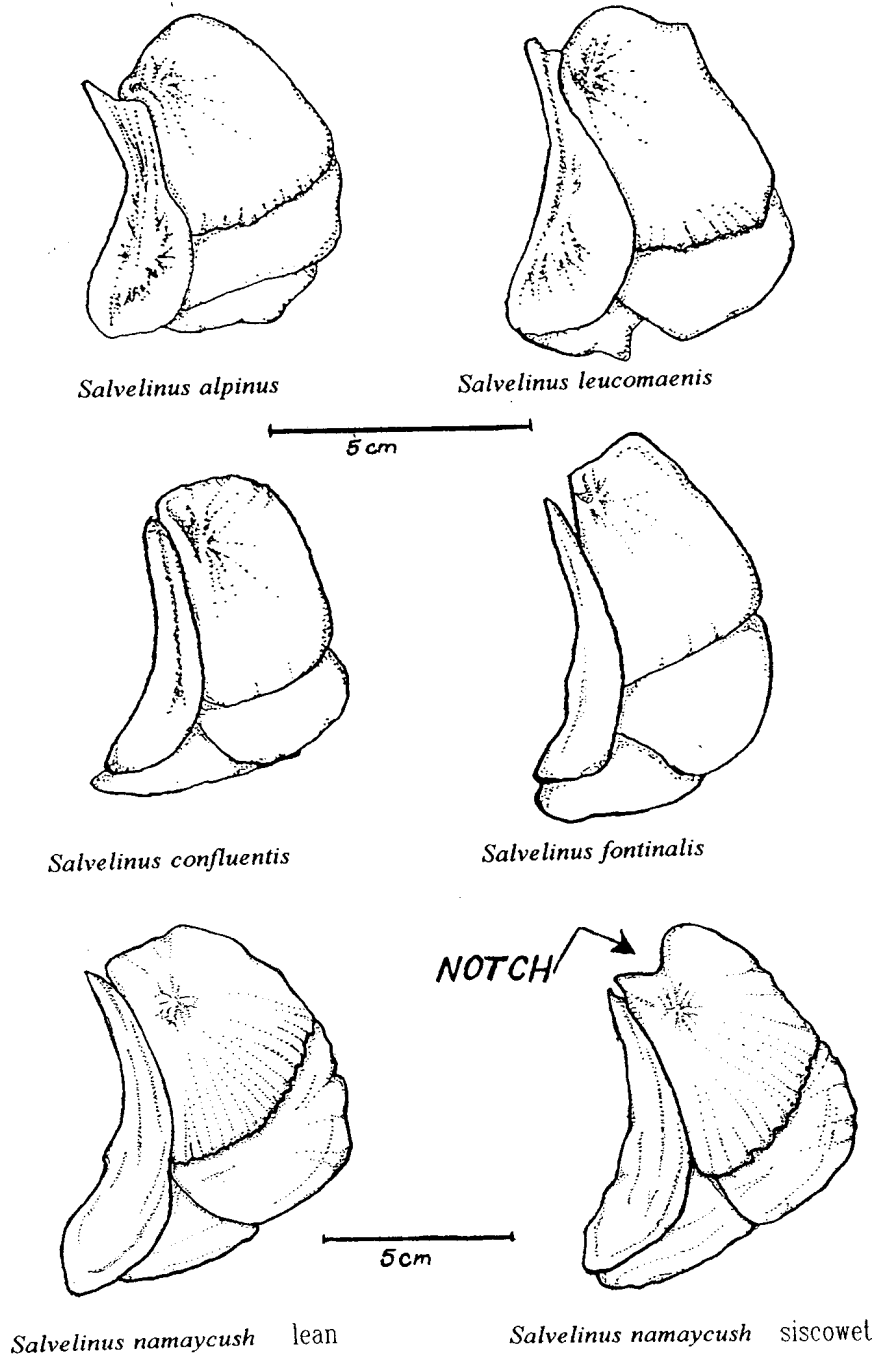
Figure 3.3. Opercle series of representative salmonid species.
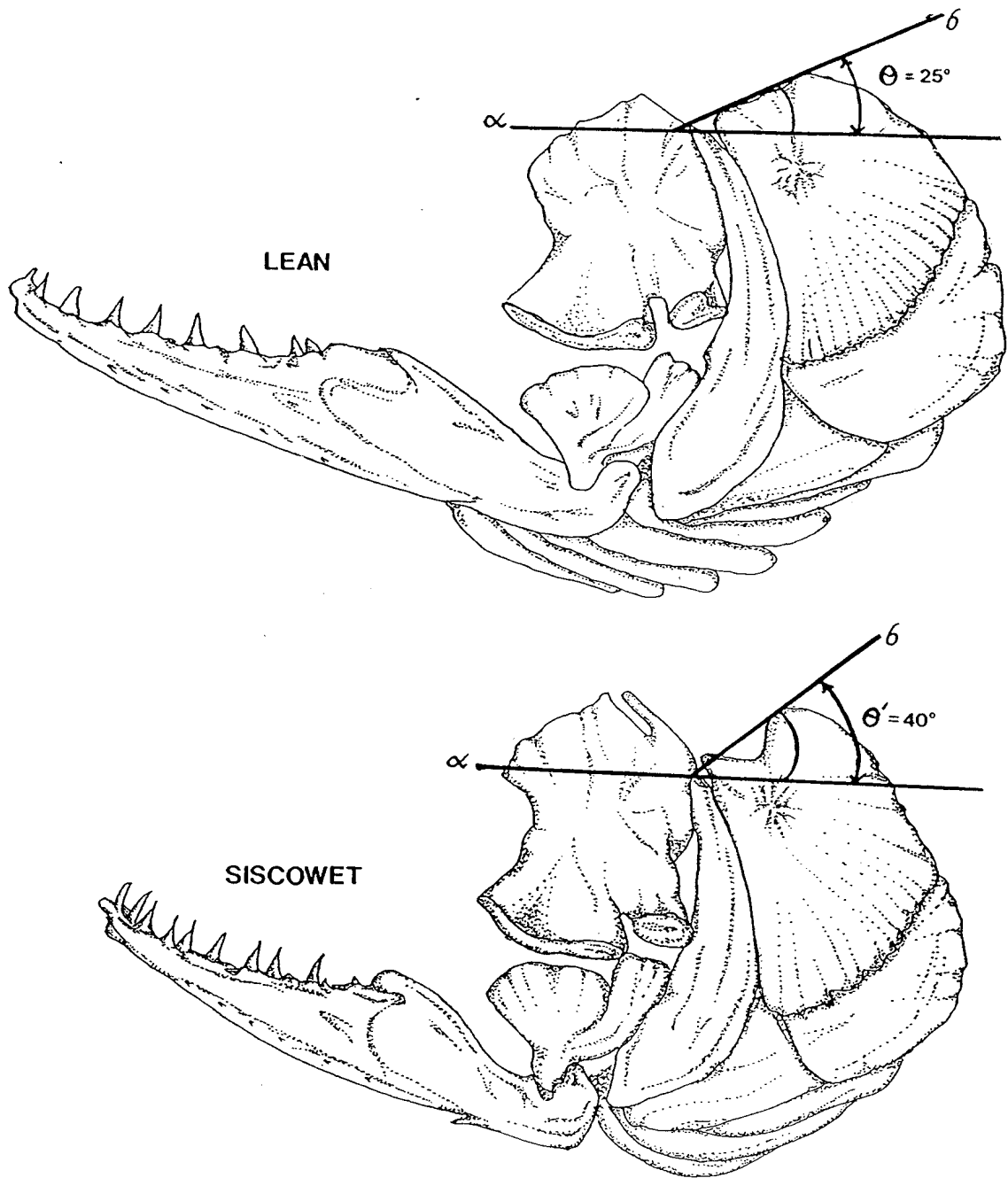
Figure 3.4.   Dermal cranial bones of lean and siscowet lake trout from Lake Superior. An angle formed by a line parallel to the top of the head at the top of the preopercle with a line drawn from the top of the opercle to the top of the preopercle is more acute on specimens which lack the opercular notch.
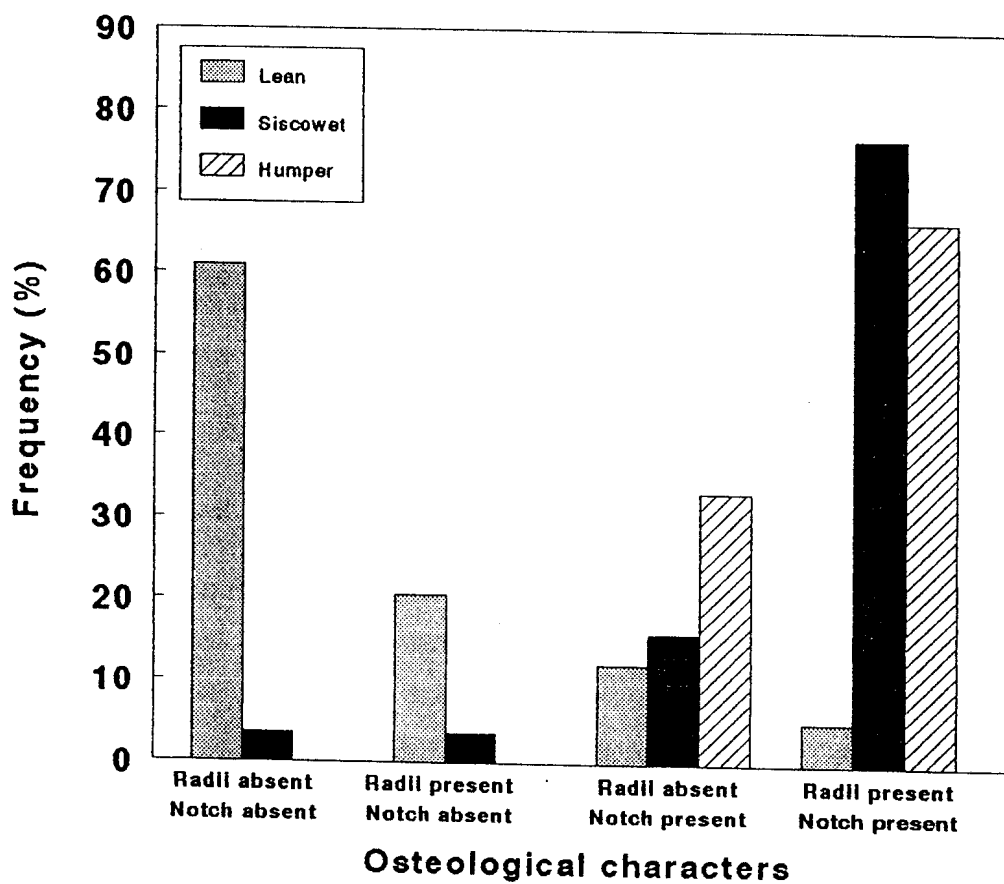
Figure 3.5. Frequency distribution of supraethmoid radii and opercular notch characters among lean, siscowet, and humper lake trout from Lake Superior. The phenotypes were identified using external morphological criteria.

Presence of Supraethmoid Radii			
	N	Percent	Stand. Dev.
Lean	24	26.1	0.2
Siscowet	111	80.4	0.1
Humper	32	66.7	0.5

Presence of Opercular Notch			
	N	Percent	Stand. Dev.
Lean	17	18.5	0.2
Siscowet	128	92.7	0.1
Humper	48	100	0

Supraethmoid radii absent, Opercular notch absent			
	N	Percent	Stand. Dev.
Lean	56	60.9	0.3
Siscowet	5	3.6	0.02
Humper	0	0	0

Supraethmoid radii present, Opercular notch present			
	N	Percent	Stand. Dev.
Lean	6	65.2	0.1
Siscowet	106	76.8	0.1
Humper	32	66.7	0.5

Supraethmoid radii absent, Opercular notch present			
	N	Percent	Stand. Dev.
Lean	11	11.9	0.1
Siscowet	22	15.9	0.1
Humper	15	31.3	0.5

Supraethmoid radii present, Opercular notch absent			
	N	Percent	Stand. Dev.
Lean	19	20.6	0.2
Siscowet	5	3.6	0.02
Humper	0	0	0

Table 3.1. Frequency of occurrence of supraethmoid radii and opercular notch among wild Lake Superior S. namaycush.

CHAPTER IV

MITOCHONDRIAL DNA VARIATION IN

LAKE SUPERIOR <u>Salvelinus namaycush</u>

Abstract

The hypothesis that lean, siscowet, and humper lake trout phenotypes are reproductively isolated was tested using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis. The results of the Thompson Hatchery study (Stauffer and Peck 1981: Chapter 2) and the consistent differences in dermal cranial osteology between lean and siscowet phenotypes (Chapter 3) provided evidence that some morphological characters have a genetic basis. MtDNA RFLP analysis revealed a hypervariable genome that showed no correspondence between mtDNA genotype and either phenotype or geographic locality. Estimates of sequence divergence based upon both fragment pattern haplotypes and mapped restriction sites ranged from 0.5% - 1.7%, similar to published estimates of intraspecific levels of divergence in other salmonids. A restriction site map is presented for Lake Superior *Salvelinus namaycush*. Cladistic analysis could not unambiguously resolve the historical relationships among mtDNA clonal lineages. Lake Superior is geologically young; the basin formed about 20,000 ybp, but in its present form was established only 8000 ybp. It is likely that mtDNA lineage sorting is incomplete among Lake Superior populations. The lack of genetic substructure and the hypervariability of the mtDNA genome in lake trout is attributed to hybridization and introgression combined with a relatively slow evolutionary rate. Accumulated genetic

differences among lean, siscowet, and humper lake trout are subtle, and are probably located in nuclear genes governing metabolism, fat storage, and growth.

Introduction

Morphological variability among populations of lake trout in Lake Superior has been shown to have a genetic basis (Eschmeyer and Phillips 1965, Stauffer and Peck 1981; Chapter 2). Although heritable differences exist in hatchery-raised progeny, it is still not clear whether the genetic differences are species-level or stock differences. Ihssen et al. (1981) defined a stock as an intraspecific group of randomly mating individuals with temporal or spatial integrity. Implicit in a stock concept is the assumption of genetic continuity within a population. An analysis of allozyme variation among putative stocks of Lake Superior lake trout (Dehring et al. 1981; Ihssen et al. 1988) failed either to provide enough resolution to detect unambiguous genetic differentiation among local populations, or to justify the application of a genetic stock concept to the divergent lake trout phenotypes. Lake trout have occupied the Superior Basin for about 8000 years since the final retreat of the Pleistocene glaciation.

The lack of extant siscowet and humper lake trout in any other deep freshwater lake throughout the range of lake trout suggests that siscowets and humpers are the products of incipient speciation. If this is true, then genetic discontinuities corresponding to a speciation event should be detectable. This hypothesis is tested by analyzing mitochondrial DNA (mtDNA) variation among populations of lean, siscowet, and humper lake trout. MtDNA variation among conspecific populations of mammals and other vertebrates has been useful in detecting genetic discontinuities due to reproductive isolation (Avise and Lansman 1983, Brown 1983, Avise 1987). MtDNA evolves virtually independently from the nuclear genome and it has a high natural mutation rate (Brown et al. 1979). These two features enable mtDNA to accumulate differences between recently

diverged populations prior to detectable differentiation of more obvious genetic characters.

The question of genetic divergence among Lake Superior *S. namaycush* was examined on two levels. The first analysis was at the level of the phenotype. Genetic differences could be either fixed differences in mtDNA fragment patterns or differences in the presence or absence of mtDNA restriction sites. If divergence has occurred consistent with phenotypic boundaries, then genetic differences may accurately reflect the hypothesized historical relationships among the lean, siscowet, and humper phenotypes. The second analysis was at the level of the mtDNA genome. The genetic relationships among variable mtDNA genomes interpreted from genetic distance estimates could provide insight into patterns of divergence. Differences in the frequency of closely related genomes among segregated populations tested alternative hypotheses of ecophenotypic divergence or isolation by distance.

Populations and species exchange mtDNA in ways that can be modelled, and each mtDNA carries the history of its lineage without complication from recombination (Wilson et al. 1985). The maternal inheritance of mtDNA provides insight into periods of introgression, hybridization, or reproductive isolation (Avise et al. 1984, Meyer et al. 1990, Meagher and Dowling 1991). MtDNA also has a stable (though not fixed) gene content and order (Anderson et al. 1981, Brown 1983, Mortiz et al. 1987); it is relatively homoplastic in somatic cells within an organism (Avise et al. 1984); it shows sequence polymorphism among conspecifics (Anderson et al. 1981); and it transmits copies maternally to progeny (Hutchinson et al. 1974, Brown et al. 1979, Lansman et al. 1981). Mutations occur randomly at a high rate, mostly due to the inefficiency of DNA repair mechanisms (Cann and Wilson 1983). Consistent with the hypothesis of inefficient DNA repair in mtDNA is the high rate of nucleotide transitions to transversions, and the high incidence of length mutations (Cann and Wilson 1983). In spite of a high rate of mutation, mtDNA shows a remarkable conservatism in gene order and function not just across species but also across phyla (Anderson et

al. 1981). MtDNA is easy to isolate due to its high copy number and its extranuclear organelle localization. MtDNA also has a buoyant density enabling it to be easily separated from nuclear DNA. The mtDNA molecule is small (in lake trout 16500 ± 200 base pairs (bp)) relative to nuclear DNA and lacks introns and repetitive sequences common in nuclear DNA (Brown 1983).

Random mutations in the mtDNA genome could have become uniquely fixed in different populations of lake trout in Lake Superior as a result of genetic drift or assortative mating. As outlined by Avise et al. (1979), each complex restriction phenotype characterized is unique, and the chances of an identical phenotype arising by convergence is remote. Shared composite mtDNA profiles are an indication of a shared evolutionary history because the mtDNA phenotypes are transmitted intact from mother to offspring such that sequence changes only arise by mutation. Mutations that become fixed in an individual result in a new phenotype that is associated with its maternal progenitor. The stability of gene order and function in mtDNA contrasts with its rapid rate of evolution at the nucleotide level and provides a unique framework for statistical analysis of intra- and interspecific genetic variation and cladistic analysis of shared, derived genetic characters.

Enzyme cleavage sequences in mtDNA have been demonstrated to be consistent among different organisms (Avise et al. 1979). Differences in the sizes of fragments obtained by digesting homologous DNA's with a specific enzyme are assumed to be an accurate reflection of sequence differences present at the restriction site (Avise et al. 1979). MtDNA can be compared using a variety of methods with varying ability to resolve evolutionary questions. Analysis of restriction enzyme fragments is useful for comparing closely related mtDNA genomes (Avise et al. 1979), but the use of fragments alone reduces the power to analyze genealogical relationships due to the uncertainty of fragment homology. Relative locations of restriction sites can be mapped using the technique of digesting the DNA with two restriction enzymes (Maniatis et al. 1982). The

position of mapped sites are unambiguous and can be interpreted as homologous characters in a genealogical or cladistic analysis.

Variation in the base composition of lake trout mtDNA has been demonstrated (Ferris and Berg 1986, Grewe and Hebert 1988, Grewe et al. 1990). Some mtDNA genotypes are more closely related to others by virtue of the number and type of base substitutions that occur. The quantitative analysis of nucleotide changes produces divergence estimates representing the degree of dissimilarity between two genomes. Under the assumption that closely related genomes have similar base sequences, pairwise comparisons of a dissimilarity index among mtDNA genotypes should reveal patterns of genetic relatedness corresponding to the relative amount of time since the genotypes diverged. Genetic dissimilarity may indicate relationship patterns among genomes but it does not adequately represent biological relationships resulting from the physical transmission of mtDNA across generations. Where phenetic analysis of genetic dissimilarity will provide information about genetic relationships, cladistic analysis based on shared, derived similarities will answer the question of historical relationships among the mtDNA lineages.

Hypotheses of Genetic Divergence

If lean, siscowet, and humper phenotypes diverged prior to recolonization of Lake Superior after the Pleistocene glacial retreat, sequence divergence estimates might reflect the amount of change since divergence over 20,000 years ago. The expected amount of change is about 0.5% per million years in salmonid fishes (Smith 1992). This would result in minimum sequence divergence estimates of about 1.0%. The different phenotypes should show corresponding fixed differences in their mtDNA, and each phenotype should have a unique sister group outside of the Laurentian basin. If genetic divergence corresponds to phenotypic divergence, dissimilarity between mtDNA genotypes will be lowest within a specific phenotype and greatest between two phenotypes. A cladistic analysis of mtDNA characters (restriction sites) should correspond to the within-

phenotype clustering of mtDNA genotypes to provide an estimation of historical relationships congruent with genetic relationships.

Phenotypic differentiation alternatively could have resulted from intralacustrine divergence due to lake level fluctuations (Mayr 1942, Stankovic 1960, Kohzov 1963, Smith and Todd 1984). Lowering water levels could have isolated populations in basins within which stochastic processes and localized selective forces would have then caused divergence from other isolated populations. Sequence divergence estimates would show divergence less than 12000 years ago and may reflect geographic population subdivision. Sequence divergence among populations of conspecifics would be low because of the short time since recolonization of the lake. Patterns of divergence would also show evidence of bottlenecks in the form of localized reductions in gene diversity resulting from habitat reduction and expansion (Bernatchez et al. 1989). If genetic divergence corresponds to geographic divergence, then dissimilarity between mtDNA genotypes will reflect vicariant patterns of divergence. A *vicariant* pattern of divergence (*sensu* Eldredge and Cracraft 1981, after Wagner 1869 and Romanes 1886) is a pattern of segregation in which closely related species tend to replace each other geographically as ecological "vicars". In this way, new species form as a direct result of geographic differentiation followed by reproductive isolation (similar to "allopatric speciation" of Mayr 1942). Populations in close geographic proximity should have the most similar genomes and widely separated populations should have very different genotypes. Cladistic hypotheses would correspond to vicariant patterns of isolation in which sister groups occupied adjacent basins. Analysis of mtDNA characters corresponding to this model would reveal a pattern of historical relationships influenced by isolation in separate lake basins. Sister groups would be geographic equivalents rather than ecological equivalents.

A third alternative is micro-allopatric or parapatric divergence resulting from ecophenotypic differences involving habitat choice or the efficiency of habitat use. Survival of an organism in a complex environment is enhanced by

the ability of that organism to successfully exploit available resources. The evolution of stable alternative phenotypes ("alternative adaptations" of West-Eberhard 1986) could precede genetic divergence. The alternatives may be the result of complex adaptations to different niches, but they originate from the same genome. Genetic divergence could occur later as the alternative phenotypes are gradually improved by selection and as the regulatory genes evolve under the influence of local selective forces. Restricted or specialized habitat choice would further enhance assortative mating and eventually lead to lineage evolution and to complete reproductive isolation (West-Eberhard 1986). Sequence divergence estimates would be consistent with time since divergence less than 8000 years ago. Variation present in the mtDNA genome due to either recent gene flow or ancestral polymorphism would be reflected in a lack of fixed variation or a lack of correlation between genetic and phenotypic divergence. Hypothesized cladistic relationships would indicate sister groups as ecological equivalents rather than geographic equivalents.

In this study I chose a combination of 13 polymorphic restriction enzymes with tetrameric, pentameric, and hexameric recognition sequences to evaluate the mitochondrial DNA characteristics of phenotypically divergent populations of lake trout from Lake Superior. I constructed a restriction site map for comparison with other studies of mtDNA diversity in *S. namaycush*. I estimated levels of genetic distance and phylogenetic relationships among mtDNA genotypes for various lake trout populations to discover if patterns of genetic relatedness reflect historical relatedness according to the intralacustrine speciation hypothesis.

Methods

Collection of Specimens

Lake trout were collected in variable mesh gill nets and bottom trawls. Gill net collections were made by the U.S. Fish and Wildlife Service (USFWS), Michigan Department of Natural Resources (MIDNR), Wisconsin Department of

Natural Resources (WDNR), Minnesota Department of Natural Resources (MNDNR), and commercial fishermen in U.S. waters of Lake Superior (Figure 4.1). Bottom trawl collections were made by the USFWS in spring forage assessments in U.S. and Canadian waters of Lake Superior. Bottom trawl collections were made in 15- to 150-meter depths (across-contour) using a 12-meter balloon trawl with 1x2-meter doors. Gill nets fished by USFWS, MIDNR, WDNR, and MNDNR were variable mesh, multi-filament nylon gill nets, 51- to 114-mm stretched measure. Nets were fished on the bottom in 1- to 3-night sets from 15- to 125-m depths. Commercial fishermen fished 114- to 152-mm extended measure mono- and multi-filament nylon gill nets on the bottom in 3- to 5-night sets at greater than 110-m depths.

Lengths and weights were taken in the field for all fish as conditions permitted, and livers were removed and frozen prior to mtDNA extraction. Animals were sacrificed in the field and liver samples were acquired. Liver samples taken on commercial vessels in Copper Harbor, MI and Port Wing, WI were flash frozen in liquid nitrogen prior to transport. Liver tissue taken at other locations was packed in wet ice for transport. Liver tissue was subsequently stored in a -70° C freezer at the University of Michigan Museum of Zoology until preparation for cell fractionation and mtDNA extraction. Whole fish specimens were transported on wet ice and frozen, or were preserved in 10% buffered formalin for morphological study.

Identification criteria

Lake trout specimens were assigned to "lean," "siscowet," or "humper" phenotype categories on the basis of a combination of several external morphological characteristics used by fisheries managers and commercial fishermen. Lake trout were considered to be "leans" if they had a straight, pointed snout and slender body. Lake trout were considered to be "siscowets" if they had a convex snout (bent over the eye) and a deep body. Lake trout were considered to be "humpers" if they had a disproportionately large eye and a thin

abdominal wall. Humpers had facial characteristics similar to the lean phenotype, and they lacked the excessive visceral fat of the siscowet phenotype. In some cases, identification of leans and siscowets was difficult and gross observation of the amount of visceral and intramuscular body fat was utilized as an additional criteria for identification. Siscowets had a much greater amount of visceral body fat (lining the dorsal wall of the visceral cavity) than leans. In addition, the excessive fat in the muscle tissue of siscowets was easily observed by squeezing the flesh between one's fingers. Fisheries managers and commercial fishermen often targeted siscowet populations based on depth of capture. Management regulations restricted state-licensed gill net fishing to depths greater than 60 fathoms (109.8 meters). All fish collected at depths greater than 110 meters by commercial fishermen in this study (Copper Harbor, Port Wing, Duluth) consistently possessed "siscowet" characteristics. Around Isle Royale, leans, siscowets, and humpers were taken in the same nets, set across-contour, but with only slight overlap in depths. I observed that if leans and siscowets were both taken from the deeper water, siscowets did not bloat as severely as leans when brought to the surface.

Mitochondrial DNA Restriction Analysis

Characterization of mtDNA variation was performed in this study using Type II restriction endonucleases which cleave phosphodiester bonds in both strands of the DNA at specific tetra-, penta-, and hexameric nucleotide sequences. A mitochondrially-enriched fraction of a tissue homogenate was prepared using the procedure of Lansman et al. (1981) and Maniatis et al. (1982) with some slight modifications (Appendix A). Animals were sacrificed in the field and liver tissue was removed and placed on wet ice for no more than 10 days, or flash frozen with liquid nitrogen when available. Greater yields of mitochondria were attained by keeping liver tissues on wet ice for at least 2 days before processing or freezing at -70°C. This seemed to weaken the cell membranes and enhance

release of mitochondria during homogenization. Tissues which were flash frozen in liquid nitrogen provided the purest mtDNA fractions.

Between 0.5 and 1.0-grams of liver tissue per sample were homogenized in cold buffer for two 10-second bursts. Two slow speed centrifugations were required to remove significant amounts of cellular debris and lipids. Mitochondria were lysed in detergent (20% SDS) and samples were subject to an additional centrifugation step to pellet membranes and cellular debris. In most siscowet samples the lipid content of the homogenate appeared to be much greater than comparable volumes of lean or humper tissue, and excess lipid was lifted from centrifuge tubes with a sterile wipe prior to adding lysate. Samples with high lipid content in the homogenate yielded less purified mitochondrial DNA after ultracentrifugation and were often contaminated with nuclear background when analyzed with ^{32}P end-labeling and electrophoresis. Samples were run through a clean 23-gauge hypodermic needle to shear large nuclear strands and reduce significant nuclear background. Mitochondrial and nuclear DNA fractions were separated using cesium chloride density gradient centrifugation. The best purification results were obtained by performing a velocitization prior to the second density gradient step on the mtDNA sample collected from the first density gradient step. Velocitization produced a concentration of mitochondrial and nuclear DNA to the exclusion of most of the buoyant proteins so that separation was more distinct in the second density gradient.

Mitochondrial DNA samples (2 - 10 ng/μl) were digested according to the manufacturer's recommended conditions (New England Biolabs, Beverly, MA). If electrophoresis was not performed immediately after incubation, digested samples were stored at -20°C prior to electrophoresis. The method of thawing and re-freezing samples was avoided because degradation of the mtDNA appeared to be rapid in samples in which lipid content was high.

MtDNA fragments were radioactively end-labeled with the large fragment (Klenow) of DNA polymerase I and ^{32}P-αdATP, ^{32}P-αdTTP, ^{32}P-αdGTP, and ^{32}P-

αdCTP (Brown 1980). Each sample was electrophoresed through agarose gels (0.8 - 1.2%) and polyacrylamide gels (3.5 - 4.0%) for 14-18 hours. Gel concentrations were adjusted up or down for different enzymes to best visualize smaller and larger fragments. Gels were adhered to 3-mm Whatman chromatography filter paper with heat and vacuum, and digestion profiles were visualized by autoradiography (Maniatis et al. 1982). X-ray film was exposed to the gels for 2-14 days depending upon the strength of the nucleotides and the concentration of sample DNA. Tetrameric digestion profiles were exposed at room temperature without intensifying screens to reduce blurring. Pentameric and hexameric digestion profiles were exposed at -70°C with one or two intensifying screens as needed.

Fragment and genome sizes were estimated in comparison to standard molecular weight markers provided by Hind III digestion of Λ DNA and Hae III digestion of ΦX DNA. Sequence divergence estimates were calculated from composite fragment patterns which defined fragment haplotypes (Nei and Li 1979, Nei and Tajima. 1983). Haplotype labels were assigned according to previously published data (Grewe and Hebert 1988, Grewe 1991), or in order of decreasing frequency of the fragment pattern.

Maps of DNA restriction endonuclease cut sites were constructed by preparing digests with two restriction enzymes and comparing fragment sizes to determine the relative position of restriction sites. I mapped restriction sites using double digests for Ava I, Ase I, and BspH I. I could not accurately map the sites for Acc I, Ava II, and EcoO109 because of the number of small fragments. I confirmed the position of restriction sites for BamH I, Hind III, EcoR I, BstE II, Pst I, Nco I, Xba I, Bcl I, Pvu II, Sma I, Sal I, Bgl II, Sst II, and Xho I, previously mapped by Grewe and Hebert (1988), with independent mapping using double digests. I compared mapped positions of restriction sites to published maps (Grewe et al. 1990) for congruence. The presence or absence of a composite set of restriction sites defined unique clonal haplotypes. The presence/absence data

was used as input for calculating estimates of sequence divergence and cladistic analysis.

Data Analysis

Genetic relatedness among mtDNA fragment phenotypes were estimated by calculating sequence divergence based on shared fragments with the FRAGDIFF program (Hagen, unpublished) and the equations of Nei and Tajima (1983) and Nei and Li (1979). Divergence estimates between mtDNA fragment phenotypes were examined in all pairwise combinations. Genetic relatedness among mtDNA genotypes were estimated by calculating sequence divergence based on mapped restriction sites according to the equations of Nei and Tajima (1983) using the SITEDIFF program (Hagen, unpublished).

Distance matrices calculated from fragment data were condensed with the unweighted pair-group method using an arithmetic average (UPGMA) (Sneath and Sokal 1973) to look for patterns of genetic similarity corresponding to phenotype or geography. For this I used the average distance option of PROC CLUSTER in the SAS statistical package (SAS Institute 1989). Distances from site data were also analyzed by UPGMA to look for patterns of genetic similarity in the distance measures. A cutoff level of $\delta = 0.008$ (0.8% sequence divergence) was used to define major clusters in the fragment data and a cutoff of 0.5% was used for the site data. The frequencies of the major clusters were mapped by phenotype and geographic area to illustrate geographic haplotype distribution.

Outgroup species used in the fragment and site analyses included *S. namaycush* with a lean phenotype from arctic Canada and Alaska as lake trout sister groups from outside of the Laurentian Great Lakes basin, and *S. fontinalis* as a congeneric outgroup. The outgroup species for the site analyses included a sample of *S. namaycush* from isolated freshwater lakes which have no current connection to the Lake Superior basin. This sample was composed of a single lake trout from the Kenai Peninsula, Alaska and 9 lake trout from 3 isolated lakes

in Arctic Canada (Table 4.1). The second outgroup used was *S. fontinalis*, the brook trout, based upon data which places *S. fontinalis* as the sister group to *S. namaycush* (Cavender 1980, Grewe et al. 1990, Phillips and Pleyte 1991). Restriction site positions from published data (Grewe et al. 1990) were added to the cladistic analysis for the bull trout, *Salvelinus confluentus*, to address data which place *S. confluentus* as the sister group to *S. namaycush* (Smith and Stearley 1989, Stearley 1992). Mapped sites for *S. confluentus* were interpreted from Grewe et al. (1991), and I mapped the sites for *S. fontinalis*.

The site data was analyzed cladistically to see if the genetic relationships correspond to historical relationships. If the evolutionary rate is uniform for *S. namaycush* in Lake Superior, then we might expect the phenetic and cladistic methods to produce similar trees. Presence/absence matrices of 77 mapped restriction sites were analyzed cladistically using the parsimony method (*Hennig86*; Farris 1988). Cladistic analysis used restriction site changes, relative to outgroup species, to classify taxa by the sequence of branching of lineages of genealogical descent. The classification is made by homology rather than similarity and is used in this analysis to estimate historical relationships (Hennig 1966).

Nucleon diversity and nucleotide diversity were calculated as estimates of heterogeneity for the mtDNA genome among groups defined by phenotype (i.e. lean, siscowet, and humper); by locality (i.e. north, west, southwest, south, and east); and by individual populations. Nucleon diversity (Nei and Tajima 1981, equation 6) is a measure of the heterogeneity of a group of populations based on the frequency of different nucleomorphs or clonal haplotypes. This measure provides a way to look at the genetic diversity among the populations. Haplotype frequency differences among populations reflect segregation of populations. Nucleotide diversity is another measure of heterogeneity based on the number of restriction site differences (nucleotide differences) between haplotypes under the assumption that differences are due to base substitutions (Nei and Tajima 1981, equation 17). This measure of heterogeneity provides a way to look at genome

diversity, or how many different mtDNA genomes are present in a population. Interpopulational diversity was estimated by inspecting the net restriction site differences between phenotypes, between localities, and between individual populations. If reproductive isolation occurs, the net restriction site differences between groups should be higher than the mean restriction site differences within groups.

The significance of interpopulational differences was tested using the G_{st} statistic (Takahata and Palumbi, 1985) which is similar to Wright's F_{st} (Wright 1978) as applied to haploid data. The G_{st} is an estimate of the fraction of the genetic variation within an entire population that is due to interpopulational genetic differences (Nei 1975). Identity probabilities were calculated by phenotype, by locality, and by population for within groups (I of equation 17, Takahata and Palumbi 1985) and between groups (J of equation 19, Takahata and Palumbi 1985).

Results

Size of the Mitochondrial DNA Genome

The mitochondrial DNA genome of *Salvelinus namaycush* from Lake Superior averaged 16,741 ± 219 base pairs. This estimate was similar to previously published estimates (Berg and Ferris 1984, Gyllensten and Wilson 1986, Grew and Hebert 1988). Minor length variations were observed using the tetrameric restriction enzyme HinP I. There was no evidence of heteroplasmicity in the mtDNA of Lake Superior *S. namaycush*.

Restriction Fragment Analysis

Fragment data were acquired using 13 restriction enzymes found to be polymorphic in these lake trout (Table 4.2). The restriction products entered into the fragment analysis were from the following enzymes: Sau3A I, Msp I, HinF I, α-Taq I, and Ava II. Products of enzymes Acc I, Eco0109, Nci I and HinP I were excluded from the fragment analysis because the fragment patterns were highly variable, widely dispersed, and showed multiple reversals among clonal haplotypes. Minor length variations detected with the HinF I restriction enzyme were excluded from the analysis. The pentameric and hexameric restriction enzymes were mapped and analyzed in the restriction site analysis. Restriction sites for the tetrameric enzymes, and Acc I, Ava II, and Eco0109 could not be estimated or accurately mapped using double digests because of the large number of small fragments.

The seven unmapped polymorphic restriction enzymes produced 179 unique composite restriction fragment patterns for 302 individuals. When fragment patterns for the mapped restriction enzymes were included, the number of distinct clonal haplotypes increased to 264. There were no fixed differences among the restriction fragment patterns which corresponded to phenotype or to geographic location. Many of the patterns were represented by only one individual, and were inferred to be the result of loss or gain of 1 restriction site from the most closely related fragment pattern. The composite fragment haplotypes are listed in Table 4.3. Pairwise sequence divergence estimates ranged from δ = 0.0005 - 0.0159 (0.05% - 1.59%). The average sequence divergence estimate between *S. fontinalis* and the sample of *S. namaycush* was 3.87%, supporting its position as a sister group (Grewe et al. 1990). The nine Arctic Canadian individuals shared a composite fragment haplotype which was also common to a group of six siscowets from Lake Superior. The lean phenotype from Alaska shared a composite fragment haplotype common to a group which included leans, siscowets, and humpers.

The restriction enzyme fragments revealed a hypervariable mtDNA genome in Lake Superior lake trout. Data showed neither correspondence between phenotype and fragment pattern, nor correspondence between sample locality and fragment pattern. Calculated sequence divergence estimates were comparable to similar RFLP analyses of *S. namaycush* (Grewe and Hebert 1988, Grewe et al. 1990, Grewe 1991). Figure 4.2 shows the geographic distribution of fragment haplotypes grouped by UPGMA clustering. UPGMA was used to reduce the matrix of 15,931 pairwise distance indices from 179 phenotypes into three major clusters (see Appendix B for matrix of pairwise sequence divergence estimates for fragment data). The clusters are identified by setting a cutoff for sequence divergence estimates at 0.8% ($\delta = 0.008$). Table 4.4 lists the fragment haplotypes from Table 4.3 included in the A, B, and C clusters. Frequency differences are evident between lean and siscowet populations in the east and southwestern localities, but no phenotype or locality uniquely possesses any fragment haplotype. Fragment haplotype alone is insufficient to characterize lean, siscowet, or humper lake trout.

The "D" fragment pattern derived from the tetrameric enzyme Ava II was differentially distributed geographically among North American lake trout. Frequency differences were also detected among the three Lake Superior phenotypes. Samples of *S. namaycush* from eastern North America had a higher frequency of the "C" fragment pattern and the "D" pattern was virtually non-existent (Grewe 1991). In Lake Superior, the "C" pattern was completely absent while the "D" pattern was widely distributed among all phenotypes in the lake. Fifty-four of the 179 fragment patterns (30%) were of the Ava II "D" pattern. The distribution of the Ava II "D" fragment pattern among the three phenotypes in Lake Superior is illustrated in Figure 4.3. The differences in the distribution of Ava II "D" between the three phenotypes were not statistically significant (P > 0.05). The frequency distribution of all restriction enzyme clonal haplotypes are listed in Table 4.5.

Restriction Site Analysis

Fifteen unique clonal haplotypes were produced from the mapped restriction sites (Table 4.6). Of these, ten were represented by only one to four individuals. Of 77 mapped sites, only 8 sites were polymorphic among the Lake Superior lake trout. Figure 4.4 illustrates the locations of all currently mapped restriction sites. The location of the D-loop was interpolated from the map published by Grewe et al. (1990). There was no correspondence between restriction site haplotype and either phenotype, or geographic locality. There were no fixed differences corresponding to phenotype that would enable discrimination among lean, siscowet, and humper based on mitochondrial DNA restriction site profiles. In addition, no unique clonal haplotypes existed among the arctic Canadian or Alaskan lake trout samples.

Pairwise sequence divergence estimates among mtDNA genotypes identified by mapped sites ranged from $\delta = 0.0015$-0.017 (0.15% - 1.7%). The estimated genetic distance between *S. namaycush* and the *S. confluentus* and *S. fontinalis* outgroups was 0.0425 (4.25%). The dissimilarity present among the clonal types had absolutely no correspondence to phenotype or locality. Not only were there no fixed genetic differences in mtDNA that correspond to phenotype, there were no fixed differences between the three widely allopatric populations sampled.

UPGMA clustering reduced the matrix of 136 pairwise sequence divergence estimates from 17 restriction site haplotypes into 3 major clusters (Figure 4.5). The cutoff for sequence divergence was about 0.5% ($\delta = 0.005$). Figure 4.6 shows the distribution of three major mtDNA clonal groups in Lake Superior. The frequency of the three clonal groups are similar for leans, siscowets, and humpers from the northern localities. The siscowets sampled from the western locality had a higher frequency of the "A" group than siscowets from other localities. In the southwest locality leans and siscowets showed similar frequencies of the "A" group relative to the "B" group, but no siscowets were found in the "C" group from the southwest localities. This may be due to the

small sample size of siscowets from the southwest (n=8). Frequency distributions among leans and siscowets from the southern locality were similar. The eastern locality showed the greatest differences in frequency of haplotype groups, and, as with the siscowets from the western locality, no eastern siscowets fell into the "C" haplotype group. Leans in the east had a higher frequency of the "A" group than siscowets, but the difference again may be due to small sample size (lean n=9, siscowet n=8). Overall, there is no correspondence between mtDNA genotype and phenotype or geographic locality.

Haplotype Frequency Distribution

The frequency distribution of restriction site haplotypes are presented in Figure 4.7 a-b, grouped by phenotype and locality. In these analyses, frequency distributions among phenotypes and among individual populations were not significantly different, but distributions among geographic locations were significantly different at $P < 0.005$.

Cladistic Analysis of MtDNA Genotypes

A presence/absence matrix of mapped restriction sites was analyzed cladistically to see if patterns of phylogenetic relationships could be uncovered that would clarify the above patterns of genetic relationships. Figure 4.8 represents the consensus of 108 equally parsimonious trees of length 38. Of the 77 mapped sites 42 were plesiomorphic and 9 mapped sites are assumed to have synapomorphic character states for *S. namaycush*. Within the *S. namaycush* clade, most of the clonal haplotypes formed an unresolved polytomy, and the most derived taxa were diagnosed by single restriction site changes. The unresolved polytomy may be due to at least one reversal at each of three restriction sites among the included haplotypes as terminal taxa. There was no correspondence between mtDNA genotype and phenotype in the cladistic analysis.

Restriction Site Diversity

Nucleon diversity by phenotype ranged from 0.83 to 0.87 (mean diversity = 0.86) (Table 4.7). Nucleon diversity analyzed by locality showed a range of 0.76 in the east to 0.91 in the southwest (Table 4.8). The values for nucleon diversity within individual populations ranged from 0.73 for siscowets in the southwest (Apostle Islands) to 0.90 for leans from the north (Isle Royale) with an overall mean of 0.84 (Table 4.9).

Variation within the mtDNA genomes estimated by nucleotide diversity for lean, siscowet, and humper phenotypes (Table 4.7) was low, ranging from 0.0030 to 0.0031. Nucleotide diversity was slightly lower by locality than by phenotype, but the differences were so minute as to be insignificant (Table 4.8). The nucleotide diversity estimates among individual populations showed a pattern similar to that among localities. The lowest nucleotide diversity estimates occurred for siscowets from southwest Lake Superior (Apostle Islands), while the highest estimates were for leans from southwest Lake Superior (Table 4.9). There did not appear to be any phenotypic or geographic pattern to the observed nucleotide diversity.

Interpopulation variation measured by differences in net restriction sites was highest between lean and humper lake trout (Table 4.10). The net restriction site differences between leans and siscowets was similar to that between siscowets and humpers. The greatest net restriction site differences occurred between the eastern and southern Lake Superior localities ($d^\wedge = 0.66$), and northern and western localities ($d^\wedge = 0.57$) (Table 4.11). The net restriction site differences ranged from -6.30 to 3.69 in pairwise comparisons of individual populations (Table 4.12). The results (particularly the negative values) were indicative of the close genetic relationship among lake trout populations in Lake Superior (Nei and Tajima 1983). The values for mean restriction site variations within phenotypes, within localities, and within populations are greater by an order of magnitude than the net interpopulational differences.

The overall variation attributed to interpopulational variability estimated with the G_{st} statistic was 0.0005 (0.05%) when arranged by phenotype (Table 4.13). When G_{st} was calculated by locality, 1.68% of the observed variation was attributed to geographic variation (Table 4.14). When G_{st} was calculated for the individual populations, 1.5% of the variation was allocated to interpopulation genetic differences (Table 4.15).

Discussion

The mitochondrial DNA genome of *Salvelinus namaycush* is hypervariable and lacks substructure corresponding to phenotypic differentiation. Using 13 polymorphic restriction enzymes, no fixed differences in composite restriction sites were detected which corresponded either to phenotypic groups or to geographic groups. The close genetic relationship among populations with observable morphological differences suggests that extensive gene flow has occurred either within post-glacial Lake Superior, or in the glacial refugia. Gene exchange in glacial refugia (ancestral polymorphism) is supported by the existence of common haplotypes between *S. namaycush* from isolated arctic lakes and lake trout from different geographic populations in Lake Superior. Most of the observed genetic diversity in mtDNA was present within populations, and there was little or no divergence between populations. When the results of intra- and interpopulational comparisons of heterogeneity are compared to those of Nei and Li (1979) and Nei and Tajima (1981) it is apparent that most of the variance is allocated to intrapopulation variation rather than interpopulation variation.

The lack of genetic substructuring either by phenotype, or by geographic locality forces the acceptance of the null hypothesis that lean, siscowet, and humper populations in the wild are not reproductively isolated. The levels of genetic diversity and the haplotype distribution do not allow species-level discrimination. If allopatric differentiation of the three phenotypes occurred in isolated glacial refugia, we would have expected some fixed genetic differences in

the mtDNA of the extant populations as a result of founder effect (Templeton 1981), or population bottlenecks.

The extant phenotypes showed no evidence of historically reduced diversity, and the frequency of the most common haplotypes among the three phenotypes was not significantly different. In contrast, other *S. namaycush* populations derived from a small number of hatchery founders showed decreased mtDNA diversity (DeSilva 1989, Grewe et al. 1990, Grewe 1991). The survey by DeSilva (1989) showed fixed differences among three inland populations derived from hatchery transplants. In comparison, DeSilva found much higher levels of gene diversity among wild Lake Superior *S. namaycush*. Similar discrepancies between inland lakes and the Laurentian Great Lakes were supported in a survey of mtDNA diversity by Grewe (1991), in which *S. namaycush* of hatchery origin among lakes in eastern North America (Manitou Lake, Killala Lake, Seneca Lake) showed genotype frequency differences sufficient to allow stock discrimination. Some genotypes commonly found in wild populations of the progenitor stock were absent in those transplanted populations. The differential distribution of the Ava II "D" fragment pattern among Lake Superior phenotypes suggests that the Lake Superior lineages were isolated from the lineages which recolonized eastern North America, but there is no evidence for reproductive isolation among the Lake Superior phenotypes.

The occurrence of common mitochondrial DNA clonal haplotypes based on mapped restriction sites in all phenotypes and geographic locations could be consistent with four models: (1) Sampled populations were subdivisions of a panmictic spawning population; (2) MtDNA diversity arose from *in situ* substitutions (Gyllensten and Wilson 1986); (3) Widespread ancestral polymorphism was retained in recently subdivided populations (within the last 10,000 years). Lineage sorting in this model is incomplete because population sizes during colonization were large enough to support co-occurrence of ancestral composites and their derivatives. Thus widespread diversity developed prior to genetic isolation (Kornfield and Bogdanowicz 1987); or (4) Recent or ongoing

gene flow occurred among localized spawning populations through migration and interbreeding.

The first model is unlikely because there are documented behavioral differences among phenotypes in time and place of spawning (Eschmeyer 1957, Eschmeyer and Phillips 1965, Rahrer 1975). The absence of fixed differences among populations of *S. namaycush* in Lake Superior does not itself support the conclusion that the samples were taken from one genetically homogeneous population (Utter 1981). The number of females necessary to maintain effective mtDNA gene flow is not particularly large (Allendorf 1983), and random hybridization due to straying among spatially or temporally segregated populations could have produced the observed pattern. The second alternative is unlikely because even widely allopatric populations have a number of haplotypes common to Great Lakes populations. The occurrence of convergent complex composite restriction site profiles by random mutation alone is unlikely (Avise et al. 1979). Shared composite restriction site haplotypes reflect a shared history.

The data in this study are not sufficient to provide a basis for selecting between the last two models which are related to historical contact. It is neither necessary, nor sufficient to invoke retained ancestral polymorphism or gene flow as the sole source of mtDNA variation among Lake Superior *S. namaycush*. The 8000 years since colonization of the Lake Superior basin has not been long enough to allow lineage sorting and significant divergence of the mtDNA genomes that corresponds to morphological and ecological divergence. Current estimates of the rate of evolution of salmonid mitochondrial DNA are 0.5% per million years (Smith 1992). Estimates of sequence divergence calculated from lake trout mtDNA restriction fragments average around 0.01 to 0.05%. If the rate of divergence is near 0.5% per million years, the observed levels of diversity support the recent evolutionary history of the Great Lakes *S. namaycush* lineages. The divergence of Lake Superior lake trout populations has occurred within the last 20,000 years.

The lack of correspondence of unique clonal haplotypes to location or population is consistent with contemporary gene exchange. Interbreeding and backcrossing could easily explain the widely shared mtDNA genotypes among lean, siscowet, and humper phenotypes. In Lake Superior, sizes of inshore and offshore populations in the 1960's and 1970's declined far enough that natural reproduction was inadequate to sustain many stocks in U.S. waters except in limited areas (Swanson and Swedberg 1980). One explanation for this is that the abundance of spawners remained below some threshold level necessary to ensure sufficient reproduction (Curtis 1990). It is reasonable to assume that if wild population sizes were reduced severely, a bottlenecking effect may be detected in the mtDNA. Lake trout of hatchery origin and populations of lake trout transplanted in inland lakes show reduced levels of diversity directly attributable to a small number of founders (DeSilva 1989, Grewe 1991). There was no evidence of any bottleneck or reduction in diversity among any of the *S. namaycush* populations sampled. Although spawning times and places are different, they do overlap, and there is ample opportunity for random interbreeding to occur. Overlap during spawning and interbreeding could have occurred as populations expanded during periods of recovery. Although behavioral and temporal-spatial differences in spawning exist, straying is known to occur in homing fish.

Heterozygosity measures reflect the partitioning of most of the mtDNA variation into the within-population component. The distribution of diversity into between- and within-population components complements a study of allozyme variation in Lake Superior lake trout which allocated 94% of the observed variation in protein loci to within-population variation (Dehring et al. 1981). Though slightly smaller in magnitude, allozyme variation in salmonids produced similarly high measures of heterozygosity in which over 98% of the observed variation in mtDNA was attributed to within-population variation (Gyllensten 1985, Gyllensten and Wilson 1987). The variation in this study represented the variability of only 8 of 77 mapped restriction sites. In samples from outside Lake

Superior, heterozygosity declined sharply. No variability was detected among lake trout samples from three isolated Arctic lakes. *Salvelinus namaycush* from lakes in northeastern North America showed low levels of heterozygosity with similar levels of sequence divergence (Grewe 1991). Selection on ecophenotypic traits did not significantly affect the distribution of mtDNA clonal haplotypes among Lake Superior leans, siscowets, and humpers. The pattern of diversity represented by mapped restriction sites in the mtDNA genome was primarily due to random mutation and lineage survival, yet the distribution of the Ava II "D" fragment pattern suggests either differential selection or, more likely, clinal dispersal of phenotypes.

Ecophenotypic Variation and Mitochondrial DNA Diversity

The distribution of mtDNA clonal haplotypes based on mapped restriction sites among different lake trout phenotypes indicates that an incompatibility barrier has not yet developed between the mtDNA and nuclear genotypes. The Thompson Hatchery study provided evidence that phenotypic differences have a genetic basis. Fat content is clearly different between leans and siscowets as shown by Eschmeyer and Phillips (1965) and Stauffer and Peck (1981). However, those differences are not reflected in the mtDNA genome. The accumulated genetic differences are few, and are probably located in the nuclear genome.

Phenotypic variability that corresponds to environmental differences is often considered to be a form of phenotypic plasticity. Some forms of plasticity can be correlated to trophic differences (Turner and Grosse 1980, Wimberger 1991), while others are considered alternative phenotypes with a variety of causes and interactions (West-Eberhard 1989). Phenotypic divergence among *S. namaycush* in Lake Superior is not due to plasticity. The characters of interest-- fat content, body depth, spawning time--are transmitted intact across generations, and are not affected within a generation by environmental alterations. These characters have been shown to be heritable in lake trout (Eschmeyer and Phillips 1965, Stauffer and Peck 1981: Chapters 2 and 3). Despite the hypervariability in

the lake trout mtDNA genome, there is no evidence of reproductive isolation among lean, siscowet, and humper lake trout phenotypes in the wild.

Morphological variation accompanied by low genetic diversity is common among fishes. The anadromous salmon of the Pacific Northwest (Aro and Shepard 1967; Atkinson et al. 1967; Aspinwall, 1974; Chilcote et al. 1980; Utter 1981; Taylor and McPhail 1985; Wilson et al. 1985; Wehrhahn and Powell 1987; Utter et al. 1989; Beacham 1990; Kartavtsev 1992) and the arctic char of Iceland, northern Europe, and Scandinavia (Friend 1959; Frost 1965; Kornfield et al. 1981; Jonsson and Hindar 1982; Hindar et al. 1986; Magnusson and Ferguson 1987; Sandlund et al. 1988; Jonsson et al. 1988; Sigurjonsdottir and Gunnarson 1989; Skulason et al. 1989; Danzmann et al. 1991) are two examples of salmonid species in which phenotypic and genetic diversity conflict. Differences among populations of Atlantic and Pacific herring (*Clupea harengus* and *C. pallasi*) (Grant 1984, 1986, Ryman et al. 1984, Kornfield and Bogdanowicz 1987) provide additional evidence that population subdivision can result from ecophenotypic and behavioral differences which lead to reproductive isolation in the absence of significant levels of genetic divergence. The subdivision of the populations in some cases is geographic (Kornfield et al. 1982, Grant 1984, and Currens et al. 1990) and in others is temporal (Frost 1965, Chilcote et al. 1980, and Sinclair and Tremblay 1984). Discrete populations are electrophoretically indistinguishable but adapt differently to their respective environments (Utter 1981). This sets up conditions that favor morphological evolution and assortative mating in the absence of genetic differentiation. The transmission of characteristics from one generation to the next, in these sometimes widely divergent populations, supports hypotheses of speciation in progress (Utter et al. 1989).

The magnitude of genetic variability may influence the survival of a population over ecological time, or the survival of a lineage over evolutionary time. In populations of poeciliids in Arizona (Vrijenhoek et al. 1985; Quattro and Vrijenhoek 1989) the highest amount of variability was recorded in wild self-sustaining populations near the center of the species range. Lowest variability was

recorded in peripheral populations, or in transplanted populations. When representatives of high, intermediate, and low variability populations were raised in the laboratory, highest fitness (high fecundity and survival) occurred in the populations with the highest variability. The maintenance of genetic variation is important to the fitness of higher vertebrates as well (Wildt et al. 1987). The high level of variability found among wild populations of *S. namaycush* in Lake Superior (DeSilva 1989, Grewe 1991, this study) contrasts sharply with the low mtDNA variability found in transplanted or hatchery populations and populations in lakes at the periphery of the range of *S. namaycush*. Maintenance of high levels of genetic diversity, or low rates of lineage extinction may be contributing to the survival and persistence of morphological diversity among lake trout in Lake Superior.

Conclusion

Variation in mitochondrial DNA among populations of *Salvelinus namaycush* in Lake Superior is widespread, and no fixed genetic differences exist which correspond to the lean, siscowet, and humper phenotypes. The level of sequence divergence among mtDNA clonal haplotypes based on mapped restriction sites supports a conclusion that genetic isolation of phenotypically differentiated populations is incomplete. The hypothesis that lean, siscowet, and humper lake trout have been diverging only 8000 years and are members of the same species cannot be rejected.

Cladistic analysis of restriction site characters showed a high level of homoplasy, and showed that lineage relationships within Lake Superior *S. namaycush* remain unresolved. Forty-two of 77 mapped sites are plesiomorphic, and only 5 of 8 polymorphic sites are phylogenetically informative. The high level of homoplasy among restriction site characters can be the result of one of two models. The first explains high levels of variation as ancestral polymorphism

retained in recently diverged populations. Lineage sorting is incomplete because of the evolutionarily short time since divergence. The second model explains the high level of homoplasy as the result of introgression among formerly divergent populations. MtDNA is inherited as a unit, and transmission of composite characters may contradict traditional patterns of inheritance of characters controlled by the nuclear genome.

There are heritable genetic differences among lean, siscowet, and humper lake trout phenotypes, but these differences are stock differences and not species differences. The accumulated genetic differences are small, and are probably located in the nuclear genomes. The most obvious phenotypic differences are in fat storage and growth. The corresponding genetic differences must involve genes regulating metabolism and growth rather than genes coded for in the mitochondrial DNA.
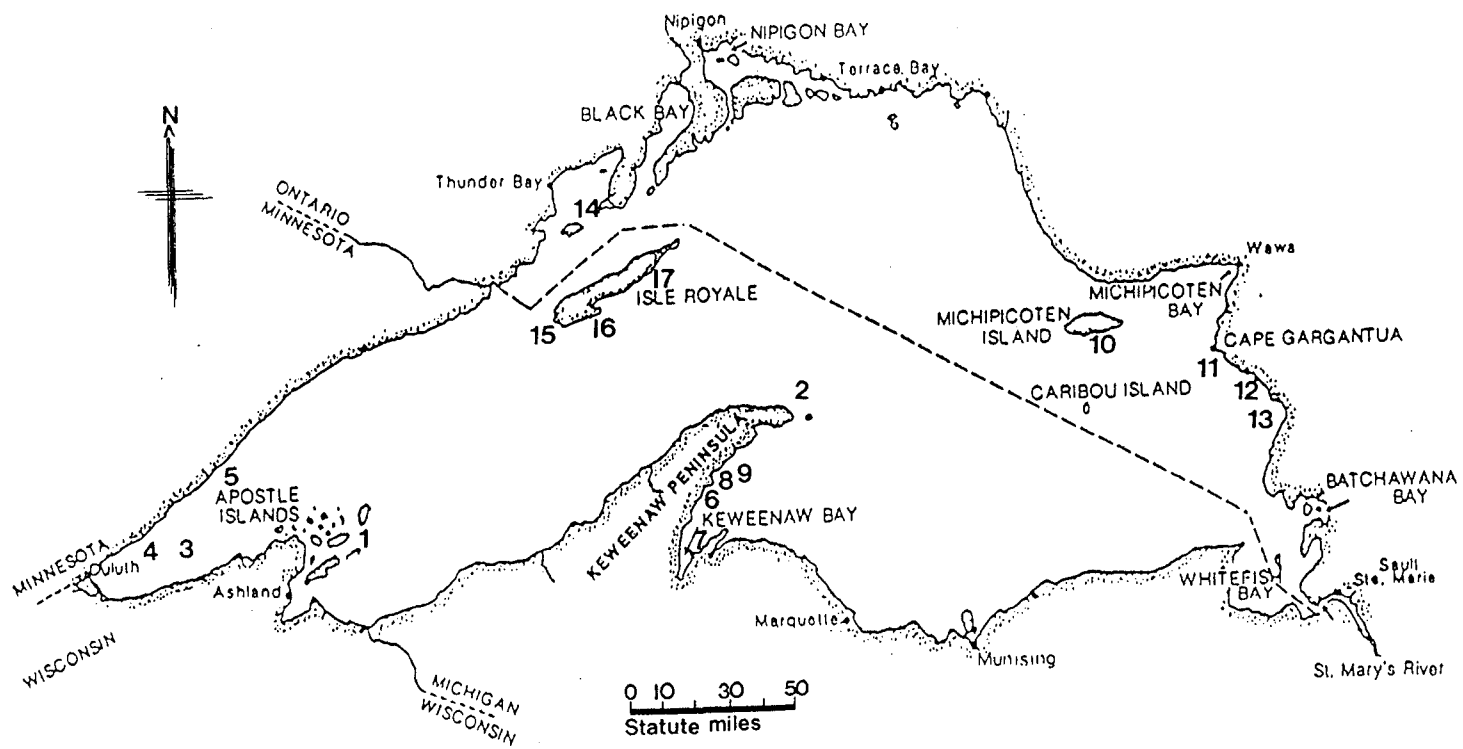
Figure 4.1. Map of Lake Superior. Locations of sample collections are indicated by numbers corresponding to Table 4.1.
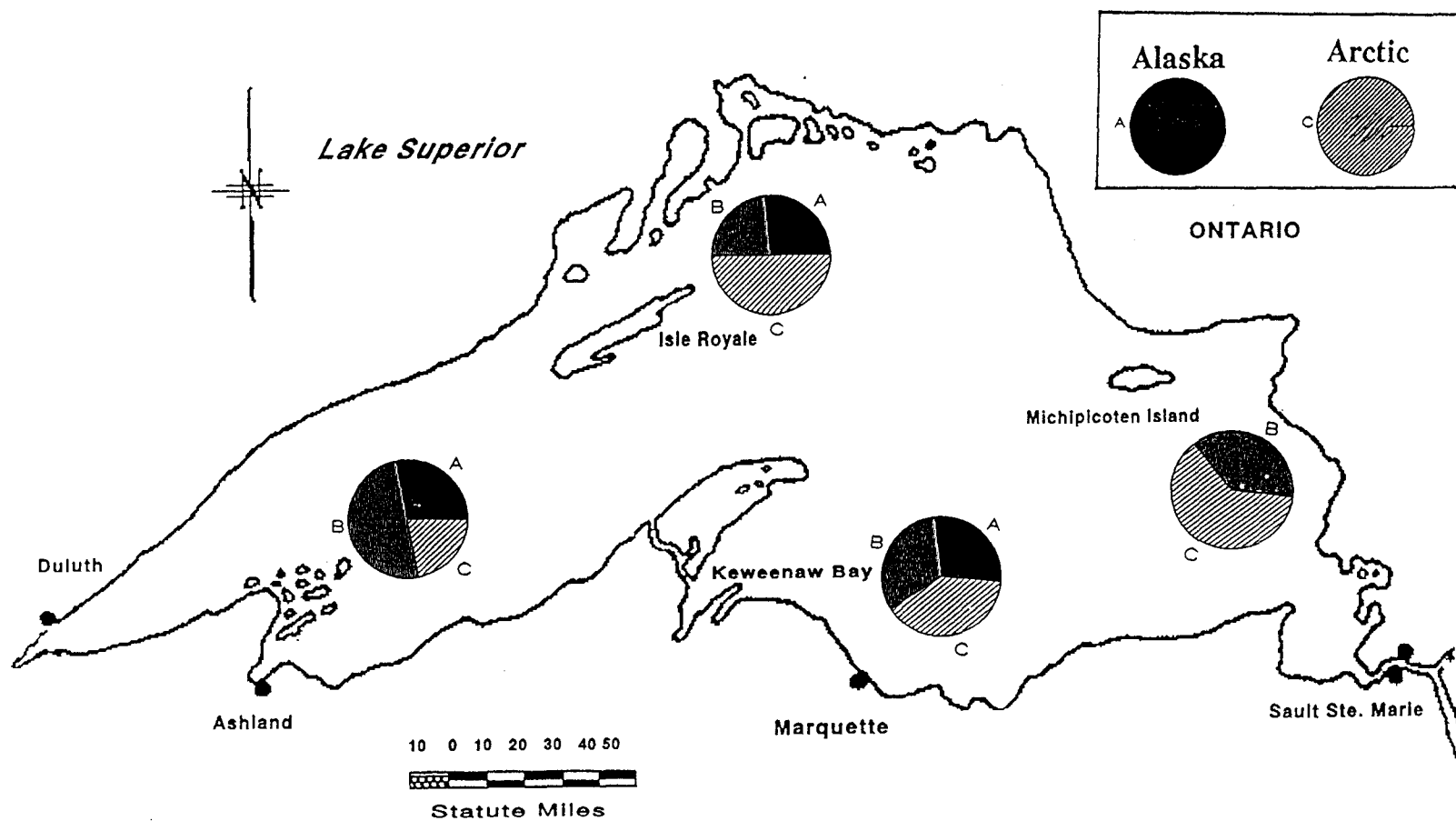
Figure 4.2a. Geographic distribution of restriction enzyme fragment phenotypes for Lake Superior lean lake trout. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.08%.
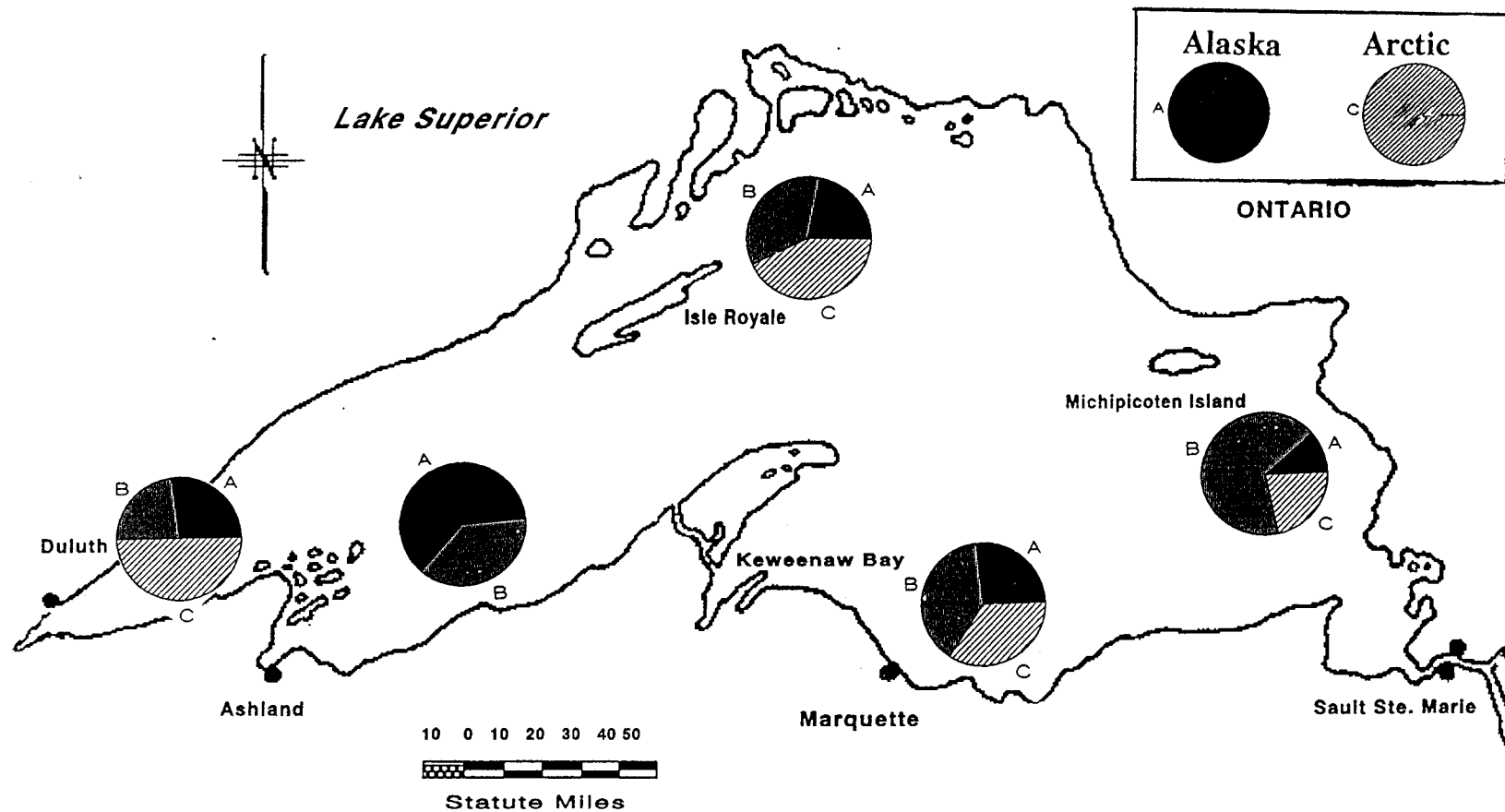
Figure 4.2b. Geographic distribution of restriction enzyme fragment phenotypes for Lake Superior siscowet lake trout. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.08%.
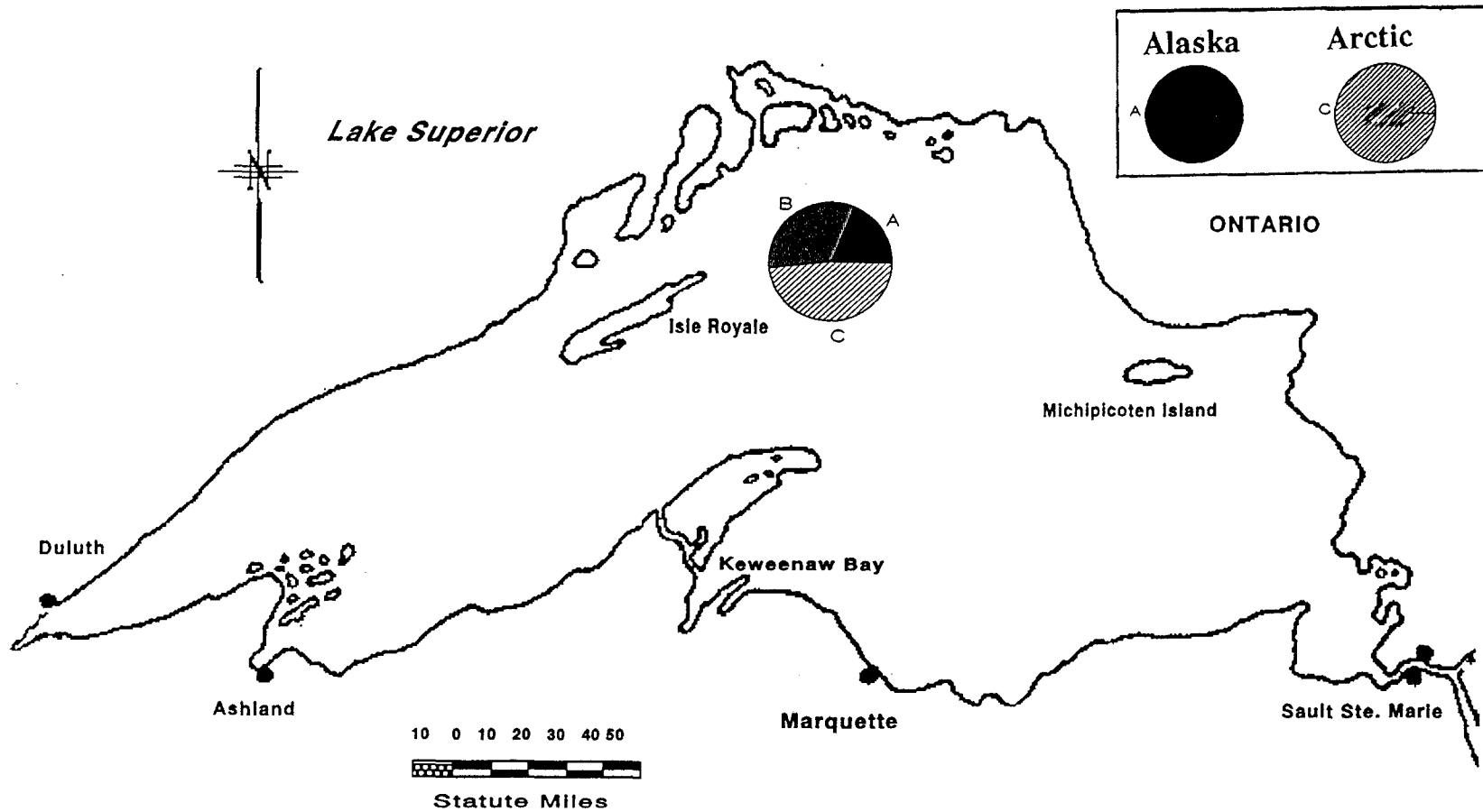
Figure 4.2c. Geographic distribution of restriction enzyme fragment phenotypes for Lake Superior humper lake trout. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.08%.
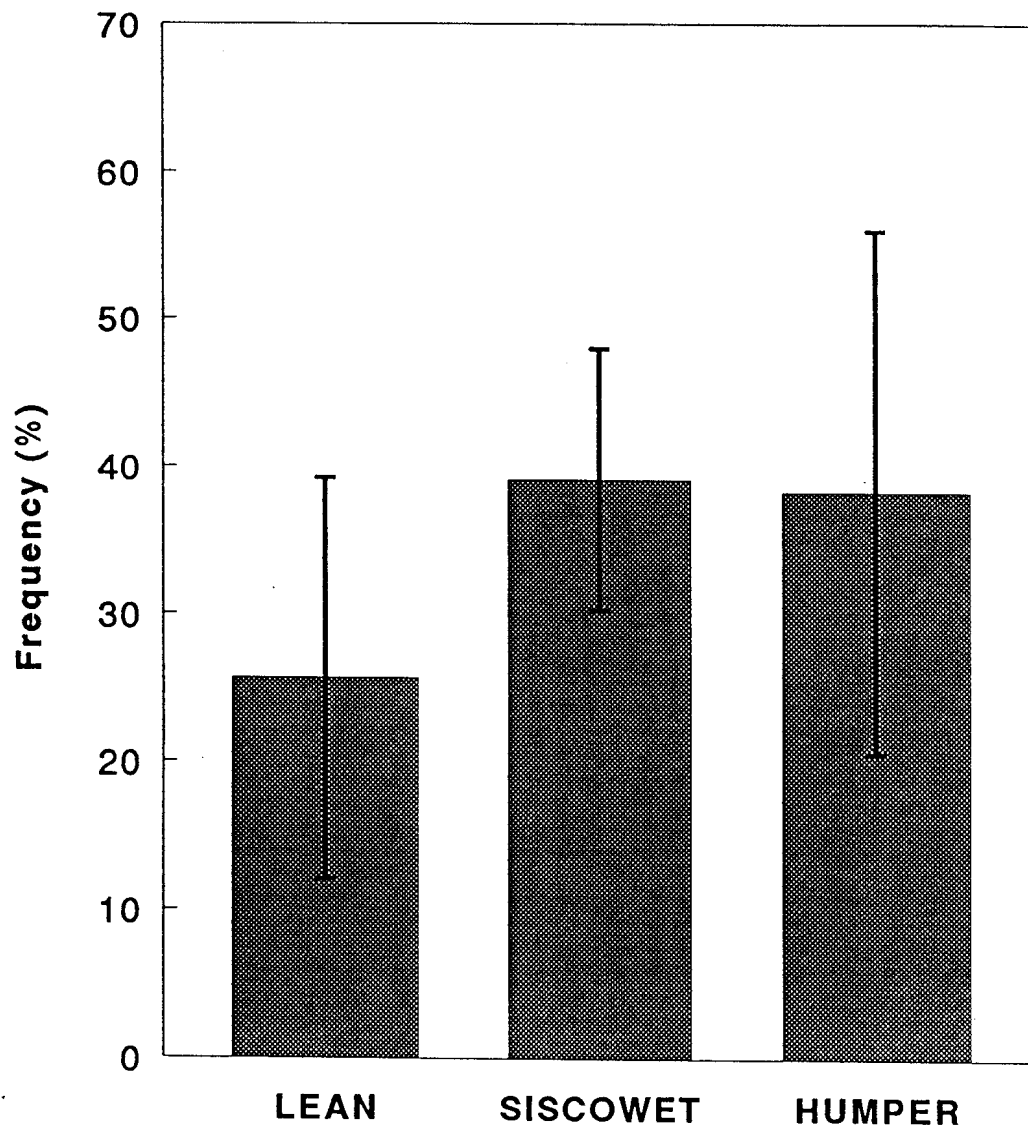
Figure 4.3. Frequency and 95% confidence limits of restriction enzyme Ava II fragment pattern "D" for lean, siscowet, and humper Lake Superior lake trout.
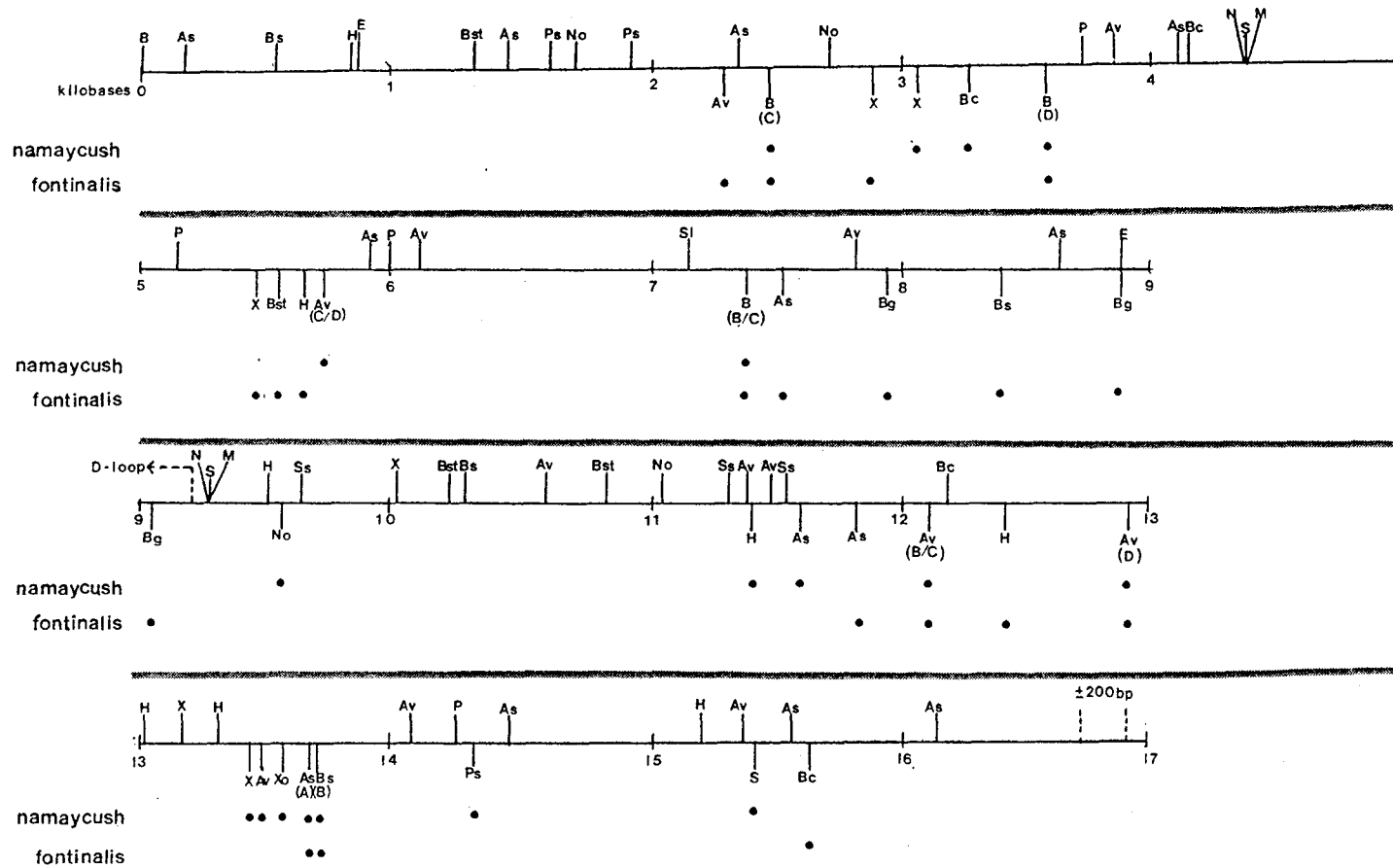
Figure 4.4. Restriction enzyme site map for Salvelinus namaycush and S. fontinalis mitochondrial DNA. Invariant sites are labeled above the line, variant sites are below the line. Sitres present in either species are indicated with a dot. Letters in parentheses indicate unique sites in S. namaycush clonal groups A, B, C, and D. Nineteen enzymes are mapped as follows: B=BamH I, As=Ase I, Bs=BspH I, H=Hind III, E=EcoR I, Bst=BstE II, Ps=Pst I, No=Nco I, Av=Ava I, X=Xba I, Bc=Bcl I, P=Pvu II, N=Nci I, S=Sma I, M=Sau 3A I (Mbo I), Sl=Sal I, Bg=Bgl II, Ss=Sst II, Xo=Xho I. MtDNA size is estimates at 16741±219 bp.
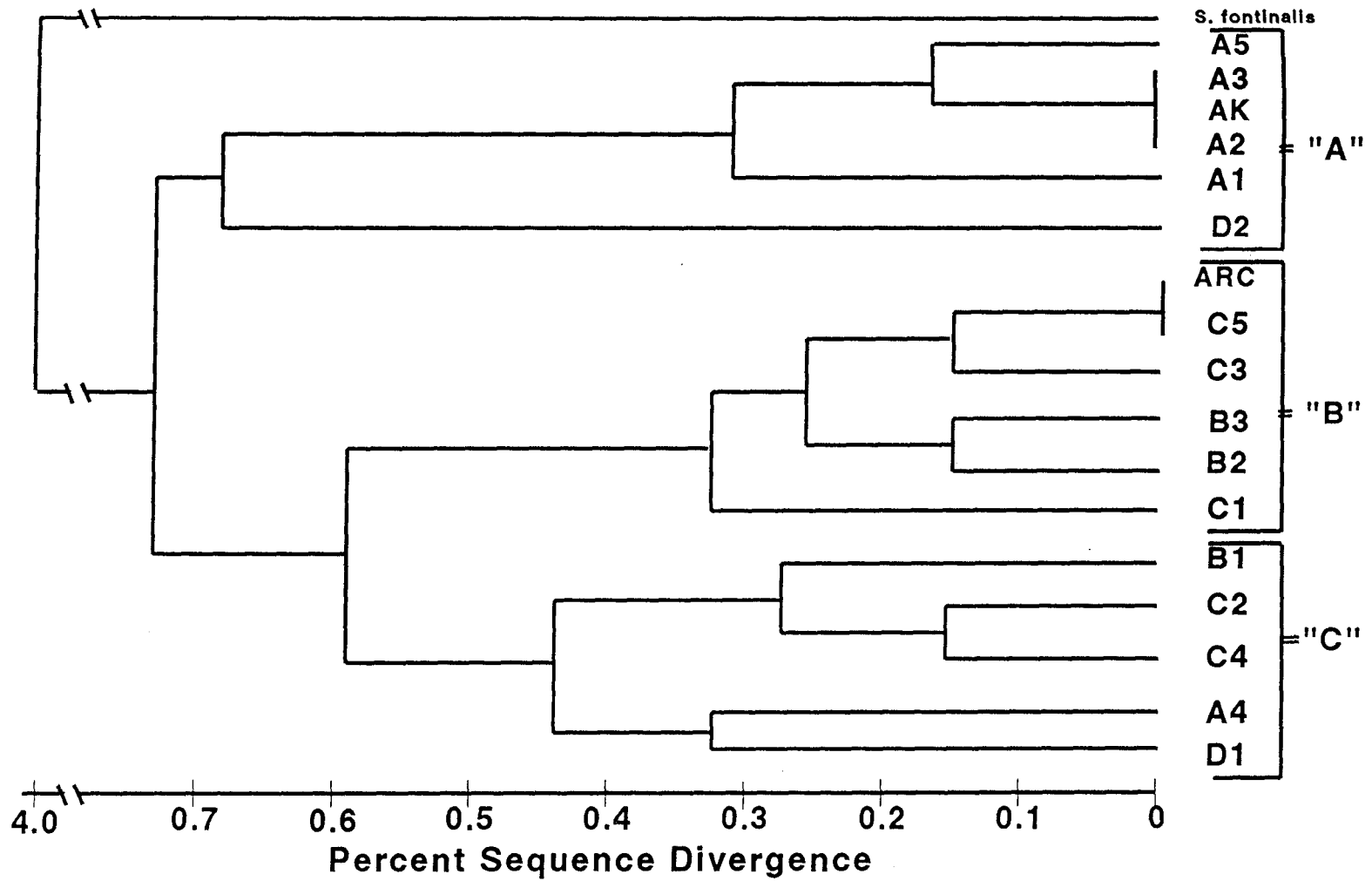
Figure 4.5. UPGMA dendrogram calculated from a matrix of pairwise sequence divergence estimates for mtDNA clonal haplotypes based on mapped restriction sites. Three major clusters can be identified at about 0.5% sequence divergence.
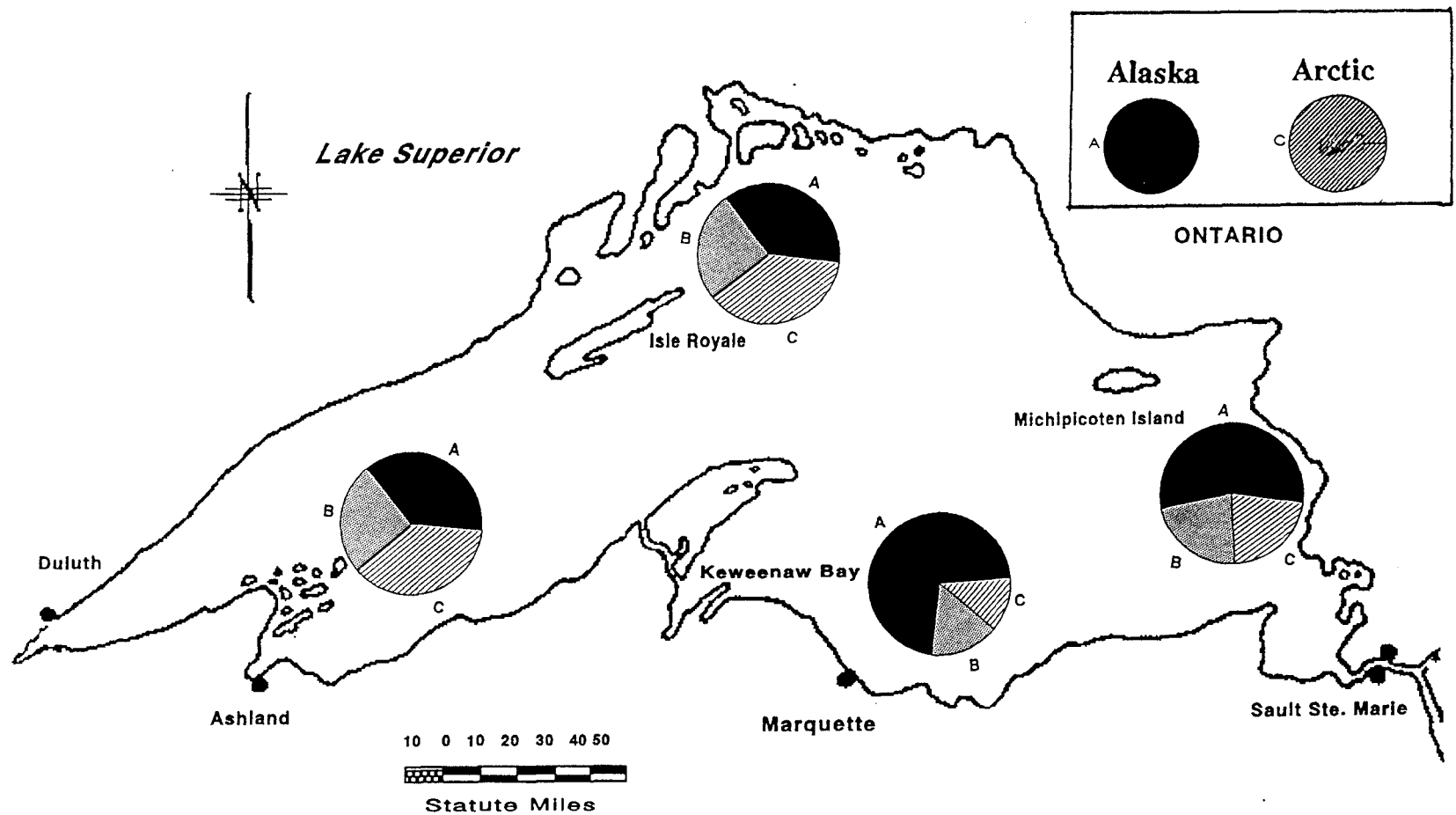
Figure 4.6a. Geographic distribution of mitochondrial DNA haplotypes for Lake Superior lean lake trout based on mapped restriction sites. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.05%.
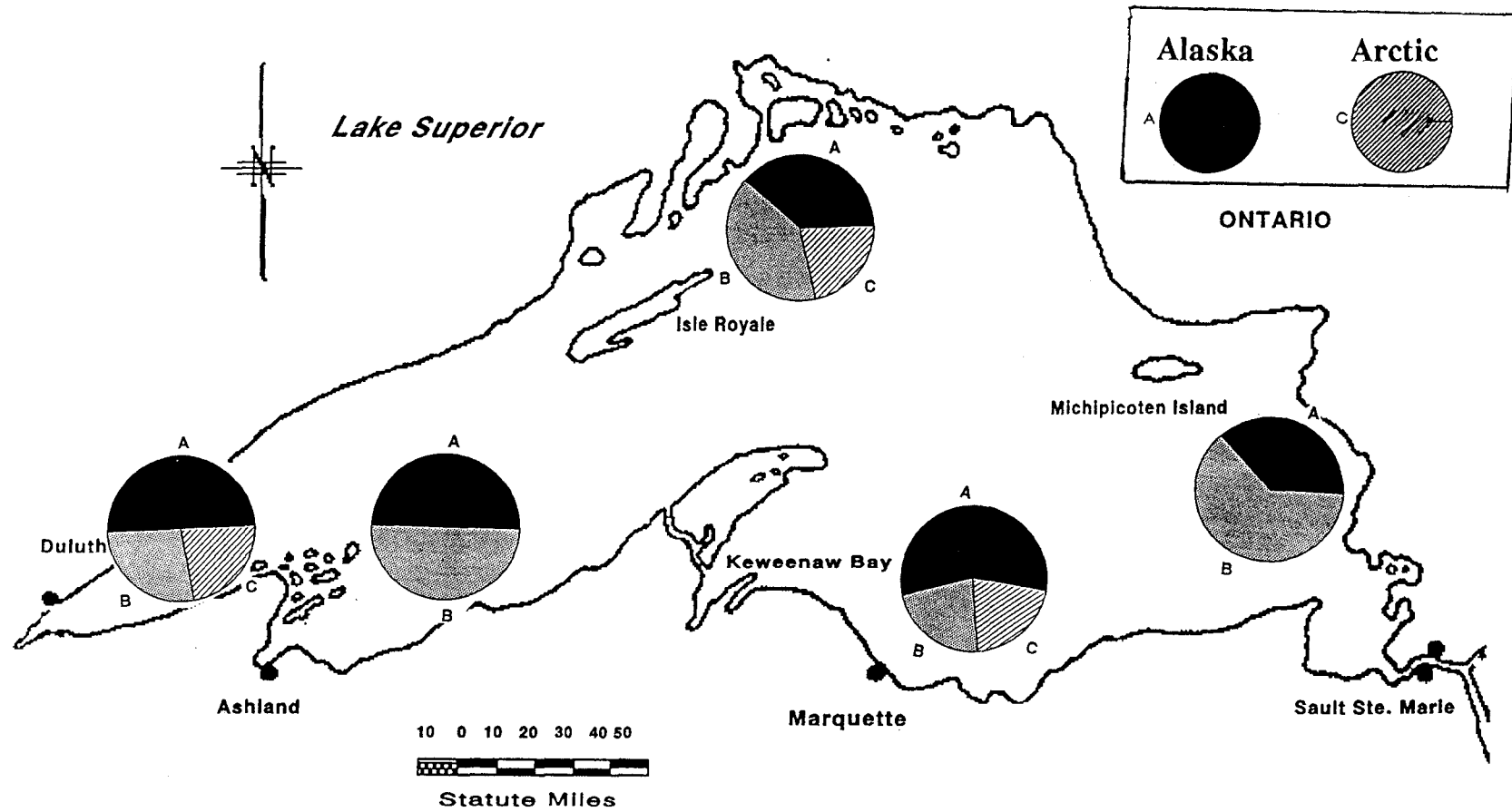
Figure 4.6b. Geographic distribution of mitochondrial DNA haplotypes for Lake Superior siscowet lake trout based on mapped restriction sites. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.05%.
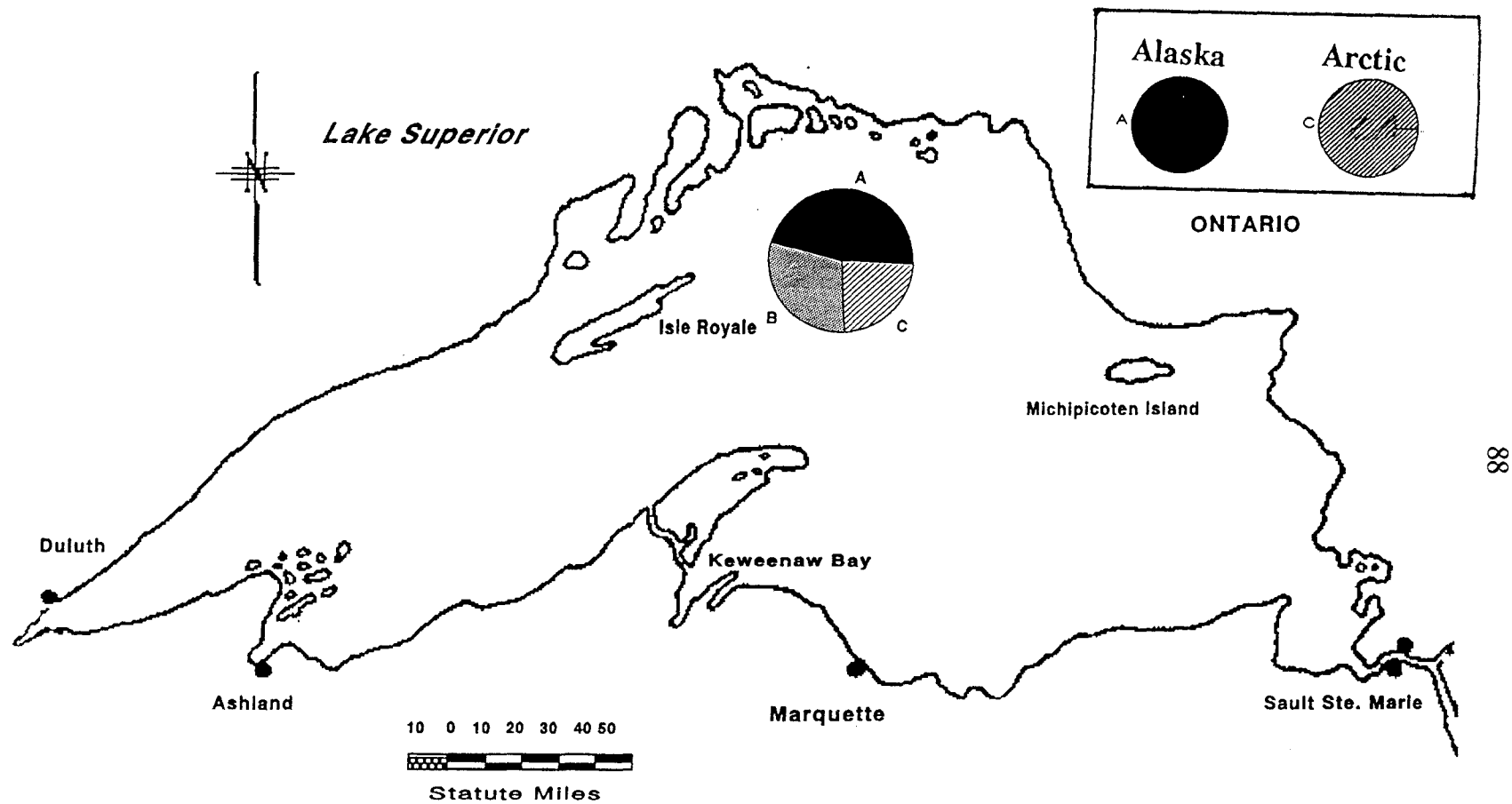
Figure 4.6c. Geographic distribution of mitochondrial DNA haplotypes for Lake Superior humper lake trout based on mapped restriction sties. Phenotypes "A," "B," and "C" are defined by UPGMA clustering of pairwise sequence divergence estimates into 3 major clusters. Sequence divergence cutoff for defining clusters was 0.05%.
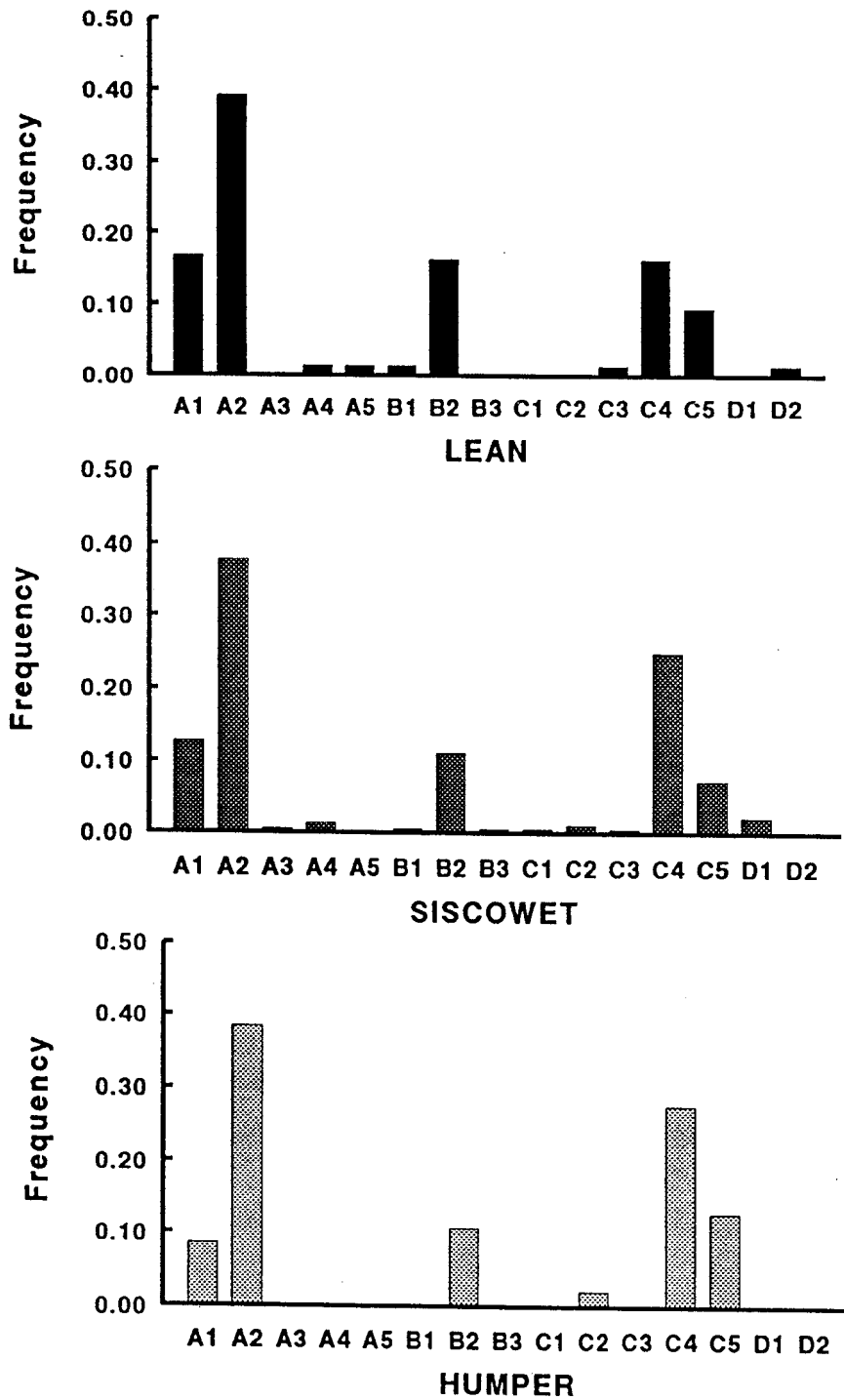
Figure 4.7 a. Frequency distribution of restriction site clonal haplotypes by phenotype. Haplotypes were based on 77 mapped restriction sites. "A," "B," "C," and "D" designations refer to Ava I/BamH I clonal groups defined by Grewe and Hebert (1989).
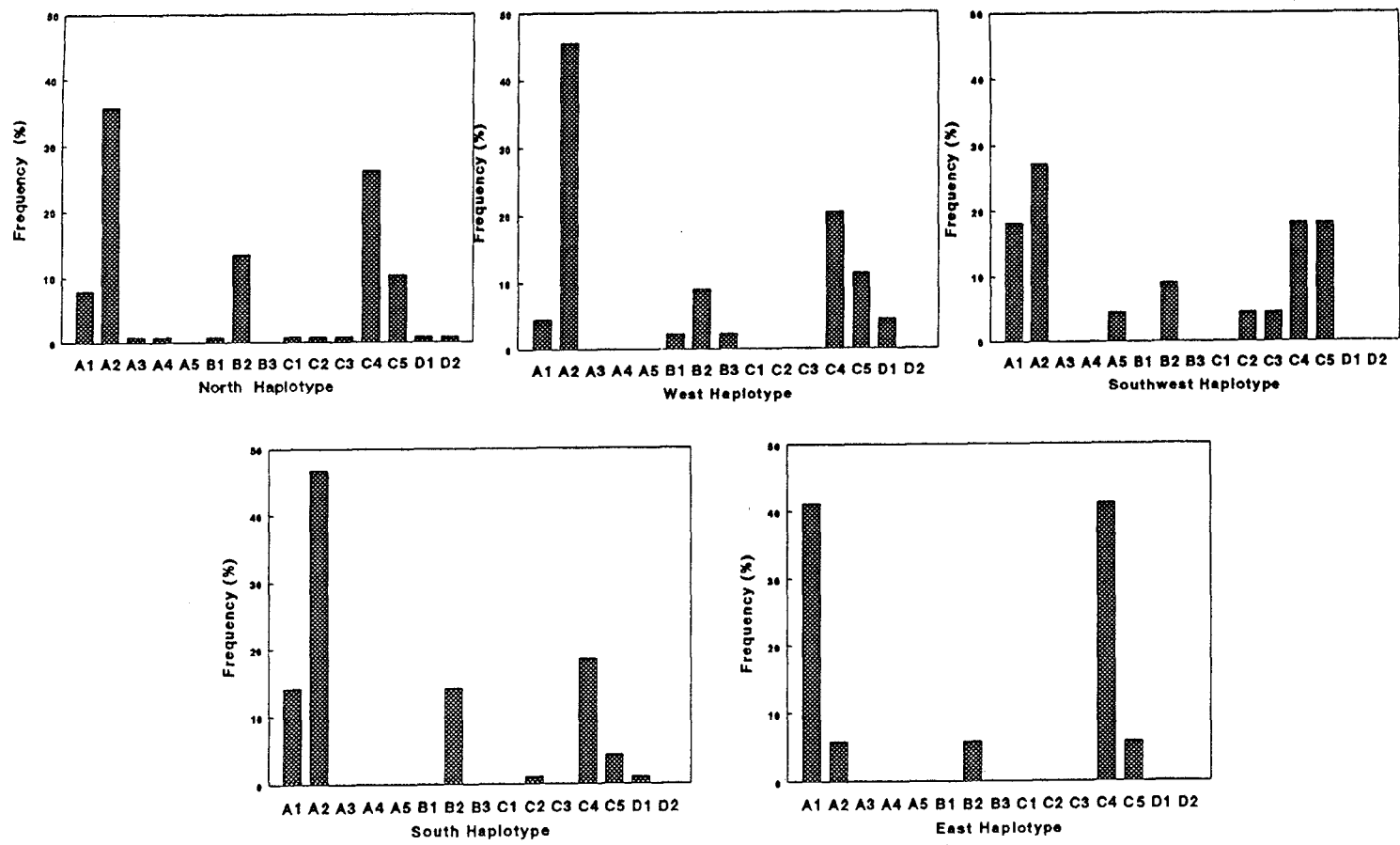
Figure 4.7b. Frequency distribution of mtDNA restriction site clonal haplotypes by geographic locality for Salvelinus namaycush from Lake Superior.

	LEAN	SISCOWET	HUMPER
ARC	1	-	-
C5	7	13	6
C3	1	1	-
B3	-	1	-
B2	12	20	5
AK	9	-	-
A2	28	68	18
C1	-	1	-
A5	1	-	-
D2	1	-	-
A3	-	1	-
A1	12	23	4
B1	1	1	-
C2	-	2	1
C4	14	50	13
A4	1	-	-
D1	-	4	-

S. fontinalis
S. confluentis

Consensus tree (108 trees)

Length 47

ci 74; ri 82
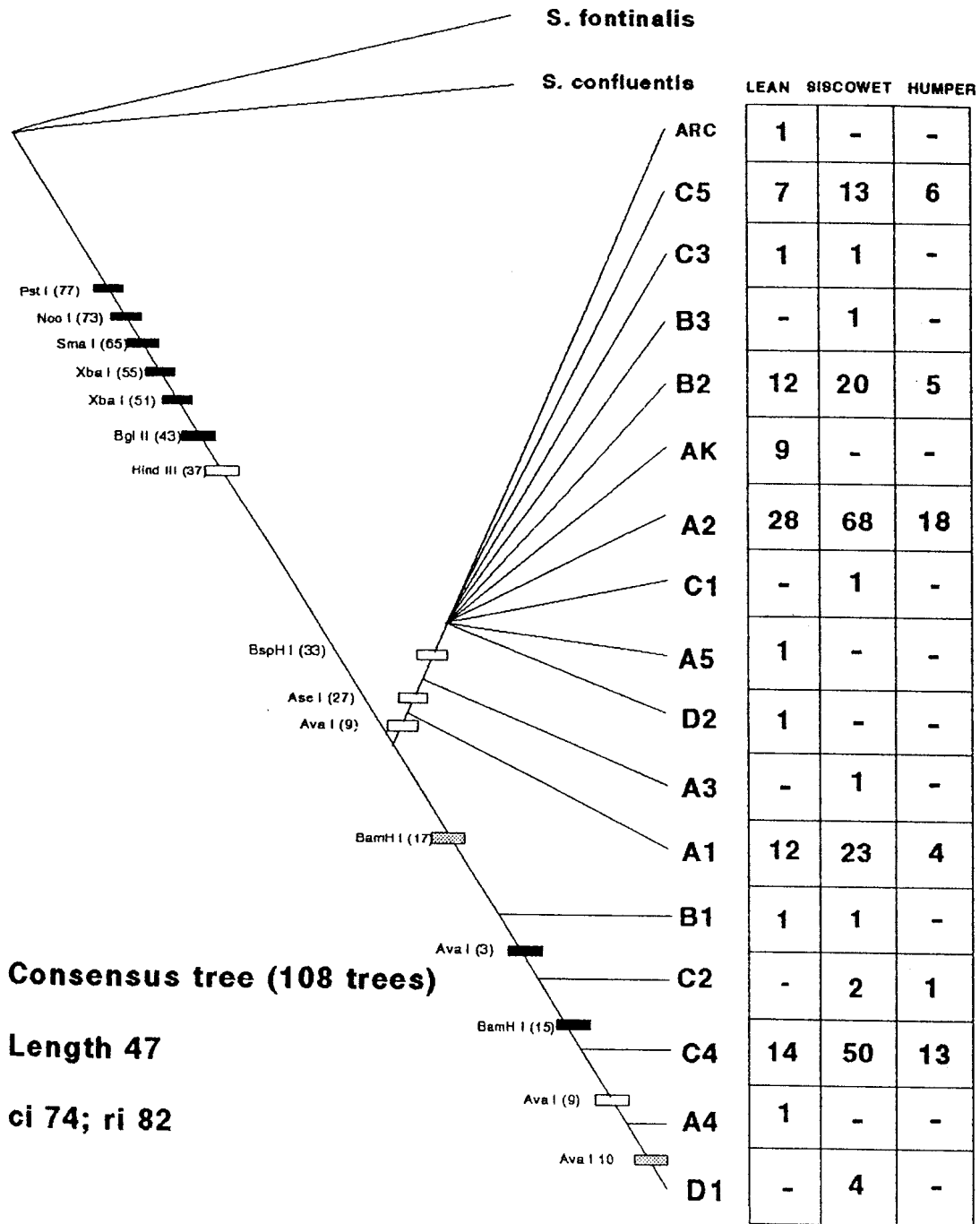
Figure 4.8. Cladogram of mtDNA clonal haplotypes based on mapped restriction sites. Solid boxes represent unambiguous site losses; open boxes represent unambiguous site gains; hatched boxes represent site reversals. Numbers in parentheses refer to the restriction site position from Figure 4.4. The table at right lists the number of individuals possessing the clonal haplotype. There is no correspondence between mtDNA haplotype and either phenotype or geographic locality.

LOCATION	Area	Latitude / Longitude	Depth fished	Types Sampled	Gear
1. Apostle Islands, WI	SW	46° 46' N / 90° 47' W	15-100 m	Lean, Siscowet	TR
2. Copper Harbor, MI	S	47° 31' N / 87° 40' W	145-160 m	Siscowet	GN
3. Port Wing, WI	W	46° 54' N / 91° 19' W	145-160 m	Siscowet	GN
4. Duluth, MN	W	46° 47' N / 92° 06' W	145-160 m	Siscowet	GN
5. Silver Bay, MN	W	47° 17' N / 91° 15' W	100-160 m	Siscowet	GN
6. Jacobsville, MI	S	46° 58' N / 88° 23' W	15-65 m	Lean, Siscowet	TR
7. Sand Bay, MI	S	46° 53' N / 88° 19' W	15-138 m	Lean, Siscowet	TR
8. Traverse Island, MI	S	47° 03' N / 88° 16' W	32-90 m	Lean, Siscowet	TR
9. Traverse Bay, MI	S	47° 09' N / 88° 13' W	15-100 m	Lean, Siscowet	TR
10. Michipicoten Island, Ontario	E	47° 46' N / 85° 42' W	15-100 m	Lean, Siscowet	TR
11. Gargantua Bay, Ontario	E	47° 33' N / 84° 57' W	15-110 m	Lean, Siscowet	TR
12. Agawa Bay, Ontario	E	47° 19' N / 84° 38' W	19-85 m	Lean	TR
13. Alona Bay, Ontario	E	47° 09' N / 84° 42' W	20-130 m	Lean, Siscowet	TR

Table 4.1. Sampling localities. Area refers to geographic area classification used in morphometric analysis. Gear used as follows: GN = multifilament nylon gill net; TR = bottom trawl; rod = caught by rod and reel. Collections in all areas except 2, 3, and 4 were made "across-contour" (across all depths indicated). Collections in areas 2, 3, and 4 were commercial sets. (continued)

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LOCATION	Area	Latitude / Longitude	Depth fished	Types Sampled	Gear
14. Sawyer Bay, Ontario, Canada	N	48° 22' N / 88° 52' W	15-70 m 60-87 m	Lean Siscowet	GN
15. Thompson Island, Isle Royale, MI	N	47° 53' N / 89° 13' W	48-85 m 76-95 m 76-95 m	Lean Siscowet Humper	GN
16. Isle Royale Light, MI	N	47° 55' N / 88° 45' W	53-77 m 53-116 m 53-116 m	Lean Siscowet Humper	GN
17. Mott Island, Isle Royale, MI	N	48° 08' N / 88° 30' W	69-94 m 69-124 m 69-94 m	Lean Siscowet Humper	GN
Localities outside Laurentian Great Lakes basin :					
Alaska, Kenai Peninsula (river)					rod
Prince Lake, Northwest Territory					GN
Grinnell Lake, Northwest Territory					GN
Cambridge Lake, Northwest Territory					GN

Table 4.1. Continued.

6 Base Enzymes		5 Base Enzymes	
Acc I *	GT ↓(A/C)(G/T)AC	HinF I *	G ↓ANTC
Ase I *	AT ↓TAAT	Nci I *	CC ↓(C/G)GG
Ava I *	C ↓(Py)CG(Pu)G		
BamH I *	G ↓GATCC	**4 Base Enzymes**	
Bcl I	T ↓GATCA	Ava II *	GG ↓CC
Bgl II	A ↓GATCT	BstU I *	CG ↓CG
BstE II	G ↓GTNACC	HinP I *	G ↓CGC
BspH I *	T↓CATGA	Msp I[a] *	C ↓CGG
Eco0109 *	(Pu)G ↓GNCC(Py)	Sau3A I[a] *	↓GATC
EcoR I	G ↓AATTC	α-Taq I *	T ↓CGA
Hind III	A ↓AGCTT		
Nco I	C ↓CATGG		
Pst I	CTGCA ↓G		
Pvu II	CAG ↓CTG		
Sal I	G↓TCGAC		
Sma I	CCC ↓GGG		
Sst II	CCGC ↓GG		
Xba I	T ↓CTAGA		
Xho I	C↓TCGAG		

Table 4.2. Type II restriction enzymes and recognition sites. Arrows indicate cut sites, and asterisiks indicate enzymes with polymorphic restriction products for Lake Superior *Salvelinus namaycush*.

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[a] Sau3A I is an isoschizomer of Mbo I; Msp I is an isoschizomer of Hpa II.

Haplotype	Ava II	Sau 3AI	Msp I	HinF I	Taq I	Total Obs
A1	A	A	A	A	A	6
A2	A	A	A	A	B	6
A3	A	A	A	A	C	1
A4	A	A	A	A	D	1
A5	A	A	A	B	B	2
A6	A	A	A	C	A	2
A7	A	A	A	D	B	2
A8	A	A	A	D	C	1
A9	A	A	B	A	A	1
A10	A	A	B	A	B	2
A11	A	A	B	A	D	2
A12	A	A	B	B	A	1
A13	A	A	B	D	B	1
A14	A	A	C	A	A	1
A15	A	A	C	B	A	1
A16	A	A	C	B	B	1
A17	A	A	D	C	B	1
A18	A	B	A	A	A	6
A19	A	B	A	A	C	3
A20	A	B	A	B	B	2
A21	A	B	A	B	C	2
A22	A	B	A	D	B	1
A23	A	B	A	D	E	1
A24	A	B	B	A	A	3
A25	A	B	B	B	A	1
A26	A	B	B	B	B	1
A27	A	B	B	C	A	1
A28	A	B	B	D	B	2
A29	A	B	C	A	A	2
A30	A	B	C	A	B	2
A31	A	B	C	A	C	1
A32	A	B	C	A	D	1
A33	A	B	C	B	B	1
A34	A	C	A	A	A	2
A35	A	C	A	A	B	3
A36	A	C	A	A	C	5
A37	A	C	A	A	D	1
A38	A	C	A	C	B	1

Table 4.3. Haplotypes based on composite restriction enzyme fragment profiles. Mapped enzymes have been excluded. Alphanumeric designations refer to "A," "B," and "D" fragment patterns for the restriction enzyme Ava II.

Haplotype	Ava II	Sau 3AI	Msp I	HinF I	Taq I	Total Obs
A39	A	C	A	C	C	1
A40	A	C	A	D	B	1
A41	A	C	B	A	B	2
A42	A	C	B	A	C	1
A43	A	C	B	B	A	2
A44	A	C	B	B	B	3
A45	A	C	B	D	A	1
A46	A	C	C	A	A	1
A47	A	C	C	B	B	1
A48	A	C	D	A	C	1
A49	A	C	D	B	A	1
A50	A	C	D	C	B	1
A51	A	D	A	A	A	3
A52	A	D	A	A	B	4
A53	A	D	A	A	C	2
A54	A	D	A	A	D	1
A55	A	D	A	B	B	1
A56	A	D	A	B	C	3
A57	A	D	A	C	B	1
A58	A	D	A	D	B	1
A59	A	D	B	A	B	1
A60	A	D	B	A	C	2
A61	A	D	B	A	D	1
A62	A	D	B	B	A	3
A63	A	D	B	B	B	2
A64	A	D	B	C	B	1
A65	A	D	B	C	C	1
A66	A	D	B	D	A	1
A67	A	D	B	D	B	1
A68	A	D	C	A	A	1
A69	A	D	C	A	B	1
A70	A	D	C	A	C	2
A71	A	D	C	C	B	1
A72	A	D	D	A	B	1
A73	A	D	D	C	B	1
A74	A	E	B	B	B	1
B75	B	A	A	A	A	1
B76	B	A	A	A	B	1
B77	B	A	A	D	A	1
B78	B	A	B	A	B	1

Table 4.3. Continued.

Haplotype	Ava II	Sau 3AI	Msp I	HinF I	Taq I	Total Obs
B79	B	A	B	A	C	1
B80	B	A	B	B	B	2
B81	B	A	B	B	C	1
B82	B	A	C	A	A	1
B83	B	A	C	A	B	1
B84	B	A	D	A	C	1
B85	B	A	D	D	D	1
B86	B	B	A	B	B	1
B87	B	B	A	B	C	1
B88	B	B	B	A	A	1
B89	B	B	B	A	C	1
B90	B	B	B	B	C	1
B91	B	B	B	D	B	2
B92	B	B	C	A	A	1
B93	B	B	C	A	B	2
B94	B	B	C	C	A	1
B95	B	C	A	A	A	2
B96	B	C	A	A	B	2
B97	B	C	A	B	B	2
B98	B	C	A	C	B	1
B99	B	C	B	A	A	1
B100	B	C	B	B	A	1
B101	B	C	B	B	B	3
B102	B	C	B	B	C	2
B103	B	C	B	C	B	1
B104	B	C	B	C	C	1
B105	B	C	B	D	B	1
B106	B	C	B	D	C	1
B107	B	C	C	A	B	2
B108	B	C	C	B	A	1
B109	B	C	D	A	C	1
B110	B	C	D	B	B	1
B111	B	D	A	A	A	3
B112	B	D	A	A	B	2
B113	B	D	A	A	C	5
B114	B	D	A	B	B	2
B115	B	D	A	B	C	1
B116	B	D	A	C	B	1
B117	B	D	A	D	B	1
B118	B	D	A	D	C	1

Table 4.3. Continued.

Haplotype	Ava II	Sau 3AI	Msp I	HinF I	Taq I	Total Obs.
B119	B	D	B	A	B	1
B120	B	D	B	B	A	1
B121	B	D	B	B	B	1
B122	B	D	B	C	A	1
B123	B	D	C	A	B	1
B124	B	D	C	C	B	1
B125	B	D	C	C	C	1
D126	D	A	A	C	B	2
D127	D	A	A	D	B	1
D128	D	A	B	B	B	1
D129	D	A	B	C	B	2
D130	D	A	C	A	B	1
D131	D	A	D	C	B	1
D132	D	B	A	A	C	4
D133	D	B	A	B	B	1
D134	D	B	A	C	B	3
D135	D	B	B	A	A	1
D136	D	B	B	B	B	3
D137	D	B	B	C	B	4
D138	D	B	B	C	C	1
D139	D	B	B	C	D	1
D140	D	B	C	A	B	1
D141	D	B	C	C	C	1
D142	D	B	D	A	B	1
D143	D	B	D	B	A	1
D144	D	C	A	A	C	1
D145	D	C	A	B	B	1
D146	D	C	A	C	C	1
D147	D	C	A	C	D	3
D148	D	C	A	D	C	1
D149	D	C	B	B	B	1
D150	D	C	B	B	C	1
D151	D	C	C	A	A	1
D152	D	C	C	A	B	1
D153	D	C	C	B	B	1
D154	D	C	C	C	C	1
D155	D	C	D	C	C	1
D156	D	D	A	A	A	1
D157	D	D	A	A	B	3
D158	D	D	A	A	C	2

Table 4.3. Continued.

Haplotype	Ava II	Sau 3AI	Msp I	HinF I	Taq I	Total Obs
D159	D	D	A	B	A	1
D160	D	D	A	B	B	2
D161	D	D	A	C	A	1
D162	D	D	A	C	B	1
D163	D	D	A	C	C	1
D164	D	D	B	A	B	1
D165	D	D	B	A	C	1
D166	D	D	B	B	A	3
D167	D	D	B	B	B	5
D168	D	D	B	B	C	2
D169	D	D	B	C	A	7
D170	D	D	B	C	B	14
D171	D	D	B	C	C	8
D172	D	D	B	C	D	1
D173	D	D	B	D	C	4
D174	D	D	C	C	A	1
D175	D	D	C	C	C	1
D176	D	D	C	D	B	1
D177	D	D	D	C	A	4
D178	D	D	D	C	C	1

Table 4.3. Continued.

A	A1, A2, A3, A4, A5, A6, A7, A8, A14, A15, A16, A34, A35, A36, A37, A38, A39, A40, A46, A47, A48, B75, B76, B77, B79, B82, B83, B84, B95, B96, B97, B98, B107, B108, B109, D126, D127, D130, D144, D145, D146, D147, D148, D151, D152, D153, D154, D155,
B	A18, A19, A20, A21, A22, A23, A29, A30, A31, A32, A33, A51, A52, A53, A54, A55, A56, A57, A58, A62, A68, A69, A70, A71, A72, A73, B86, B87, B92, B93, B94, B111, B112, B113, B114, B115, B116, B117, B118, B123, B124, B125, D132, D133, D134, D140, D141, D142, D143, D156, D157, D158, D159, D160, D161, D163, D175, D176, D177, D178, D179,
C	A9, A10, A11, A12, A13, A17, A24, A25, A26, A27, A28, A41, A42, A43, A44, A45, A49, A50, A59, A60, A61, A63, A64, A65, A66, A67, A74, B78, B80, B81, B85, B88, B89, B90, B91, B99, B100, B101, B102, B103, B104, B105, B106, B110, B119, B120, B121, B122, D128, D129, D131, D135, D136, D137, D138, D139, D149, D150, D162, D164, D165, D166, D167, D168, D169, D170, D171, D172, D173, D174, D180, D181, D182

Table 4.4. Distribution of fragment pattern haplotypes (from Table 4.3) as determined by UPGMA clustering. The data were grouped based on pairwise sequence divergences estimates, using a cutoff of $\delta = 0.008$ (0.8% divergence). Alphabetic haplotype designations refer to Ava II, "A," "B," and "D" fragment patterns.

		Lean	Siscowet	Humper
Acc I	A	66.22 (52.11, 77.75)	62.50 (53.84, 70.28)	65.96 (47.79, 80.15)
	B	33.78 (21.52, 47.01)	37.50 (29.40, 45.81)	34.04 (18.75, 50.84)
Ase I	A	29.73 (18.09, 42.78)	41.30 (32.98, 49.67)	40.43 (24.05, 57.21)
	B	70.27 (56.32, 81.19)	58.70 (49.98, 66.69)	59.57 (41.48, 74.80)
Ava I	A	54.05 (38.38, 68.01)	50.54 (40.89, 59.68)	44.68 (26.63, 62.20)
	B	18.92 (8.64, 32.21)	11.96 (6.54, 18.95)	10.64 (2.22, 25.39)
	C	25.68 (13.67, 39.75)	35.33 (26.49, 44.46)	44.68 (26.63, 62.20)
	D	1.35 (0.00, 6.34)	2.17 (0.28, 6.34)	---- NA
Ava II	A	42.06 (31.26, 52.83)	46.67 (27.95, 64.43)	36.36 (12.84, 62.39)
	B	23.81 (15.09, 33.82)	13.33 (3.44, 29.21)	40.91 (15.95, 66.54)
	D	34.13 (24.02, 44.76)	40.00 (22.29, 58.10)	22.73 (4.86, 48.73)
Bam HI	A	54.05 (38.38, 68.01)	51.09 (41.83, 59.87)	44.68 (26.63, 62.20)
	B	18.92 (8.64, 32.21)	13.04 (7.58, 19.94)	12.77 (3.29, 28.09)
	C	25.68 (13.67, 39.75)	35.87 (27.35, 44.66)	42.55 (60.18, 24.80)
	D	1.35 (0.00, 8.86)	---- NA	---- NA
Bsp HI	A	29.73 (18.09, 42.78)	41.85 (33.50, 50.22)	40.43 (24.05, 57.21)
	B	70.27 (56.32, 81.19)	58.15 (49.44, 66.17)	59.57 (41.48, 74.80)

Table 4.5. Frequencies and 95% CI for products of restriction enzyme analysis of *Salvelinus namaycush* mitochondrial DNA. Alphabetic designations refer to designations by Grewe and Hebert (1989) for previously tested enzymes, or to different fragment patterns in decreasing frequency for prevously untested enzymes.

		Lean	Siscowet	Humper
Eco 0109	A	29.73 (16.23, 44.81)	29.35 (20.83, 38.49)	38.30 (20.06, 57.13)
	B	44.59 (28.79, 59.79)	52.72 (42.70, 62.03)	46.81 (27.14, 65.21)
	C	6.76 (1.08, 17.69)	5.98 (2.22, 11.77)	8.51 (1.00, 23.60)
	D	8.11 (1.68, 19.34)	5.98 (2.22, 11.77)	---- NA
	E	1.35 (0.00, 9.31)	---- NA	2.13 (0.00, 13.97)
	F	9.46 (2.34, 21.34)	5.98 (2.22, 11.77)	4.26 (0.01, 17.45)
Hiu FI	A	36.49 (22.50, 51.09)	41.85 (32.55, 51.08)	42.55 (24.08, 60.78)
	B	35.14 (21.35, 49.71)	20.11 (13.08, 28.25)	21.28 (8.01, 38.80)
	C	14.86 (5.86, 27.45)	28.26 (20.12, 37.08)	34.04 (17.19, 52.43)
	D	13.51 (4.99, 25.81)	9.78 (4.92, 16.35)	2.13 (0.00, 13.60)
Hin PI	A	4.05 (0.34, 12.85)	0.54 (0.00, 3.77)	2.13 (0.00, 13.60)
	B	59.46 (44.29, 72.44)	50.54 (40358, 59.93)	55.32 (35.35, 72.40)
	C	36.49 (23.05, 50.56)	39.13 (29.72, 48.61)	40.43 (22.31, 58.74)
	D	---- NA	1.63 (0.09, 5.63)	---- NA
	E	---- NA	8.15 (3.65, 14.55)	2.13 (0.00, 13.60)
Msp I	A	45.95 (30.83, 60.41)	42.39 (33.06, 51.63)	25.53 (10.89, 43.51)
	B	41.89 (27.20, 56.48)	37.50 (28.49, 46.68)	48.94 (29.58, 66.73)
	C	8.11 (1.90, 18.94)	16.59 (7.79, 20.86)	17.02 (5.36, 33.88)
	D	4.05 (0.27, 13.24)	6.52 (2.66, 12.30)	8.51 (1.12, 23.17)

Table 4.5. Continued.

		Lean	Siscowet	Humper
Nci I	A	59.46 (43.60, 72.88)	51.09 (41.42, 60.20)	61.70 (41.38, 77.80)
	B	25.68 (13.67, 39.75)	39.67 (30.51, 48.89)	29.79 (13.96, 48.04)
	C	10.81 (3.36, 22.45)	4.35 (1.33, 9.43)	4.26 (0.03, 17.05)
	D	4.05 (0.27, 13.24)	4.89 (1.64, 10.17)	4.26 (0.03, 17.05)
Sau 3AI	A	12.16 (4.16, 24.15)	20.65 (13.54, 28.85)	10.64 (1.84, 26.44)
	B	21.62 (10.60, 35.28)	21.20 (14.00, 29.45)	17.02 (5.10, 34.36)
	C	32.43 (19.09, 46.93)	17.93 (11.28, 25.83)	17.02 (5.10, 34.36)
	D	33.78 (20.22, 48.33)	40.22 (31.02, 49.44)	53.19 (32.78, 70.94)
	E	---- NA	---- NA	2.13 (0.00, 13.97)
α-Taq I	A	22.97 (11.30, 37.18)	23.37 (15.85, 31.83)	34.04 (17.19, 52.43)
	B	45.95 (30.37, 60.79)	44.57 (35.13, 53.80)	46.81 (27.71, 64.78)
	C	24.32 (12.31, 38.67)	27.72 (19.64, 36.50)	17.02 (5.36, 33.88)
	D	5.41 (0.63, 15.53)	4.35 (1.33, 9.43)	2.13 (0.00, 13.60)
	E	1.35 (0.00, 9.11)	---- NA	---- NA

Table 4.5. Continued.

Haplotype	Ava I	Bam HI	Ase I	Bsp HI	No. Observed
A1	A	A	A	A	36
A2	A	A	B	B	109
A3	A	A	B	A	1
A4	A	C	A	A	1
A5	B	A	B	B	1
B1	B	B	A	A	2
B2	B	B	B	B	37
B3	B	C	B	B	1
C1	C	A	B	B	1
C2	C	B	A	A	3
C3	C	B	B	B	2
C4	C	C	A	A	77
C5	C	C	B	B	26
D1	D	C	A	A	4
D2	D	D	B	B	1

Table 4.6. Clonal haplotypes for restriction enzyme sites mapped for *Salvelinus namaycush* from Lake Superior.

	N	Nucleon diversity	Nucleotide diversity	Mean restriction site differences
LEAN	74	0.87	0.003	10.2
SISCOWET	180	0.86	0.003	9.1
HUMPER	47	0.83	0.003	8.7
N = 301		mean = 0.86		

Table 4.7. Nucleon diversity and mean number of restriction site differences for lean, siscowet, and humper lake trout based on Nei and Tajima (1981, eq. 6 and 10).

	N	Nucleon diversity	Nucleotide diversity	Mean restriction site differences
North	126	0.85	0.003	9.1
West	44	0.85	0.003	9.1
Southwest	22	0.92	0.004	10.7
South	92	0.84	0.003	9.1
East	17	0.76	0.002	7.7
	301	mean = 0.85		

Table 4.8. Nucleon diversity, nucleotide diversity, and restriction site differences for lake trout grouped by locality.

	N	Nucleon diversity	Nucleotide diversity	Mean restriction site differences
North Lean	24	0.90	0.003	9.3
North Siscowet	55	0.85	0.003	8.8
North Humper	47	0.83	0.003	8.7
West Siscowet	44	0.85	0.003	9.1
Southwest Lean	16	0.87	0.005	13.8
Southwest Siscowet	6	0.73	0.002	7.1
South Lean	25	0.81	0.003	8.8
South Siscowet	67	0.86	0.003	9.2
East Lean	9	0.814	0.002	8.1
East Siscowet	8	0.75	0.003	7.4
	N = 301	mean = 0.84		

Table 4.9. Nucleon diversity, nucleotide diversity, and restriction site differences for lake trout grouped by deme (locality and phenotype).

	Lean	Siscowet	Humper
Lean	10.2		
Siscowet	0.96	9.1	
Humper	2.07	0.90	8.7

Table 4.10. Net restriction site differences (d^) among lean, siscowet, and humper lake trout. Mean number of restriction site differences within phenotype are on diagonal, based on Nei and Tajima (1981, eq. 24).

	North	West	Southwest	South	East
North	**9.1**				
West	0.57	**9.1**			
Southwest	0.14	-0.37	**10.7**		
South	-1.83	-2.37	-2.05	**9.1**	
East	-0.55	-0.78	-1.12	0.66	**7.7**

Table 4.11 . Net number of restriction site differences (d^) for lake trout grouped by locality. Mean number of restriction site differences within each locality are on the diagonal.

	North Lean	North Siscowet	North Humper	West Siscowet	Southwest Lean	Southwest Siscowet	South Lean	South Siscowet	East Lean	East Siscowet
North Lean	9.3									
North Siscowet	-1.24	8.8								
North Humper	-0.41	0.86	8.7							
West Siscowet	-0.23	1.28	0.13	9.1						
Southwest Lean	0.28	1.68	0.62	0.55	13.8					
Southwest Siscowet	-1.52	-1.72	-1.73	-1.40	-3.12	7.1				
South Lean	-4.20	-2.53	-3.77	-3.95	-6.29	2.43	8.8			
South Siscowet	-2.04	-0.55	-1.72	-1.79	-3.69	3.14	2.34	9.9		
East Lean	-2.92	-2.36	-2.39	-2.46	-4.08	-0.32	-0.33	-1.61	8.1	
East Siscowet	1.42	1.43	1.53	0.99	0.44	2.44	3.62	2.33	2.31	7.4

Table 4.12. Net number of restriction site differences (d^) for lake trout grouped by deme (locality and phenotype). Mean number of restriction site differences within each locality are on the diagonal.

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	Lean	Siscowet	Humper
Lean	**0.87**	0.87	0.87
Siscowet	0	**0.88**	0.88
Humper	0	0.001	**0.89**

G st = 0.0005

Table 4.13. Identity probabilities and Nei's D for lean, siscowet, and humper lake trout. Top traingle is the average conditional identity probability, diagonal is within-deme identity probability, and bottom triangle is Nei's D (Takahata and Palumbi 1985, eqs. 17 and 19, and Nei 1972).

	North	West	Southwest	South	East
North	**0.88**	0.87	0.88	0.87	0.88
West	0.003	**0.87**	0.87	0.87	0.88
Southwest	0.001	0.001	**0.87**	0.87	0.88
South	0.001	0.003	0.001	**0.87**	0.87
East	0.004	0.005	0.005	0.01	**0.89**

Gst = 0.0168

Table 4.14. Identity probabilities and Nei's D for lake trout grouped by locality. Top triangle is average conditional identity probability, diagonal is within-deme identity probability, and bottom triangle is Nei's D (Takahata and Palumbi 1985, eqs. 17 and 19, and Nei 1972).

	North Lean	North Siscowet	North Humper	West Siscowet	Southwest Lean	Southwest Siscowet	South Lean	South Siscowet	East Lean	East Siscowet
North Lean	**0.88**	0.88	0.88	0.87	0.87	0.89	0.87	0.87	0.88	0.89
North Siscowet	0	**0.88**	0.88	0.87	0.87	0.89	0.87	0.88	0.88	0.89
North Humper	0	0.003	**0.88**	0.87	0.88	0.88	0.87	0.87	0.88	0.88
West Siscowet	0.002	0.003	0.0001	**0.87**	0.87	0.88	0.87	0.87	0.87	0.88
So-West Lean	0	0.002	0	0.002	**0.87**	0.87	0.87	0.87	0.87	0.88
So-West Siscowet	0.004	0.002	0.005	0.01	0.01	**0.90**	0.88	0.88	0.89	0.90
South Lean	0.002	0.003	0.001	0.001	0.0002	0.01	**0.87**	0.87	0.87	0.88
South Siscowet	0.001	0.002	0	0	0	0.01	0	**0.87**	0.87	0.88
East Lean	0.002	0.001	0.004	0.008	0.01	0	0.01	0.005	**0.89**	0.89
East Siscowet	0.004	0.001	0.002	0.005	0.005	0	0.01	0.004	0.002	**0.90**
$G_{st} =$	0.015									

Table 4.15. Identity probabilities and Nei's D for lake trout grouped by locality and phenotype. Top triangle is average conditional identity probability, diagonal is within-deme identity probability, and bottom triangle is Nei's D (Takahata and Palumbi 1985, eqs. 17 and 19, and Nei 1972).

CHAPTER V
MORPHOMETRIC ANALYSIS OF PHENOTYPIC VARIATION IN
<u>Salvelinus namaycush</u> IN LAKE SUPERIOR

Abstract

Principal component analysis of morphometric and meristic variables was applied to test the hypothesis that wild lean, siscowet, and humper lake trout (*Salvelinus namaycush*) are reproductively isolated. Previous investigations provided evidence that morphological characters related to fat storage were heritable in lake trout (Eschmeyer and Phillips 1965, Stauffer and Peck 1981). My investigation focused on aspects of morphology related to differences in fat content (shape differences). Analysis of covariance of morphometric characters with total length indicated significant differences in body depth. Although principal components have been used to discriminate between reproductively isolated but closely related species (Bookstein et al. 1985), it was unable to provide discrimination between wild lean, siscowet, and humper lake trout. Lake Superior lake trout show some stock differentiation rather than species level differentiation. Ecological segregation will preserve the integrity of genetic differences in genes involved in regulation or expression of metabolism and growth.

Introduction

Morphological analysis of hatchery raised lean and siscowet lake trout, *Salvelinus namaycush*, showed that distinctive parental traits (fat content and body depth) were transmitted to offspring raised under controlled environmental

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conditions (Stauffer and Peck 1981; Chapter 2), but discrimination of leans and siscowets in the wild remains a formidable task. I used principal component analysis of morphological characters to investigate differentiation corresponding to size and shape differences among wild lake trout phenotypes.

In Lake Superior, *Salvelinus namaycush* is represented by at least three divergent morphological forms. The lean phenotype has a straight snout and a slender fusiform body shape with a low body fat content. The siscowet phenotype has a convex snout and a robust body shape with a high body fat content. A third phenotype, the "humper" or "paperbelly," has a slightly convex snout and its body shape and fat content are intermediate to that of the lean and siscowet phenotypes. Historical reports referred to as many as 12 unique lake trout phenotypes prior to the early 1900's (Agassiz 1850, Goode 1884, Sweeny 1890, Monpetit 1897, Marr 1957, Rakestraw 1967, Parks Canada 1980, Cochrane 1982 and Isle Royale Oral History). Predation by the parasitic sea lamprey (*Petromyzon marinus*) since the 1950's on populations stressed by over-exploitation by man was implicated in the decline of lake trout numbers throughout the Great Lakes as well as in the elimination of some of the different lake trout phenotypes in Lake Superior (Brown et al. 1981, Goodier 1981, Eshenroder et al. 1984).

The presence of the lake trout more than any other fish species epitomizes North American oligotrophic lakes (Ryder 1972). The many diverse habitats in Lake Superior-- tributary rivers, littoral zones, offshore shoals--were at one time occupied by local, discrete, often morphologically and behaviorally differentiated populations or "stocks" (Lawrie 1973, Brown et al. 1981). The deepwater siscowet lake trout, extant only in Lake Superior, represents possibly the greatest degree of morphological divergence from the ubiquitous lean lake trout. Some have argued that the differences observed are due to environmental influence and that leans and siscowets are two ends of a continuum of phenotypes (Jordan and Evermann 1911, Eddy and Surber 1943, Scott and Crossman 1973). Persistent physiological differences that correspond to phenotype support the existence of reproductive isolation (Thurston 1962, Crawford 1966, Eschmeyer and Phillips 1965, Dehring

et al. 1981, Stauffer and Peck 1981, Phillips and Ihssen 1986, Karahadian and Lindsey 1989).

The lean lake trout is a slender fish with a straight pointed snout. It is slightly compressed laterally, and has a low body fat content ranging from 12% to 42% of its ash-free dry weight (Eschmeyer and Phillips 1965). Leans inhabit inshore waters from 15 to 80 meters deep. The siscowet lake trout is a robust fish with a rounded and slightly convex snout. It has a rounded body, and its body fat content ranges from 32% to over 85% of its ash-free dry weight. The fat in the siscowet is found not only in the visceral cavity, but interlaced throughout the muscle tissue (Thurston 1962). The lower body profile of the siscowet appears to be deeper than a lean of comparable size, and its anal fin base appears to be more posteriorly angled than the anal fin of the lean (Figure 5.1). Siscowets inhabit offshore waters from 50 to over 150 meters deep. The humper lake trout is a deep-bodied form with a slightly rounded snout and relatively large eyes. It is also distinguished from the lean and siscowet by the possession of a thin abdominal wall. The humper's body fat content is intermediate to that of the lean and siscowet, and most of the fat is located in the visceral cavity. Humpers inhabit waters around offshore deepwater shoals surrounded by greater than 100-meter depths. Evidence from fat content analysis and breeding studies suggest that the differences among the three phenotypes have a genetic basis. Principal component analysis was used as an application of traditional multivariate statistical methods to test the hypothesis that the three phenotypes are different species. Principal Component Analysis is used to determine whether observed morphological differences among extant lake trout are due to variation among character correlations in size and shape which correspond to the lean, siscowet, and humper phenotypes. If changes among correlated characters correspond to phenotypic boundaries, then PCA will discriminate the three forms. If genetic divergence corresponds to isolation by distance, then phenotypes may be discriminated by geographic locality rather than phenotype.

Chemical and fat content analyses supported the distinction between lean and siscowet phenotypes (Thurston 1962, Crawford 1966, Eschmeyer and Phillips 1965), but traditional morphological criteria provided little justification for the consideration of the different phenotypes as subspecies or full species (Eschmeyer 1957, Khan and Qadri 1970, Lawrie and Rahrer 1973). Protein electrophoretic investigations unsuccessfully attempted to uncover genetic correlates to observed phenotypic diversity (Dehring et al. 1981, Phillips and Ihssen 1986). These studies suggested that populations of lake trout in Lake Superior were geographically localized; phenotypic variants were more closely related to other lake trout in the same area of the lake than to similar phenotypes from other locations (Dehring et al. 1981). Mitochondrial DNA studies also argued that genetic diversity in *S. namaycush* is correlated to geography (Grewe and Hebert 1989). These observations suggest that some localized demographic factors that cannot be detected with traditional morphological methods must be contributing to the maintenance of stable phenotypic diversity among lake trout populations (Lande 1988). Principal component analysis and analyses of covariance provided methods by which correlations among characters that contribute to distinctions among phenotypes could be partitioned. Partitioning may help to determine if these characters have a genetic basis and if patterns of occurrence correspond to populations with phenotypic or geographic distinctions.

Methods

Sample collection

The fish used in this analysis were limited to wild lean, siscowet, and humper lake trout from Lake Superior. Collection localities are listed in Table 5.1. Lake trout were collected using variable mesh gill nets and bottom trawls. Gill net collections were made by the U.S. Fish and Wildlife Service (USFWS), Michigan Department of Natural Resources (MIDNR), Wisconsin Department of Natural Resources (WDNR), Minnesota Department of Natural Resources

(MNDNR), and commercial fishermen in U.S. waters. Bottom trawl collections were made by the USFWS in spring forage assessments in U.S. and Canadian waters of Lake Superior. Bottom trawl collections were made in 15- to 150-meter depths, across-contour using a 12-meter balloon trawl with 1x2-meter doors. Gill nets fished by USFWS, MIDNR, WDNR, and MNDNR were variable mesh, multi-filament nylon gill nets, 51- to 114-mm stretched measure. Nets were fished on the bottom in 1- to 3-night sets from 15- to 125-m depths. Commercial fishermen fished 114- to 152-mm stretched measure mono- and multi-filament nylon gill nets on the bottom in 3- to 5-night sets at greater than 110-m depths. Some specimens were retained by individual fishermen and only meristic measurements were acquired from those.

Identification criteria

Lake trout specimens were assigned to "lean," "siscowet," or "humper" phenotype categories on the basis of a combination of several external morphological characteristics used by fisheries managers and commercial fishermen. Lake trout were considered to be "leans" if they had a straight, pointed snout and slender body. Lake trout were considered to be "siscowets" if they had a convex snout (bent over the eye) and a deep body. Lake trout were considered to be "humpers" if they had a disproportionately large eye and a thin abdominal wall. Humpers had facial characteristics similar to the lean phenotype, and they lacked the excessive visceral fat of the siscowet phenotype. In some cases, identification of leans and siscowets was difficult and gross observation of the amount of visceral and intramuscular body fat was utilized as an additional criteria for identification. Siscowets had a much greater amount of visceral body fat (lining the dorsal wall of the visceral cavity) than leans. In addition, the excessive fat in the muscle tissue of siscowets was easily observed by squeezing the flesh between one's fingers. Fisheries managers and commercial fishermen often targeted siscowet populations based on depth of capture. Management regulations restricted state-licensed gill net fishing to depths greater than 60

fathoms (110 meters). All fish collected at depths greater than 110 meters by commercial fishermen in this study (Copper Harbor, Port Wing, Duluth) consistently possessed "siscowet" characteristics. Around Isle Royale, leans, siscowets, and humpers were taken in the same nets, set across-contour, but with only slight overlap in depths. I observed that if leans and siscowets were both taken from the deeper water, siscowets did not bloat as severely as leans when brought to the surface.

Data analysis

Lengths and weights were taken on specimens in the field as conditions permitted. Morphometric and meristic measurements were taken in the laboratory. Length measurements were made to the nearest millimeter and weights to the nearest gram. Morphometric measurements taken included total length, weight, body depth, head length, head depth, predorsal length, preorbital length, postorbital length, suborbital length, postmaxillary length, dentary length, dentary tooth row length, mandible length, premaxillary length, and premaxillary height. Meristic measurements included ventral pores, gill rakers, branchiostegal rays, fin rays, lateral line pores, scales in diagonal rows, scales in vertical rows, scales around caudal peduncle, and pyloric caeca. Morphometric and meristic measurements are listed in Table 5.2 and illustrated in Figure 5.2. These measurements were chosen to concentrate on size and shape features most directly related to the gross phenotypic differences among Lake Superior lake trout. Other traditional morphological and meristic measurements were found to contribute little to discrimination among phenotypes (Khan and Qadri 1970, 1971; Qadri 1967).

Differences in individual measurements within and between geographic areas were tested by grouping fish from sampling areas roughly corresponding to sub-basins within Lake Superior. The "north" area included Isle Royale and Sawyer Bay samples; "west" included samples from the western trench (Minnesota north shore and Port Wing); "southwest" included the Apostle Islands to the

Keweenaw Peninsula; "south" included Copper Harbor through Keweenaw Bay to Grand Marais; and "east" included areas from Michipicoten Island south and eastward into Whitefish Bay along the Canadian shoreline (Figure 5.3). Samples from the western basin only included siscowets because there were no wild lean populations in that area of the lake. The limited distribution of humpers in Lake Superior restricted samples of this phenotype to the northern basin.

Measurements were transformed to natural logarithms to render variances less dependent on size. I analyzed the data using univariate statistics and two methods of multivariate analysis. The first was principal component analysis (PCA) (Blackith and Reyment 1971, Bookstein et al. 1985) and the second was analysis of covariance (ANCOVA) (Sokal and Rohlf 1981). Traditional multivariate analyses are often used to identify size and shape differences among individuals and between groups. In a multivariate statistical framework, size and shape become linear combinations of variables where size as a factor is used to predict distance measurements (Humphries et al. 1981). Shape may be represented by factors that express geometric relationships among correlated characters. Shape factors are not free of size, because of the effect of allometric growth, but may be represented as independent by analytical methods (Humphries et al. 1981, Bookstein et al. 1985) . Regression methods alone are not adequate for removing the effect of size because only the effect of one size variable (usually a measure of body length) would have been removed. Discriminant analysis was not adequate to remove size effects or discriminate groups because it required *a priori* group assignments and it would have been difficult to interpret the results without circular reasoning (Humphries et al. 1981, Winans 1985; and see Appendix C, Figure C.6 and C.7).

Principal component analysis was performed using the SYSTAT statistical package FACTOR module (Wilkinson 1981, ver. 4.01). PC analysis was performed on a correlation matrix of all variables to see which morphometric and meristic characters had the greatest influence on the observed variation. Principal component analysis summarizes many variables into a smaller number of

dimensions containing the major trends of variation in the original data. The components are linear combinations of variables orthogonal to other components. They are calculated from either a covariance matrix (morphometrics), or a correlation matrix (meristics). The principal component scores for individuals are graphed two at a time to observe patterns of groups of phenotypes present in the samples. The eigenvalues of each component measure the variability explained by each PC. The loadings of each character (variable) onto individual PC's measured the correlation of that character with the component. Each of the first several components is expected to have characters with high coefficients, which suggest biological interpretations. Components in which all character loadings were positive were generally interpreted as size components and bipolar components were generally treated as measures of shape (Jolicoer and Mosimann 1960, Mosimann 1970). The data were subdivided by phenotype and geographic area for separate PC analyses to examine whether the ability to discriminate among the three phenotypes was confounded by the interaction between geographic areas.

The analysis of covariance (ANCOVA) supplemented the principal component analysis to determine if regression lines of natural log-transformed morphometric measurements on total length were similar among phenotypes or locations. ANCOVA was used to examine individual measures of size and shape, and their pattern of covariance with total length. Total length was used as the covariate term to test the significance of differences between the means of univariate morphometric characters for *S. namaycush* by phenotype (lean vs siscowet), by locality, and by interaction of phenotype and locality. ANCOVA combines the features of ANOVA and traditional linear regression to study regressions of multiple classifications (Snedecor and Cochran 1967). The covariate used in this analysis was total length and categories were phenotype (TYPE), sampling area (LOCALITY), and population (TYPE x LOCALITY). Homogeneity of residual variances was tested to see if the variation among groups was similar. If variances were similar, then the differences between categories

were tested by looking for intercept differences. Finally, homogeneity of slopes was tested to look for length by category interactions. Variation between and within classes was also examined.

Results

Univariate Statistics

The three phenotypes differ significantly from each other (with overlap) in most measurements (Table 5.3). All 95% intervals fall within the overall ranges for all samples combined (Table 5.4). The distribution of morphometric measurements calculated as ratios of total length (Figures 5.4 a-l) illustrate the overlap among sample means as well as sample ranges. There is considerable overlap in meristic measurements as well (Figure 5.4 m-p). A chi-square test of homogeneity of group variances by phenotype (Bartlett's test) showed that group variances were similar for all meristic characters except ventral pores. Group variances by phenotype were significantly different at $P < 0.05$ for all morphometric characters.

A Tukey's HSD test performed for between-phenotype differences to test the homogeneity of group means (Sokal and Rohlf 1981) showed that the differences between the comparison of leans against siscowets and leans against humpers were much greater than those between siscowets against humpers (Table 5.5). The greatest differences between leans and siscowets were in body depth, predorsal length, head depth, preorbital length, postorbital length, post-maxillary length, and caudal scale count. All of these reflected differences in robustness. The greatest differences between leans and humpers were in body depth, predorsal length, head depth, head length, suborbital length, postorbital length, preorbital length, dentary length, mandible length, postmaxillary length, premaxillary width, vertical scale rows, caudal scales, ventral pores, and lateral line pores. The greatest differences between siscowets and humpers were only in postorbital length, head depth, head length, and lateral line pores. Differences

between siscowets and humpers reflected head shape rather than body shape differences. The source of most of the between-phenotype differences was located in the lean-humper comparison.

There were no significant differences in morphometric measurements by geographic area (Table 5.6). Meristic characters, excluding anal rays, were not significantly different among geographic areas. Anal rays differed significantly among comparisons between north, west, and south populations. Bartlett's test of homogeneity showed that the variances of all characters among geographic areas were similar except lateral line pores and jaw bone measurements (dentary length, mandible length, premaxillary width, premaxillary height). Tests of least significant differences among group means generally showed that morphometric characters were significantly different among all areas but that samples from the north and west were similar (Table 5.7).

Differences in morphometric ratios were evident between populations from different geographic areas (but with overlap), but there were no significant differences between different phenotypes in the same area (Table 5.8). Both morphometric and meristic measures widely overlapped among populations. Interpopulation differences in variance were noted for morphometric characters, but not for meristic characters. Tests of least significant differences showed that among morphological characters whose means were significantly different between populations, the greatest differences were found between northern and southern populations.

Principal Component Analysis

Principal component analysis was performed on a correlation matrix of all variables to see which morphometric and meristic characters had the greatest influence on the observed variation. The first principal component (PC 1) from a correlation matrix accounted for 47% of the variation, and the second principal component (PC 2) accounted for 8% of the variation in the data set (Table 5.9). PC 1 was clearly a size component, although two meristic variables had relatively

high loadings. Caudal scale count and vertical scale rows loaded at 0.60 and 0.59 on PC 1. A plot of PC 1 against PC 2 revealed no correlation between phenotype and morphology (Figure 5.5). The humper phenotype clustered within a much narrower range than either the lean or siscowet. With locality projected onto the PC 1 against PC 2 plot, there appeared to be a very slight geographic sub-structuring (Figure 5.6).

Lake trout phenotypes from the Isle Royale area (including Sawyer Bay) could not be distinguished in a separate principal component analysis. PC 1 accounted for 44% of the variation and PC 2 accounted for about 9% of the variation (Table 5.10). The first 11 components accounted for 92% of the variation. PC 1 in the north subset is a size component. Fin ray counts had the highest loadings on PC 2 for Isle Royale fish. A plot of PC 1 against PC 2 in Figure 5.7 showed no correspondence between phenotype and morphometrics for the Isle Royale subset. The humper phenotype showed less dispersion among PC scores than the lean or siscowet phenotypes.

Principal component analysis of a correlation matrix from a subset of wild lake trout from the southern basin (Copper Harbor through Keweenaw Bay) also showed no correspondence between phenotype and morphometrics. PC 1 accounted for 45% of the variation and PC 2 accounted for 10% (Table 5.11). The first 10 components accounted for 92% of the variation. In contrast to the Isle Royale data, anal rays had the highest loading on PC 2. Except for lateral line pores, all other meristic characters had high but negative loadings on PC 2. Figure 5.8 shows a plot of PC 1 against PC 2 for the Keweenaw subset.

The Isle Royale and Keweenaw subsets were combined to test the effect of geographic area. Table 5.12 lists the loadings for the first five principal components from a correlation matrix of all variables. PC 1 accounted for 47% of the variation and PC 2 accounted for 8% of the variation (Figure 5.9). In a plot of PC 1 against PC 2, a slight substructuring was seen by geographic area (Figure 5.10). The northern fish were generally larger and had higher meristic counts than the southern fish. Within the northern localities the dynamics of

morphology and meristics were slightly different than within the southern localities, as reflected in the differences among characters highly correlated on PC 1. The signs on PC 1 were similar among the bipolar coordinates, but their magnitudes were different. Correlation of characters on PC 2 showed the same pattern among the different analyses.

Separate and pairwise principal component analyses were performed for lean, siscowet, and humper phenotypes to determine if geographic substructure existed within each phenotype in Lake Superior (Figures 5.11a-b). The results were similar to those of previous tests in their patterns of geographic association. Lean and siscowet scores widely overlapped. In separate and pairwise comparisons, there was no correspondence between phenotype and morphometrics. Southern populations were less widely scattered in component space than northern populations, but with some overlap. Within each cluster there was a very weak association of individuals of similar phenotype, and the humper phenotype consistently clustered within a narrower range. Morphological distinction could not be made by either phenotype or location alone. Principal component loadings for the lean, siscowet, humper, and pairwise combinations are listed in Tables 5.13 and 5.14.

The relative influences of length and body depth measurements on phenotypes were tested by performing principal component analyses with only total length, body depth, head depth, and head length. Total length and body depth could not discriminate phenotypes with PC 1 and PC 2 (Figure 5.12). The addition of head depth into the analysis did not change the clustering pattern (Figure 5.13). Further addition of head length again showed no discrimination between lean, siscowet, and humper phenotypes (Figure 5.14). When geographic locality is projected onto each of the PC 1-PC 2 plots, north and south localities show a slightly segregated but overlapping distribution of scores (Figures 5.12-5.14).

Principal component analyses performed on separate morphometric and meristic data could not discriminate lean, siscowet, or humper phenotypes.

Inspection of geographic distributions also could not discriminate populations by phenotype or by locality. The PC 1-PC 1 plots for the log-transformed data are similar in shape; there is no noticeable difference in the clustering relationships by phenotype or by location according to morphometrics. Principal component 1 for log transformed morphometric data accounted for 93% of the variation in the data set. PC 2 accounted for 2.3% (Table 5.15). The highest loadings on PC 1 for log-transformed data were head and jaw measurements, and body depth. The highest loadings on PC 2 were premaxillary measurements, predorsal length, and head depth. Meristic PC 1 accounted for 17.5% of the variation, and PC 2 accounted for 16%. Meristic PC 1 was dominated by vertical scale row and caudal scale counts. Gill raker and fin ray counts accounted for most of the variance in the remaining components. Most of the dispersion in the data was attributed to variation in head lengths and body depth, though the clusters in Figure 5.15 do not correspond to discrete lean, siscowet, or humper phenotypes. The humper population cluster again showed less dispersion than the individual samples identified by lean and siscowet phenotypes.

The data subset for the northern sampling area showed patterns similar to the whole data set in PC analysis. PC 1 accounted for 87% of the variation, and PC 2 accounted for 4% (Table 5.16). Jaw measurements and body depth had the highest correlation with PC 1, while head depth and body depth contributed most to PC 2 and PC 3. Meristic PC 1 for the northern subset accounted for 17% of the variance from a correlation matrix; and PC 2 accounted for 15% of the variation. The variables with the highest correlation on the first meristic principal component were ventral fin rays and vertical scale rows. The clustering pattern of PC 1 morphometrics against PC 1 meristics in Figure 5.16 did not correspond to lean, siscowet, or humper phenotypes.

Results for PC analysis of the southern sampling area showed similar patterns among log-transformed morphometric variables, but the variation among meristic variables differed from those of the northern area. PC 1 for morphometrics accounted for 91% of the variation, and PC 2 accounted for 6%

(Table 5.17). Jaw measurements, body depth, branchiostegal rays and anal rays had high loadings on PC 1, which accounted for 18% of the variation. PC 2 was dominated by predorsal length, and accounted for 15% of the variation. The dispersion in the data seen in Figure 5.17 was due to variation in body depth and premaxillary bone sizes. No correspondence was observed between PC score and lean or siscowet phenotype.

A plot of PC 1 morphometrics against PC 1 meristics for the north and south localities combined (Figure 5.18) showed a slight geographic substructuring of the data (with some overlap) along both axes into north and south clusters. The pattern of variation in the combination of the northern and southern data sets was similar to the PC analysis of the whole data set. PC 1 accounted for 92% of the variation in the data set, and PC 2 and PC 3 accounted for 3.2% and 1.5% (Table 5.18). As with the previous analyses, the clustering pattern in Figure 5.18 did not correspond to lean, siscowet, or humper phenotypes. While the PC 1 scores of the humper individuals overlapped lean and siscowet scores, the variability in scores among humpers was less than that among leans and siscowets.

Analysis of Covariance

Variation in body depth was significantly different by phenotype and location. There was a significant interaction between phenotype and total length, and between location and total length (Table 5.19). Variation between phenotypes was significant for head length, preorbital length, mandible length, dentary length, and premaxillary width. The effect of location on variation in morphometric characters was significant for head depth, weight, suborbital length, postmaxillary length, and premaxillary width. Although several of the morphometric variables varied significantly with total length by phenotype or location, the high amount of overlap does not allow discrimination among leans, siscowets, and humpers (Figure 5.19 a-f).

Discussion

There are no distinct morphometric or meristic characters that allow discrimination among preserved specimens of wild lean, siscowet, and humper lake trout. The wild fish were originally identified on the basis of body shape, snout shape, and visceral fat, in addition to depth of capture. No differences in morphological measurements related to the identification criteria could distinguish among the three lake trout phenotypes. The wide overlap in ranges of morphometric and meristic characters suggested that measurements whose means were statistically significant were not biologically significant. The differences in the variance of jaw measurements among geographic areas could have been a reflection of trophic differences (Wimberger 1991) and ecological character displacement (Smith and Todd 1984). Most morphometric characters differed by area, but northern and western populations had no significant differences. Isle Royale lies just south of the northeastern extension of a deep trench along the Minnesota shore, and populations from these two areas were likely to be under the influence of similar selective forces. Based upon these results, it must be concluded that lean, siscowet, and humper lake trout have not diverged in general morphology and are not reproductively isolated in the wild. The differences that do exist cannot support their distinction as separate species.

PC analysis has been used to discriminate between and to provide evidence to support the presence of reproductive isolation among closely related populations of cottids and atherinids (Bookstein et al. 1985). In this study, principal components was used as an application of traditional morphometric analyses and was unable to discriminate among lean, siscowet, and humper phenotypes. Discrimination of wild lake trout by traditional multivariate analysis is limited in contrast to the extreme differences between lean and siscowet progeny raised under controlled conditions. The inability of morphometrics of wild leans and siscowets to corroborate the discrimination observed in the

hatchery puts in question the breadth of the genetic basis for morphometric characters of wild lake trout.

Phenotype = Genotype x Environment

Differences between lean and siscowet lake trout in the wild are most pronounced for fat storage (Thurston 1962, Eschmeyer and Phillips 1965). The most conspicuous differences between leans and siscowets raised under controlled conditions were in growth-related characteristics in addition to persistent fat storage differences (Stauffer and Peck 1981). Lean progeny in the Thompson Hatchery experiment were 25% smaller than their siscowet counterparts, yet in the wild, siscowets grow slower. Wild lean lake trout averaged 14-18 inches (340-410 mm) at age 5 (Cable 1956), while 5-year-old wild siscowets averaged only 9-14 inches (220-340 mm) (Pratt and King 1980). Growth differences could be directly related to environment--siscowets raised at a stable temperature warmer than that in deep water (7°C vs <4°C) grew faster than leans. However, fat content differences between the leans and siscowets persisted under the same conditions.

It is clear that some morphological differences between leans and siscowets are heritable. Subtle genetic differences may be linked to genes regulating metabolism and growth, but their phenotypic effect may be magnified under critical environmental conditions. Natural selection does not operate directly on individual characters but on the degree of interlocking between one dimension of variation and another (Blackith and Reyment 1971, Lande and Arnold 1983). One dimension may be phenotypic variation, while the other may be genetic variation. Environmental variation will contribute to the intrinsic variation of heritable characters if the fitness of an individual is determined by its phenotype (Gillespie and Turelli 1989). Local fluctuations in resource availability and abundance in the wild affect growth processes and metabolic efficiency, but heritable differences could be under different selection pressures when depth becomes a factor.

It is not uncommon for growth in fishes to be slower at lower temperatures (Brett 1979, Dunn 1988) and metabolic differences have been shown to be associated with different habitats as well (Paloheimo and Dickie 1966a). Laboratory experiments conducted to test the effect of temperature on fish growth (Pentelow 1939, Brown 1946, Hokanson et al. 1977) ascertained that growth patterns were different when fish were raised at steady versus changing temperatures (Brett 1979, Paloheimo and Dickie 1966b). For example, under a cyclic temperature regime (below 18°C) brown trout grew at a faster rate than they grew at a constant temperature (Hokanson et al. 1977). Experiments testing the relationship of photoperiod to growth patterns suggested that day length also contributes to growth rates by influencing the release of somatotrophic hormone (Swift 1955, Hogman 1968). Growth rates of brown trout in cold water increased in spring with increasing day length, then slowed in summer and autumn (Swift 1955). Hogman (1968) determined that seasonal change in the growth rate of coregonids was more closely related to day length than to water temperature changes. It is significant that the deep basins of Lake Superior have characteristically steady cold temperatures and little light penetration. If these two factors have a profound impact on lake trout growth processes, then the lack of cyclic variation in temperature and light may result in a slower growth rate for siscowet than for lean lake trout in Lake Superior. Over time, a slower growth rate could become incorporated into the genome and become a heritable trait. This type of life history adaptation was demonstrated in *Poecilia reticulata* in response to changes in predation pressures (Reznick and Bryga 1987).

In their review of the relationship between body weight and metabolism in fishes, Paloheimo and Dickie (1966a) concluded that there is a general functional relationship between metabolic level and body weight that is maintained under a variety of environmental conditions. In a subsequent review Paloheimo and Dickie (1966b) concluded that some of the differences in growth and metabolism among fish species are due to seasonal changes in environmental conditions which affect growth efficiency. Differences in growth efficiency between leans and

siscowets could be due to changes in energy demands in the different environments and to changes in the disposition of energy (Paloheimo and Dickie 1966b). In various experiments it was shown that metabolic changes correspond to changing thermal conditions. However, if thermal conditions change then stabilize, metabolism will show corresponding changes then adjust to a new equilibrium level (Brett 1956). Leans may be living under environmental conditions which force them to repeatedly adjust their metabolism. The lack of significant changes in the deep water environment may provided the potential for siscowet lake trout to adjust their metabolism to a steady, but different rate than lean lake trout in the shallower waters.

Life history differences that result in variable growth, and metabolic characteristics related to environmental variation may also be tied to differences in energy use. A plot of body depth against length reveals a number of lake trout identified as siscowets that appear to be more slender than a majority of the lake trout identified as leans (Figure 5.19a). The siscowets were identified by the facial and body shape characteristics outlined earlier in this chapter. The geographic origins of the slender siscowets include all areas of Lake Superior. There is no correspondence between the low body depth:total length ratio and geographic location, a result that could be explained by hybridization in areas of geographic overlap. The slender siscowets identified by sex are primarily female. None of these individuals was identified as being in or near spawning condition. This suggests that one possible role of high fat deposition in the siscowet phenotype is energy storage for spawning. Fat content may be more variable in females if they require a larger amount of energy to produce eggs before the next spawning season. Not all female lean lake trout spawn every year (Eshmeyer 1954, Swanson and Swedberg 1980), and the same is suspected for siscowet lake trout (C. Bronte, pers. comm.). In addition, food resources in very deep water may fluctuate enough that storage of fat for energy is necessary. Much of the fat of the siscowet phenotype is located in the muscle. It is more efficient to store energy as lipids in the muscle because carbohydrates are more rapidly converted

there (Gee 1984). Low abundance of forage for siscowet lake trout would also explain occasional observations of siscowets in shallow water. Siscowets sampled from shallow inshore waters have been feeding on terrestrial insects and shallow water fish species, and they have been found with full stomachs (Eschmeyer 1954).

Ecological Segregation and Morphological Diversity

The observable phenotypic differences between Lake Superior lean and siscowet *S. namaycush* are the result of adaptive trophic specializations to stable diverse freshwater environments. The persistence of phenotypic differences in hatchery raised offspring provided evidence that the polymorphisms were not due to phenotypic plasticity (Stauffer and Peck 1981) because they were transmitted intact from parent to offspring under identical environmental conditions. In addition, there is little evidence that the phenotypic alternatives are reversible. If this were so, then a single individual should show evidence of switching tactics. In contrast, if the different phenotypes represent irreversible alternatives, then the alternative represent differences in life history tactics with the potential to lead to reproductive segregation (Gross 1991). Temporal and spatial differences in habitat and spawning have enhanced assortative mating, but lake trout with characteristics intermediate to the lean and siscowet phenotypes appear in the wild. The existence of "hybrid" morphologies and the inability to discriminate the three phenotypes with morphometric analysis confirm that reproductive isolation is incomplete.

A number of models have been proposed to explain the speciation process mediated by ecological divergence (Rosenzweig 1978, West-Eberhard 1986, Diehl and Bush 1990). Wimberger (1991) pointed out that a common denominator in those models was the presence of diverse but stable habitats. Both foraging habitats and spawning habitats provide mechanisms of niche separation which could ultimately lead to positive assortative mating and parapatric divergence (Endler 1977). Suites of characters rather than single characters are heritable, and the interaction of genetic and environmental factors influences the phenotypic

expression of the different genotypes. Some of the resulting phenotypic variation can be environmentally induced, such as changes in visceral and gonadal fat deposition in response to the type of food or its abundance. Some variation can be mostly genetic, such as the interstitial fat of siscowets and the abdominal musculature of the humpers. The link between the functional adaptations and the developmental process is the key to the evolution of genetic diversity. Regulatory mechanisms that develop and are integrated into the genome become key players in the speciation process. Selection in different environments will drive character divergence as well as divergence in associated developmental genetic regulatory systems. In turn, character divergence will be enhanced by philopatry and assortative mating, and mutations in the genetic regulatory mechanisms may become fixed in populations. The divergence between leans, siscowets, and humpers originated as an ecophenotypic response to ecological differences, but has become incorporated as a heritable component.

Character divergence between leans and siscowets is not merely a growth-related phenomenon. There is no question that leans and siscowets have different metabolic characteristics. Eschmeyer and Phillips (1965) documented non-overlapping differences in fat content that proved to be heritable. Thurston (1962) documented elemental differences in muscle composition as well as differences in the proportions of muscle proteins. Crawford (1966) documented differences in neutral buoyancy between leans and siscowets that suggested adaptation to different depths. Finally, Karahadian and Lindsay (1989) demonstrated differences in the oleic acid composition of the Ω-3 oils of lean and siscowet lake trout. Traditional morphometric analyses cannot discriminate leans, siscowets, and humpers, despite overwhelming evidence that morphological differences are heritable. The only real morphological differences are in the lower body profile (robustness as affected by fat content), and in the morphology of the opercle and supraethmoid bones (Chapter 3). Ecological differences support some level of segregation during spawning which has preserved the integrity of the heritable characters. Based on morphometric analyses lake trout

phenotypes in Lake Superior are not different species, but their physiological adaptation to different habitats has a genetic basis. *Salvelinus namaycush* may well be in the process of speciation in Lake Superior.

Conclusion

Principal component analysis was used to ordinate morphological variation among wild lean, siscowet, and humper lake trout from Lake Superior. PC analyses have demonstrated morphometric discrimination among reproductively isolated populations of closely related species (Bookstein et al. 1985) but could not discriminate among the three lake trout phenotypes. Analysis of covariance showed slight but not significant differences between leans and siscowets in body depth and head depth. Fat content analyses and hatchery breeding studies (Eschmeyer and Phillips 1965, Stauffer and Peck 1981) provided convincing evidence that morphological characteristics of lake trout were heritable, but the results of the PC analyses force the acceptance of the null hypothesis that the three phenotypes are not different species.

Subtle genetic differences among Lake Superior *Salvelinus namaycush* are related to fat storage and growth. Factors in the wild that affect the regulation or the expression of those genes modify the intensity of the phenotypic differences. Populations of lean, siscowet, and humper lake trout are partially segregated by time and place of spawning. The occurrence of intermediate morphologies among wild fish suggests that hybridization does occur in areas of contact. Divergence among these populations is not at the level of species, but may be at the level of stock differentiation. If assortative mating mediated by ecological segregation preserves the integrity of existing genetic differences, lean, siscowet, and humper lake trout in Lake Superior may be in the process of intralacustrine speciation.

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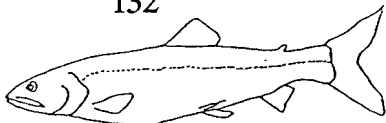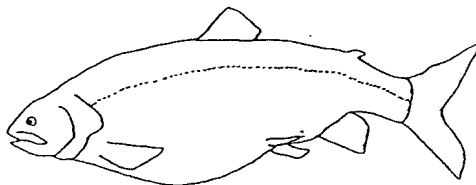
Lean
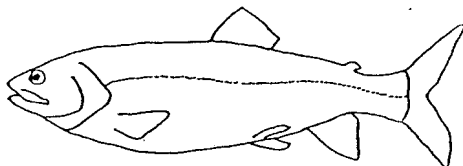
Siscowet

Humper

Lean

Siscowet
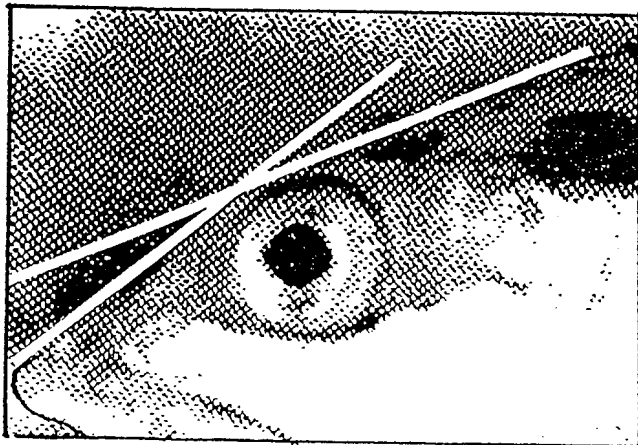
Figure 5.1. General body shapes of lean (top), siscowet (middle), and humper (bottom) lake trout phenotypes. The most obvious morphological differences are in body depth, and in the shape of the snout as indicated by the bars above the head shown in the lower picture. The anal fin of the siscowet phenotype also appears to be angle more posteriorly than the lean or humper.
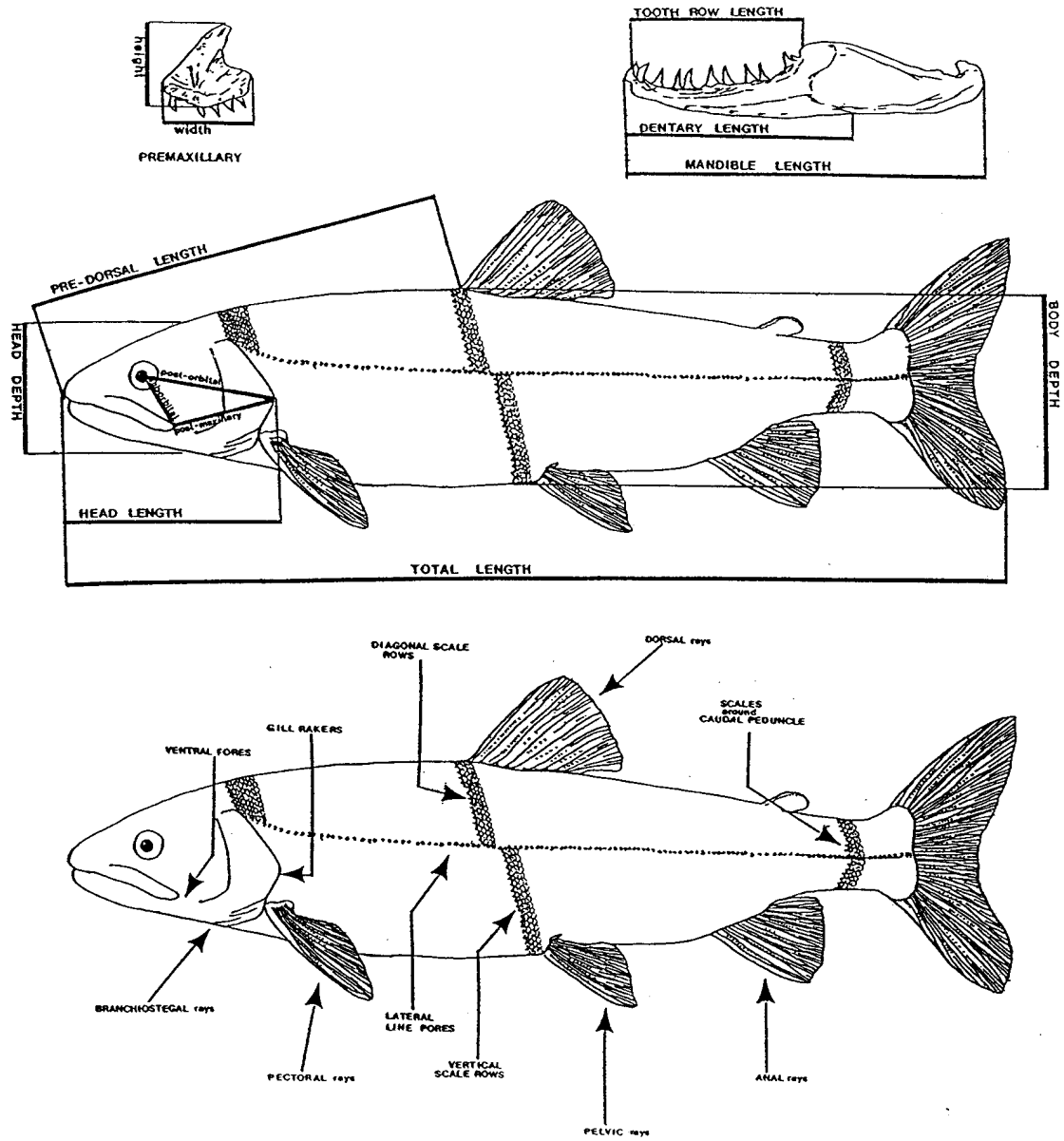
Figure 5.2. Morphometric and meristic measurements collected for wild Lake Superior *Salvelinus namaycush*. All length measurements taken in millimeters.
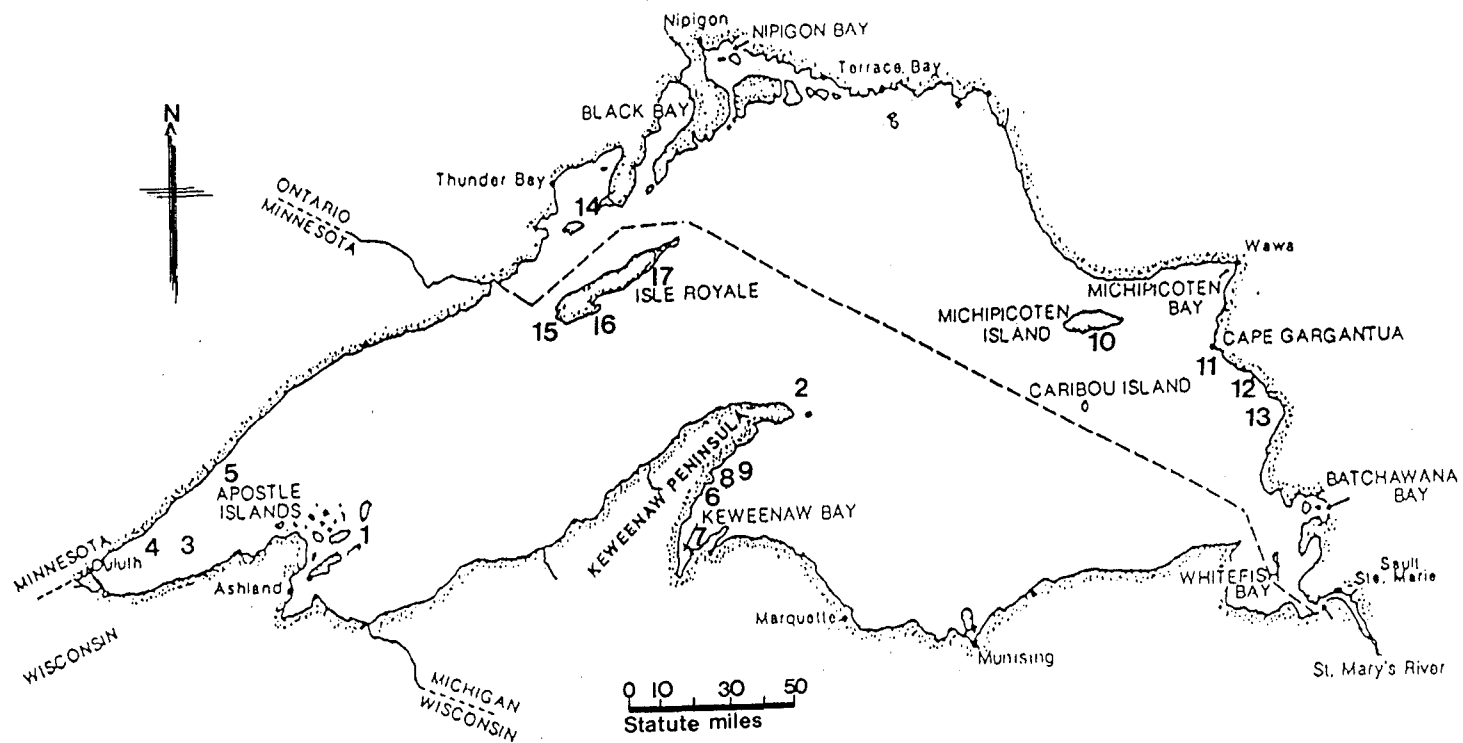
Figure 5.3. Map of Lake Superior. Locations of sample collections are indicated by numbers corresponding to Table 5.1.
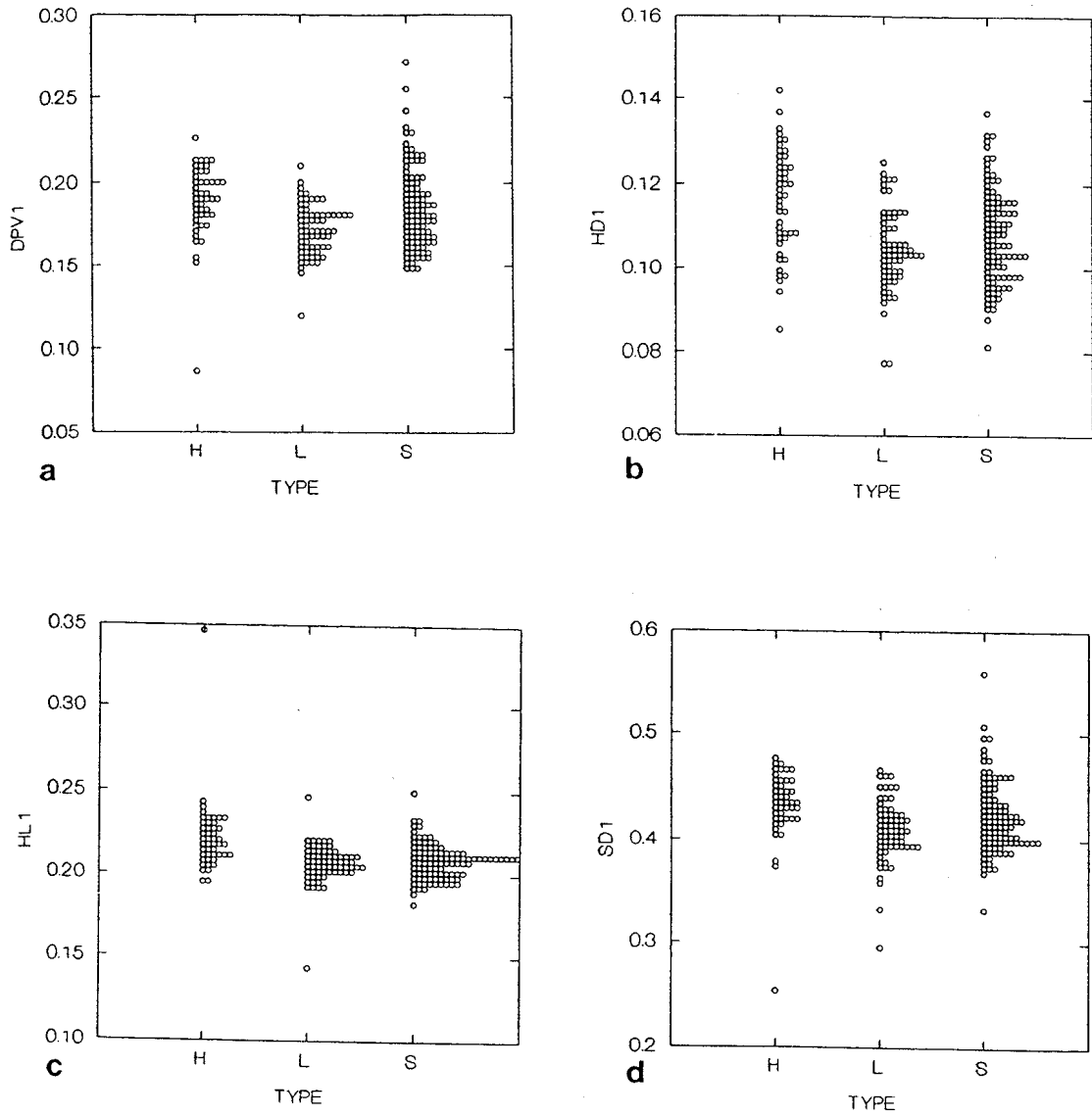
Figure 5.4 a-d. Distribution of morphometric measurements of wild lean (L), siscowet (S), and humper (H) lake trout from Lake Superior. Measurements are given as ratios of total length. DPV1 = body depth./total length (tl); HD1 = head depth/tl; HL1 = head length/tl; and SD1 = predorsal length/tl.
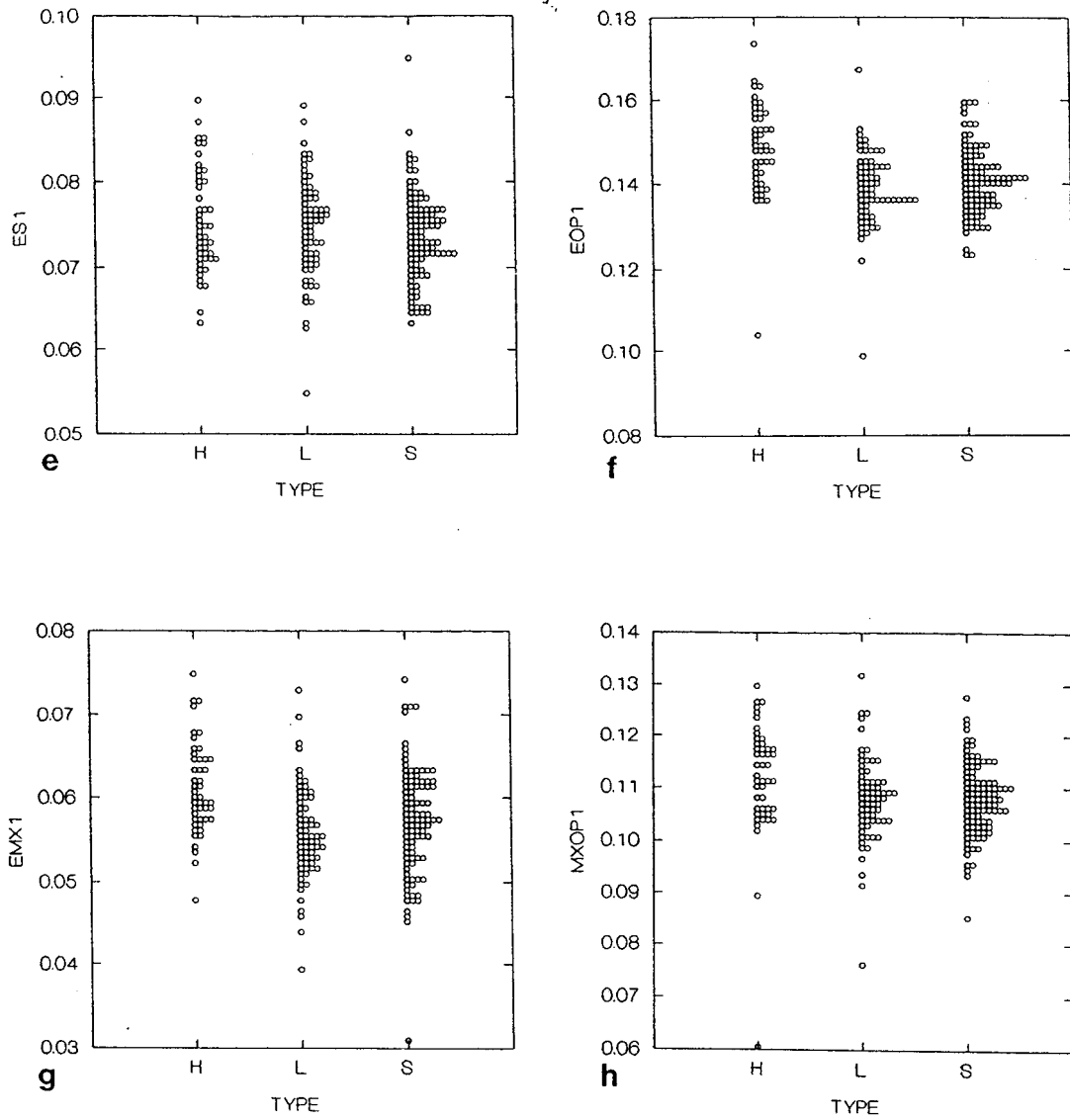
Figure 5.4 e-h. Distribution of morphometric measurements of wild lean (L), siscowet (S), and humper (H) lake trout from Lake Superior. Measurements are given as ratios of total length. ES1 = preorbital length/total length (tl); EOP1 = postorbital length/tl; EMX1 = suborbital length/tl; MXOP1 = postmaxillary length/tl.
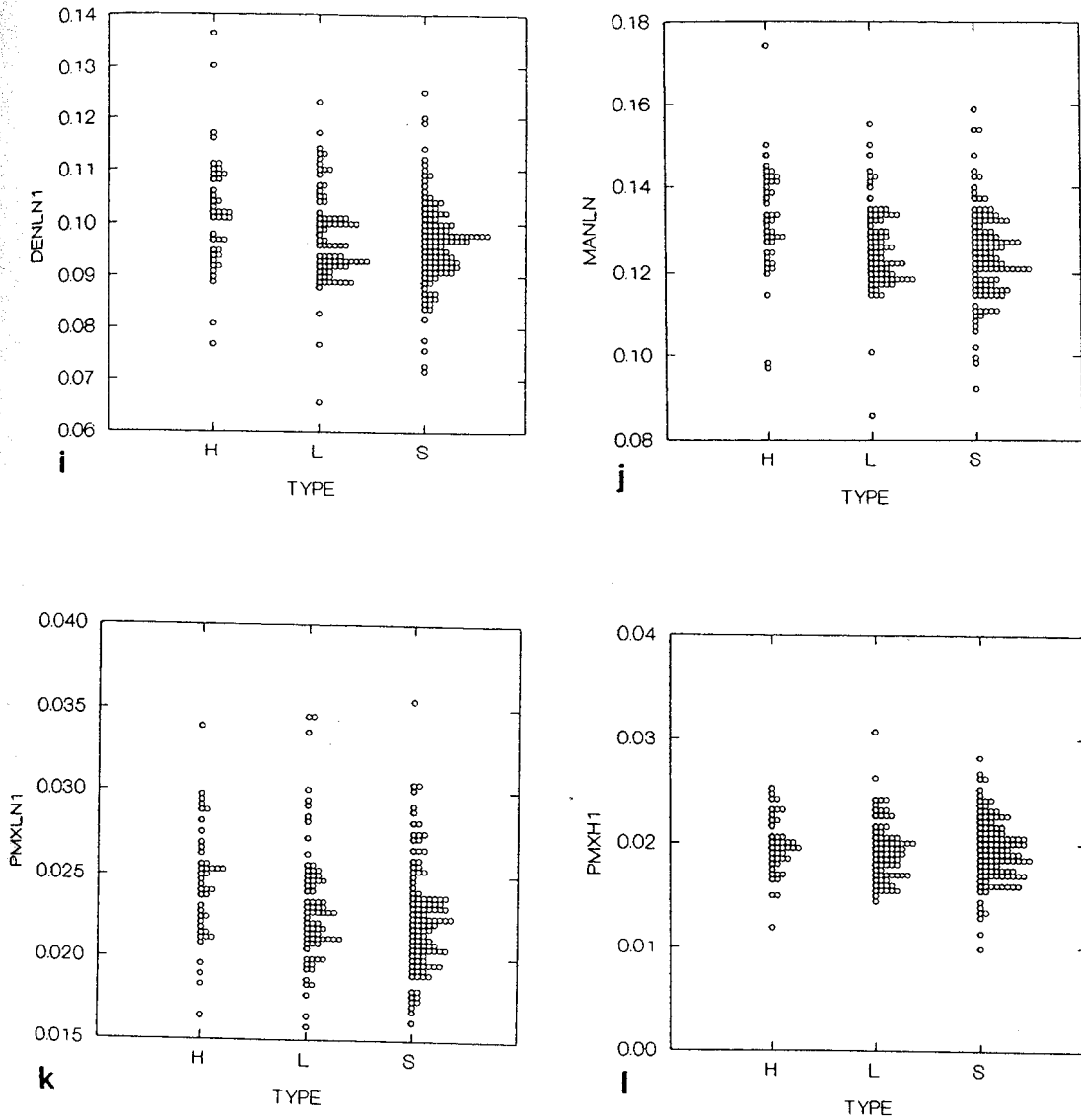
Figure 5.4 i-l. Distribution of morphometric measurements of wild lean (L), siscowet (S), and humper (H) lake trout from Lake Superior. Measurements are given as ratios of total length. DENLN1 = dentary length /total length (tl); MANLN = mandible length/tl; PMXLN1 = premaxillary width/tl; PMXH1 = premaxillary height/tl.
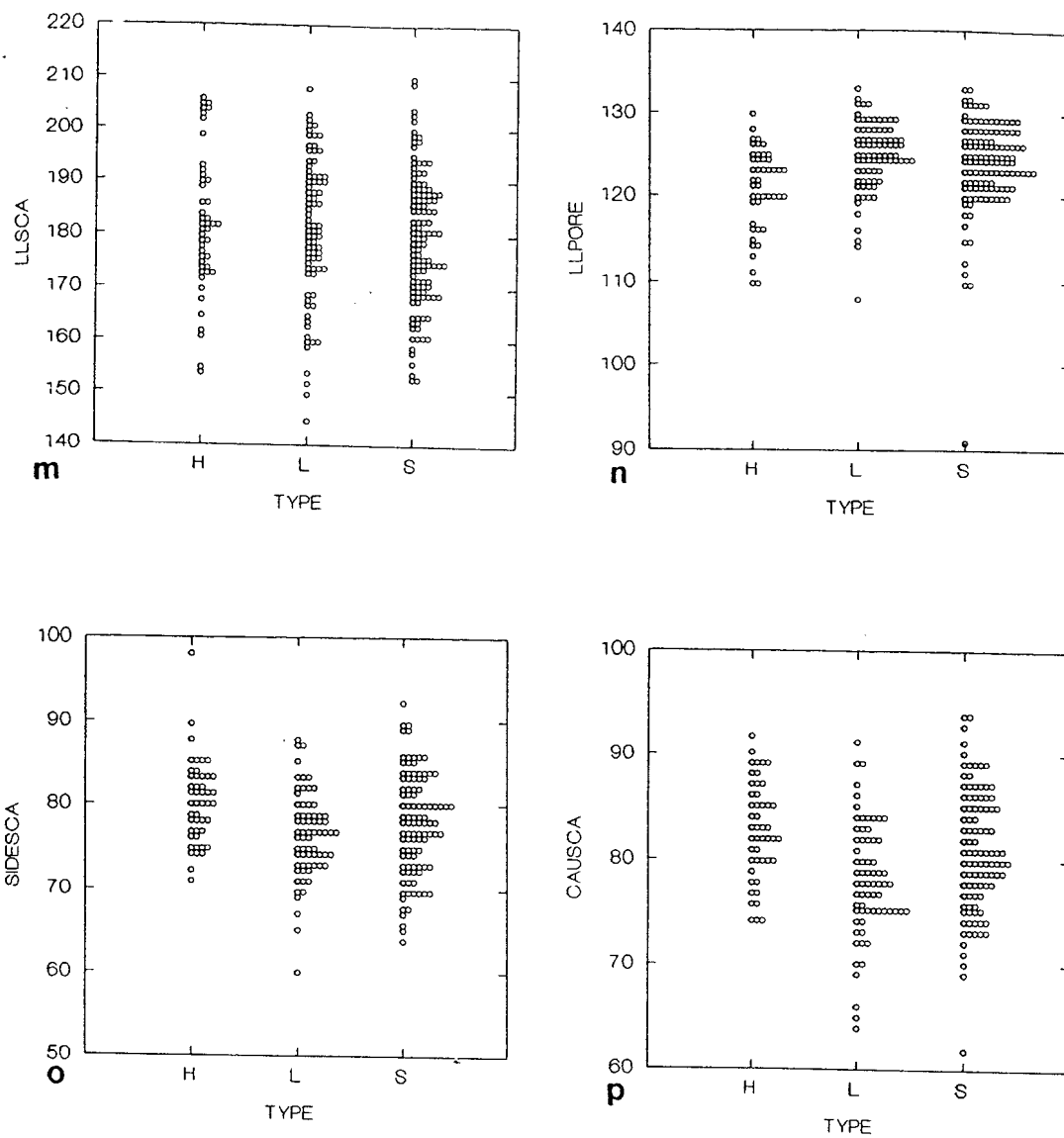
Figure 5.4 m-p. Distribution of some meristic measurements of wild lean (L), siscowet (S), and humper (H) lake trout from Lake Superior. LLSCA = scales in diagonal rows; LLPORE = lateral line pores; SIDESCA = scales in vertical rows; CAUSCA = scales around caudal peduncle.
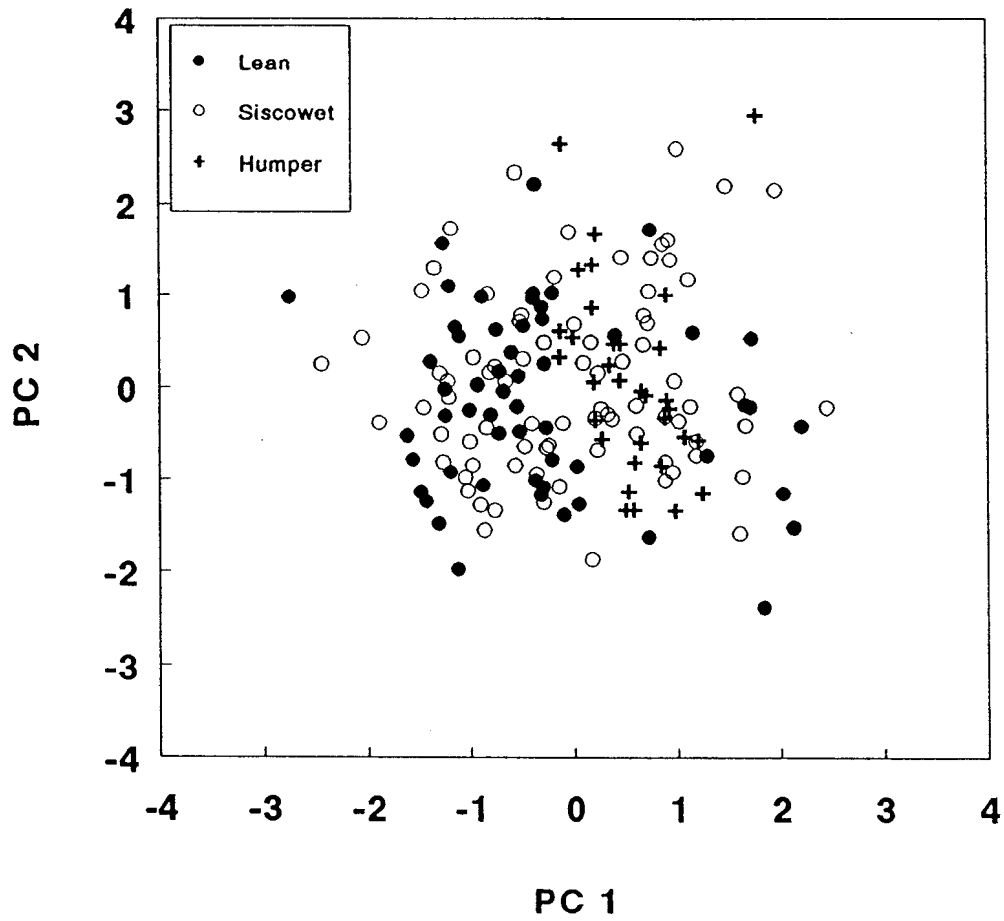
Figure 5.5. PC 1 vs PC 2 for wild Lake Superior *S. namaycush* identified by phenotype. PC scores were computed from a correlation matrix of all variables. The humper phenotype shows much less dispersal than either the lean or siscowet phenotypes in PC space.
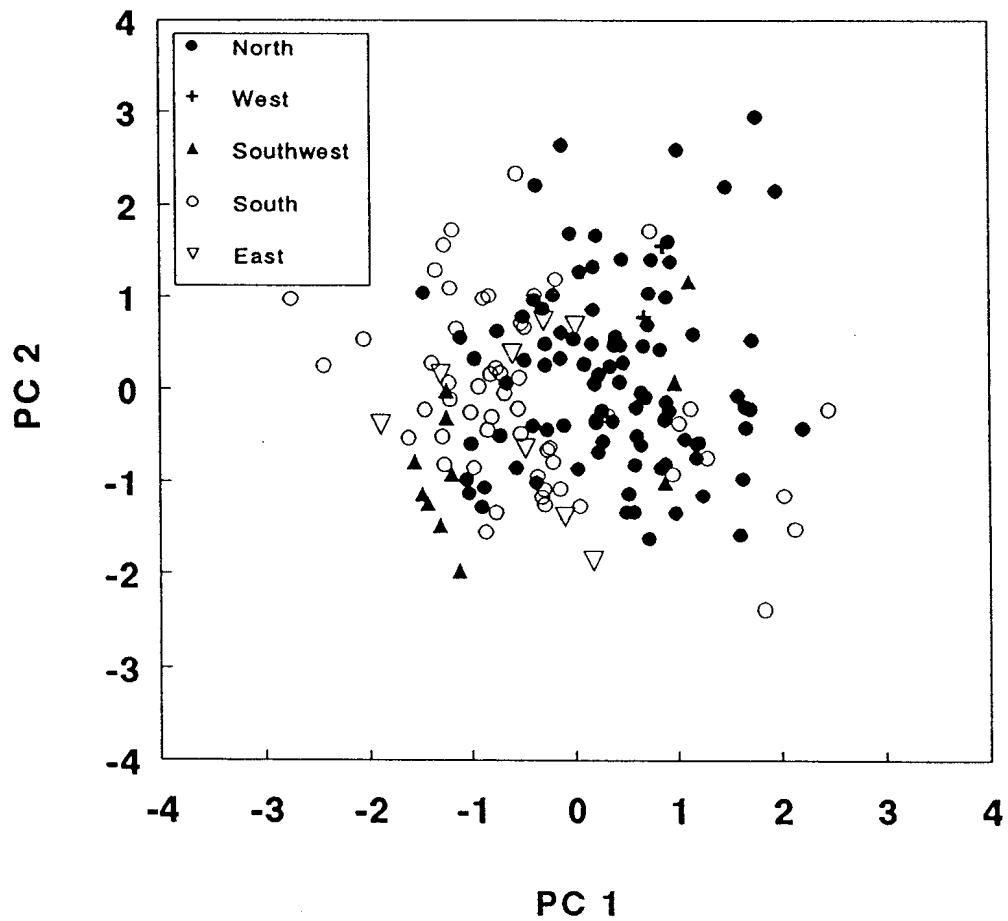
Figure 5.6. PC 1 vs PC 2 for wild Lake Superior *S. namaycush* identified by geographic locality. PC scores were computed from a correlation matrix of all variables. North and west localities appear to cluster together and south and east localities appear to cluster together in PC space.
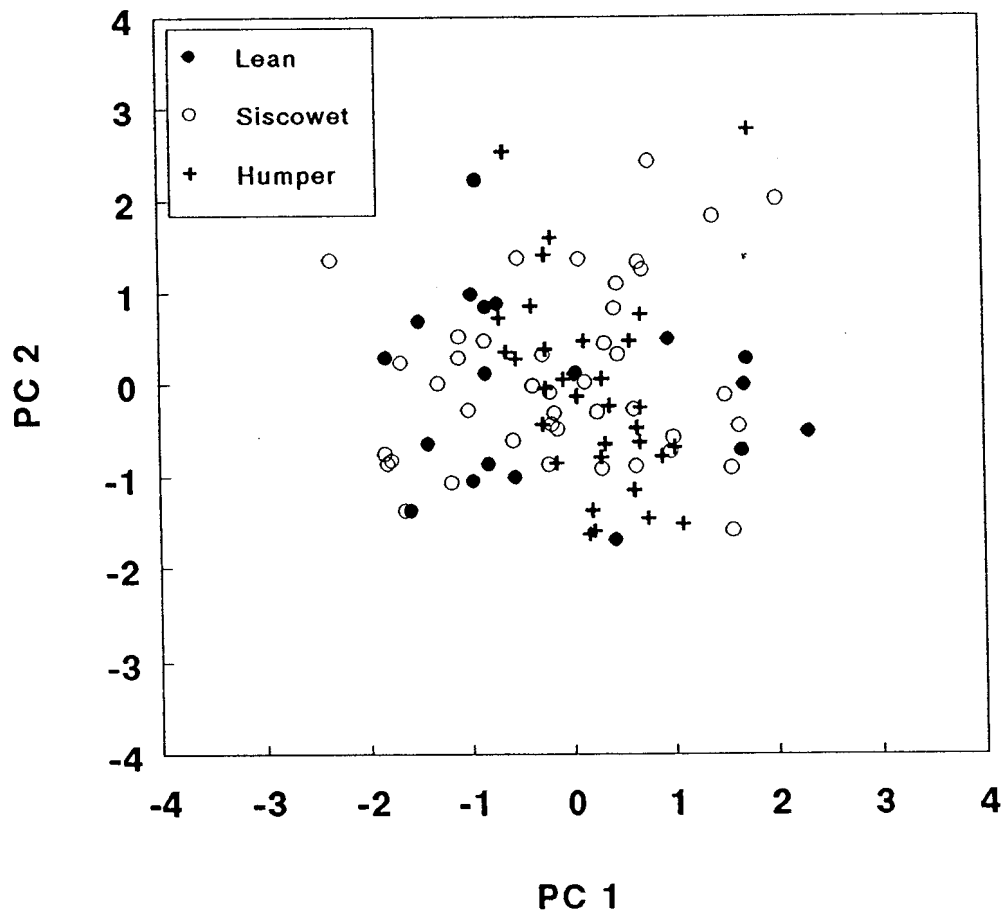
Figure 5.7. PC 1 vs PC 2 for Isle Royale *S. namaycush*. PC scores were computed from a correlation matrix of all variables. While there is no distinct clustering pattern, humpers appear to have less widely dispersed scores in PC space than leans or siscowets.
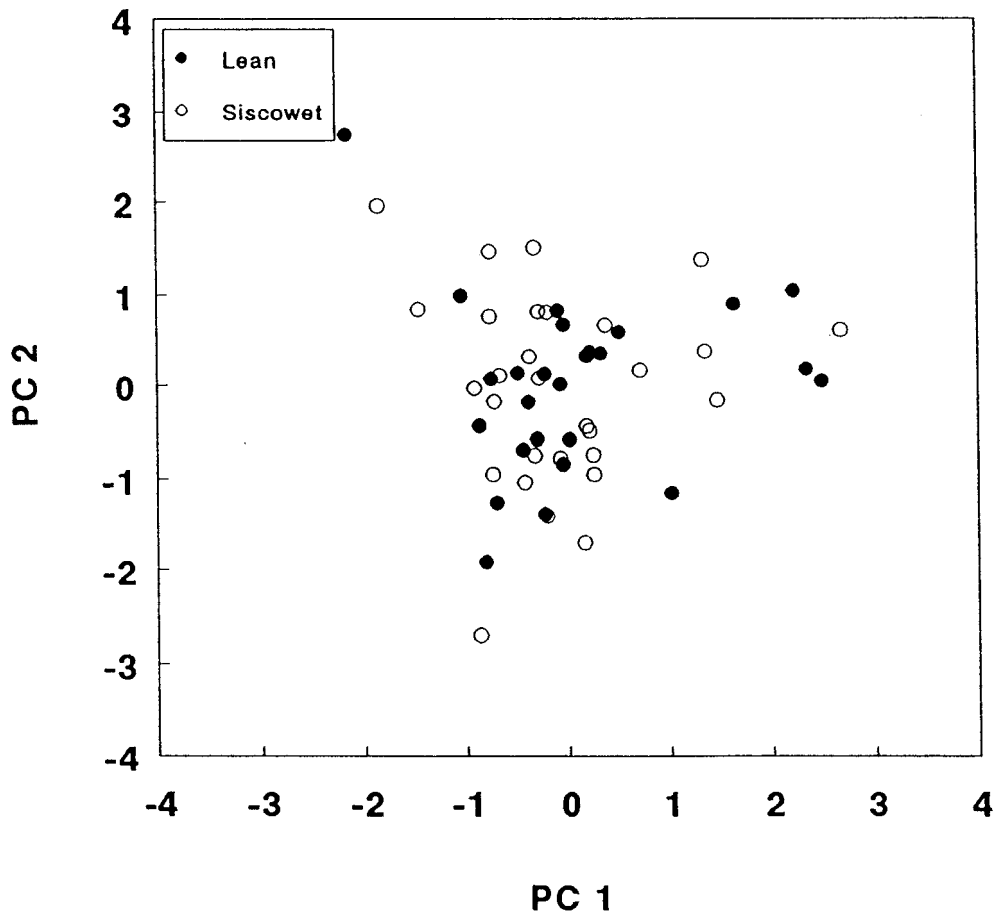
Figure 5.8. PC 1 vs PC 2 for Keweenaw Bay *S. namaycush*. PC scores were computed from a correlation matrix of all variables. There is no clustering pattern corresponding to phenotype.
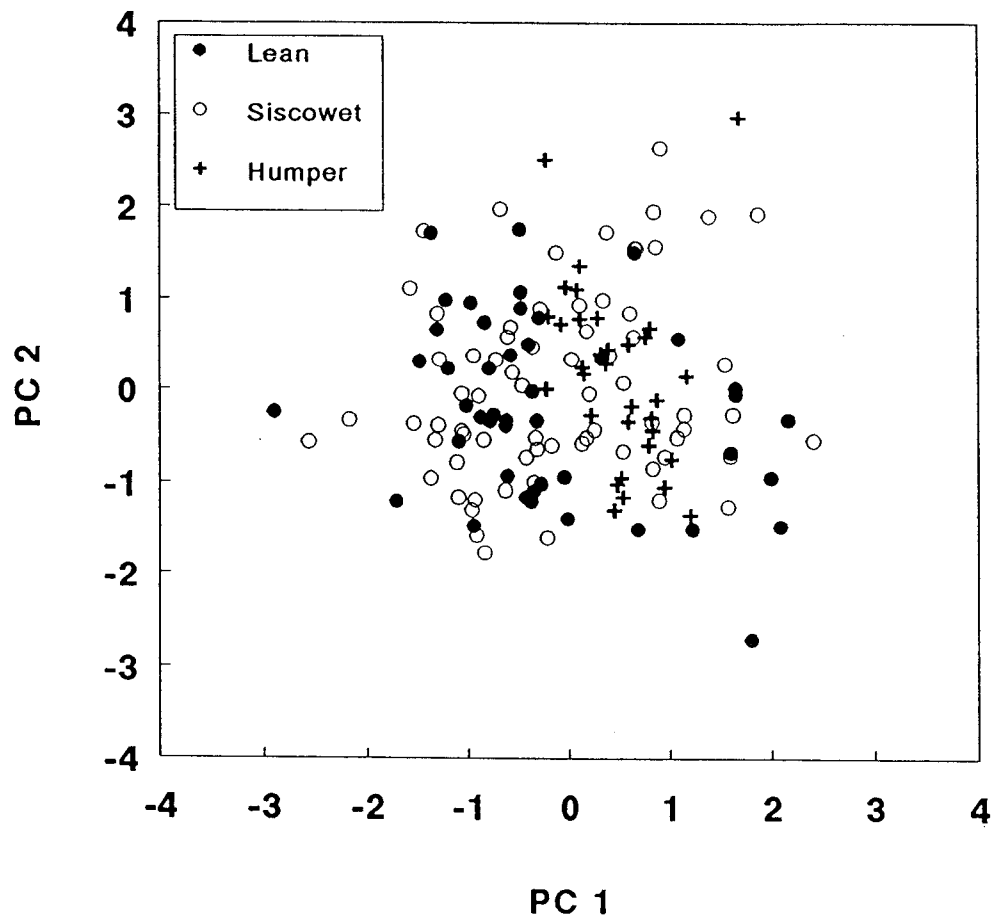
Figure 5.9. PC 1 vs PC 2 for *S. namaycush* from Isle Royale and Keweenaw Bay combined, identified by phenotype. PC scores were computed from a correlation matrix of all variables. Humpers again show a smaller dispersal pattern than leans or siscowets.
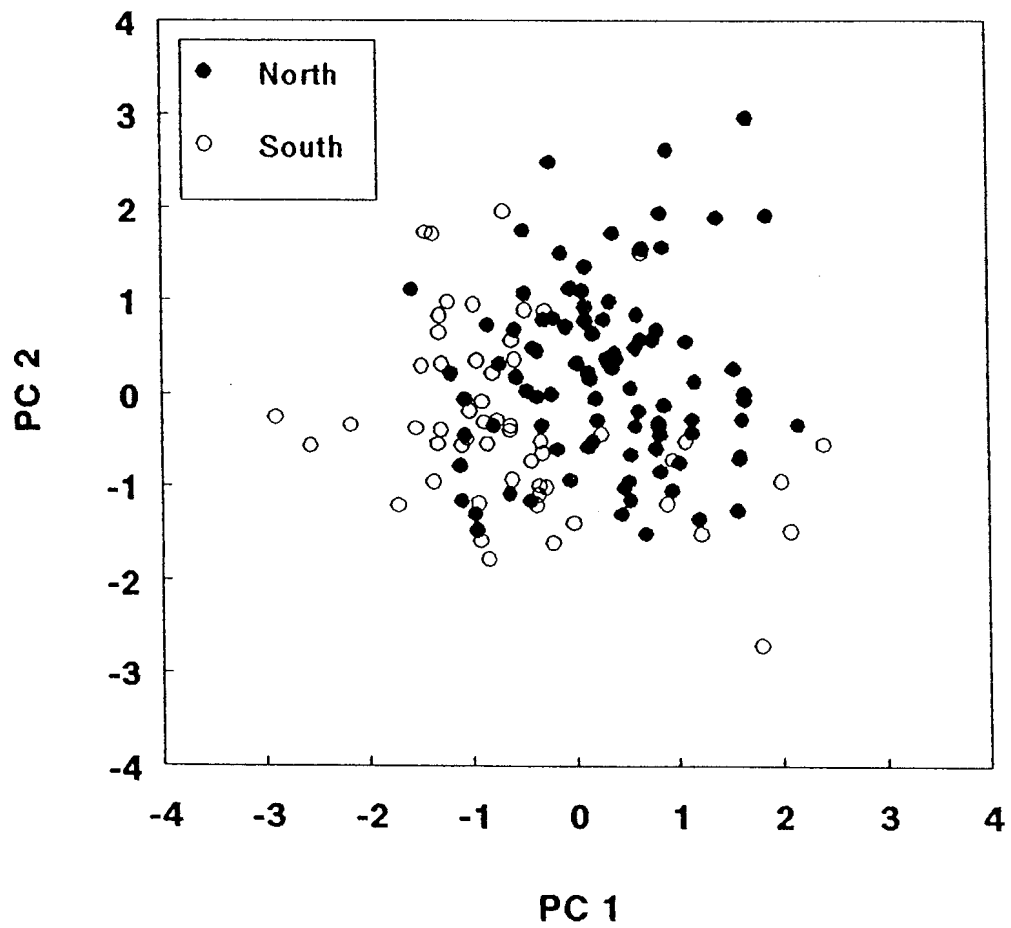
Figure 5.10. PC 1 vs PC 2 for *S. namaycush* from Isle Royale and Keweenaw Bay combined, identified by geographic location. PC scores were computed from a correlation matrix of all variables. Some slight geographic substructuring exists between north and south localities.
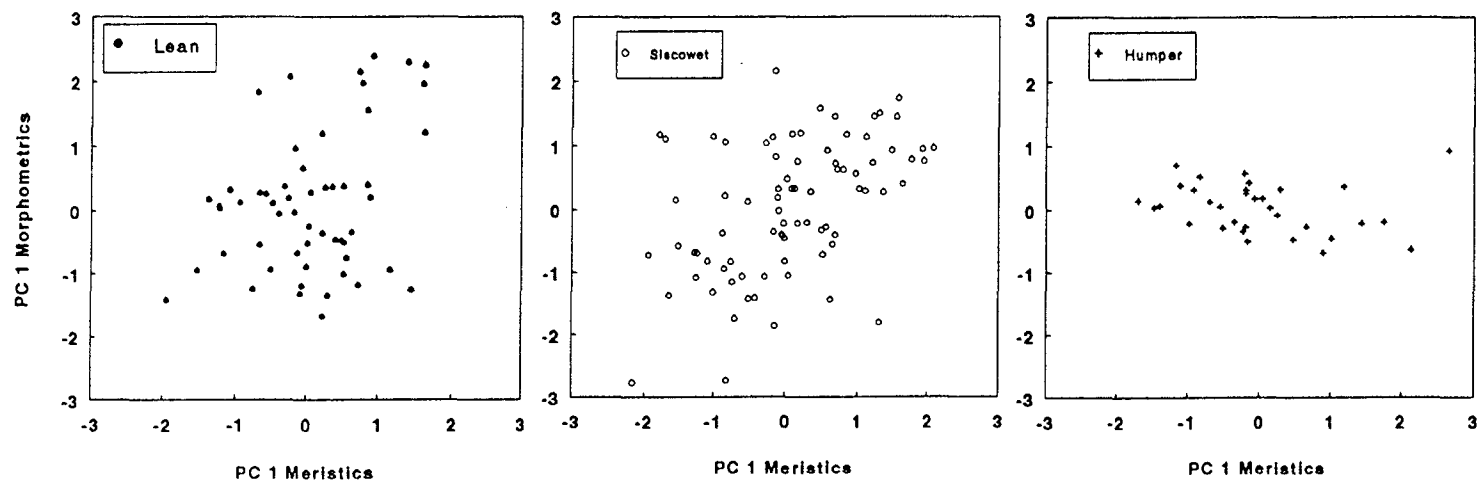
Figure 5.11a. Comparison of principal component analyses for separate lean, siscowet, and humper data. Morphometric data was analyzed in a covariance matrix and meristic data was analyzed in a correlation matrix.
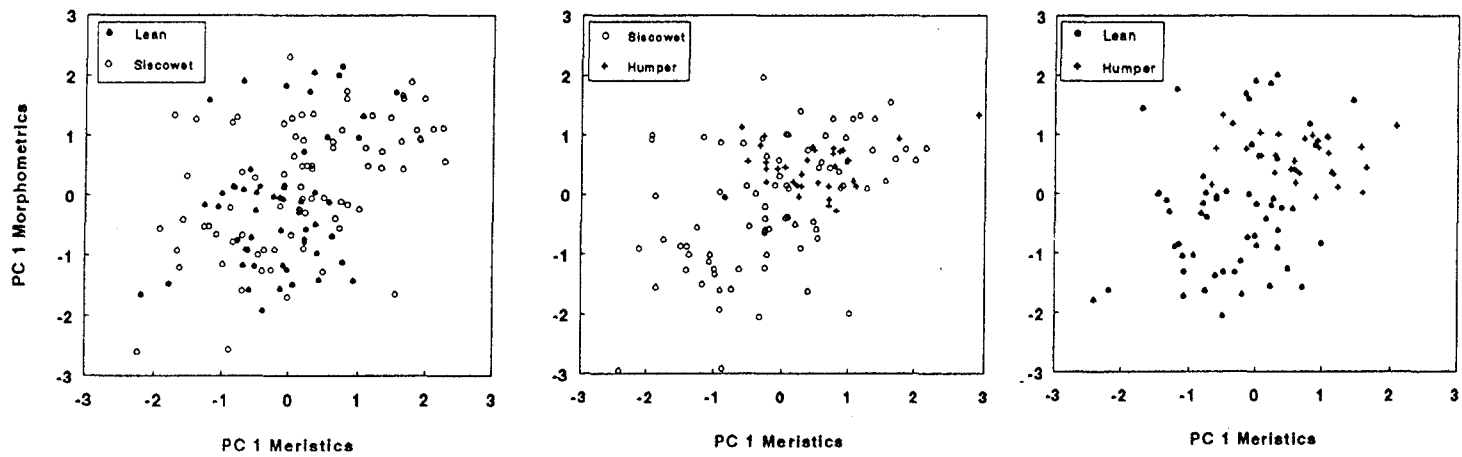
Figure 5.11 b. Comparison of principal compnent analyses for pairwise combinations of lean, siscowet, and humper data. Morphometric data was analyzed in a covariance matrix and meristic data was analyzed in a correlation matrix. The humper phenotype clusters in the same general area as leans and siscowets from the northern sampling localities.

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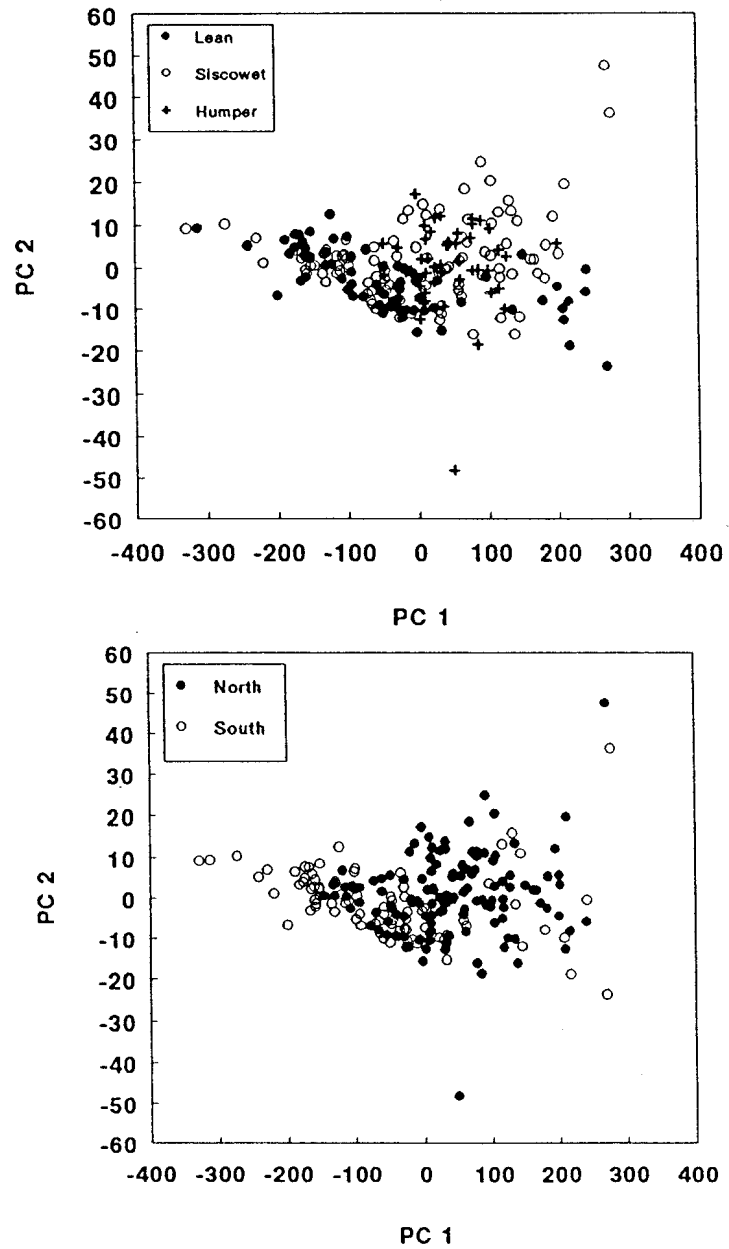
Figure 5.12. Results of principal component analysis of total length and body depth measurements. PC 1 plotted against PC 2 shows no discrimination of leans, siscowets, or humpers. There is a subtle substructuring by north and south geographic areas.
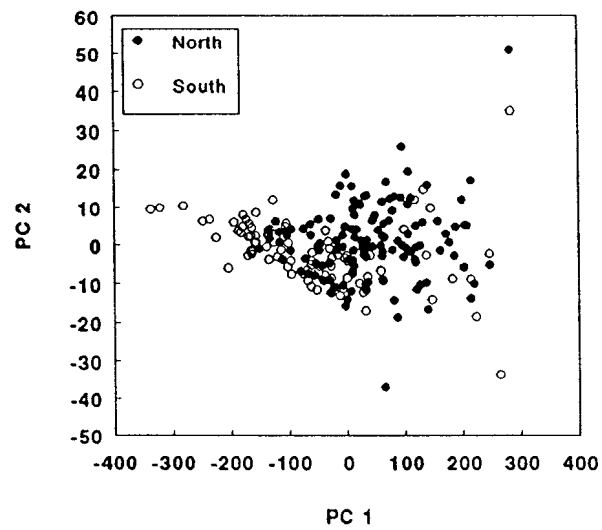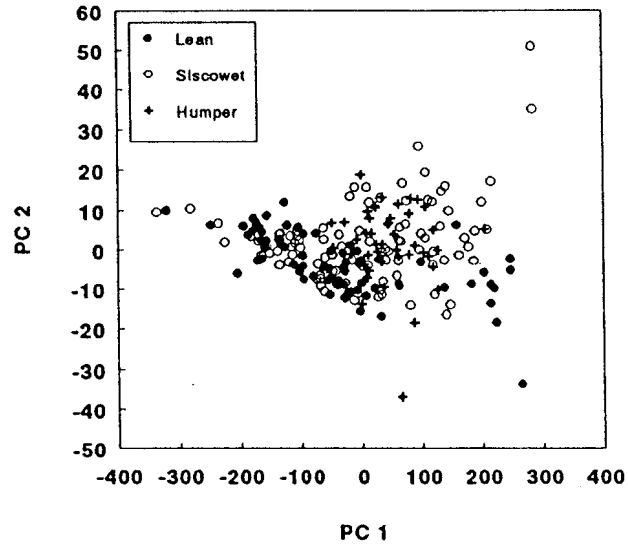
Figure 5.13. Results of PC analysis of total length, body depth, and head depth measurements. There is no discrimination of lake trout by phenotype or by geographic location.
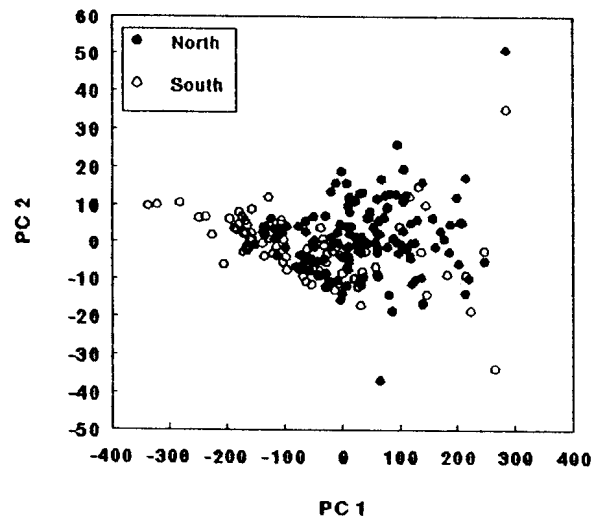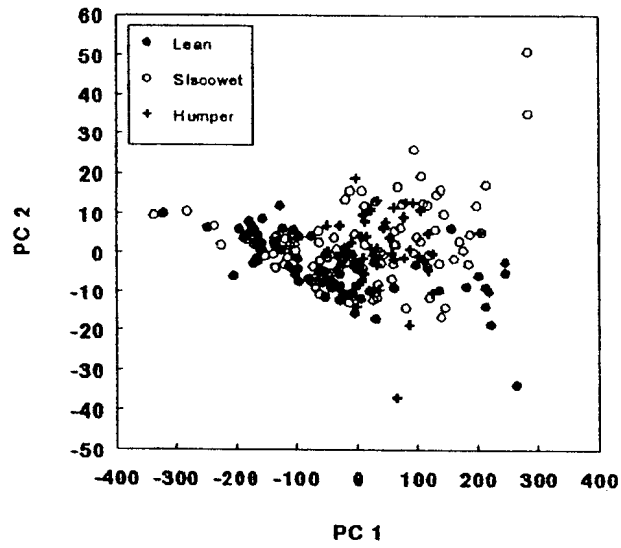
Figure 5.14. Results of PC analysis of total length, body depth, head depth, and head length measurements. Addition of specific morphometric characters representing robust shape differences does not improve the ability to distinguish lake trout phenotypes.
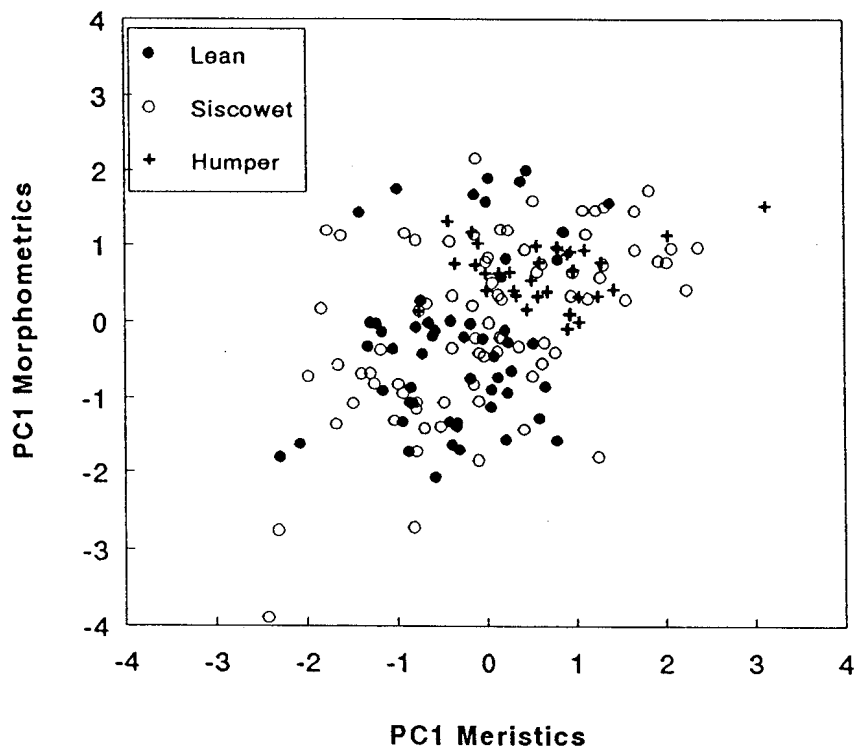
Figure 5.15. PC 1 log transformed morphometrics vs PC 1 meristics for Lake Superior S. namaycush. Morphometric PCs were calculated from a covariance matrix and meristic PCs were calculated from a correlation matrix. There is no discrimination of lake trout phenotypes by morphometrics or meristics.
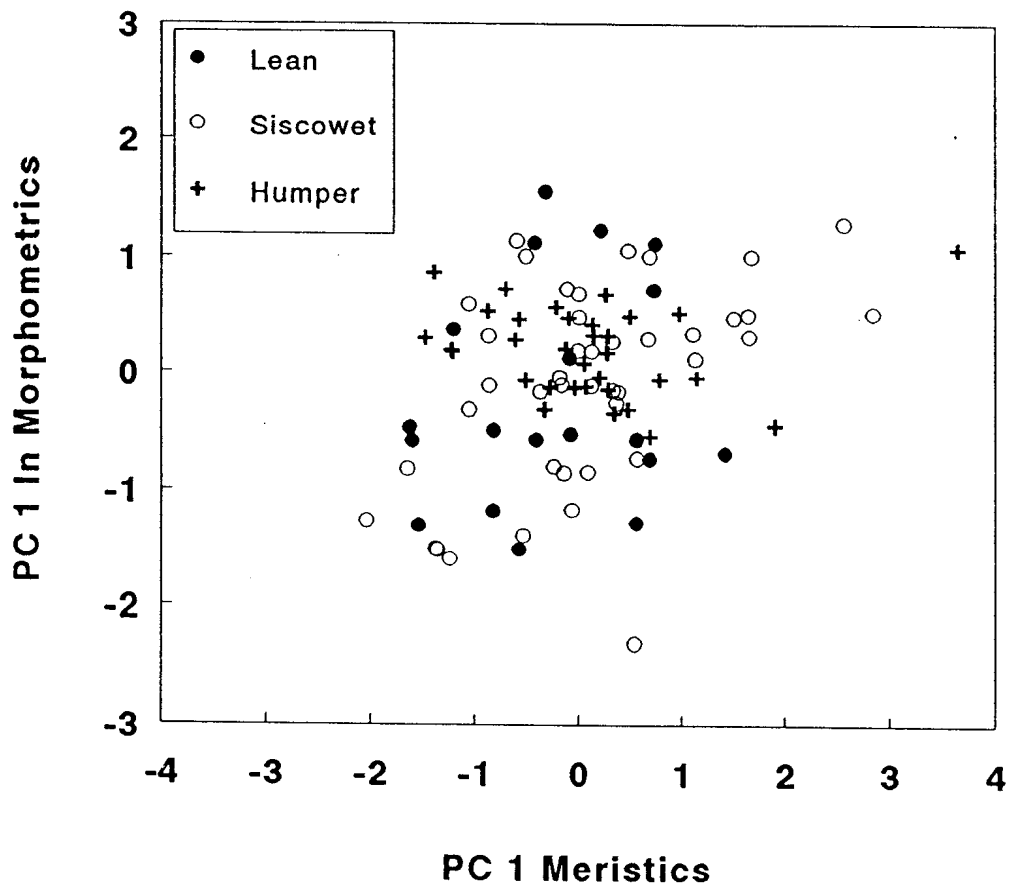
Figure 5.16. PC 1 log transformed morphometrics vs PC 1 meristics for Isle Royale populations. Morphometric PCs were calculated from a covariance matrix and meristic PCs were calculated from a correlation matrix. There is no discrimination of phenotypes within the northern sampling locality by morphometrics or meristics.
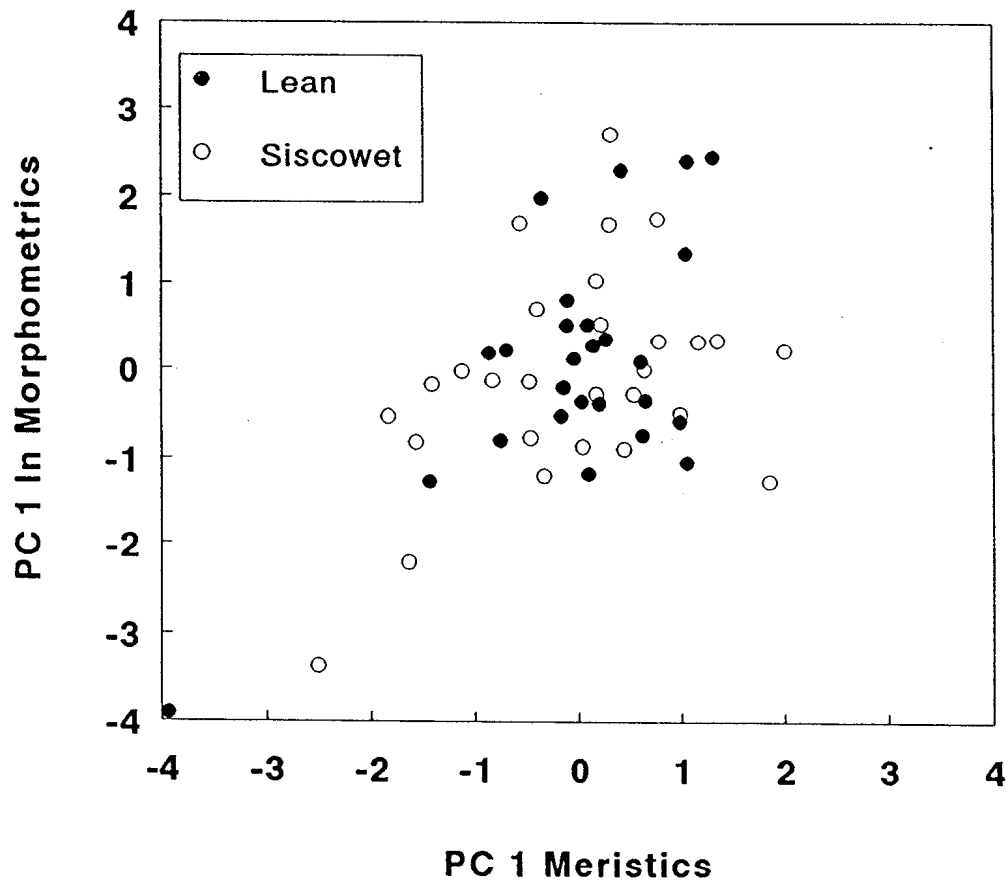
Figure 5.17. PC 1 log transformed morphometrics vs PC 1 meristics for Keweenaw Bay populations. Morphometric PCs were calculated from a covariance matrix and meristic PCs were calculated from a correlation matrix. There is no discrimination of lake trout phenotypes in the southern sampling locality by morphometrics or meristics.
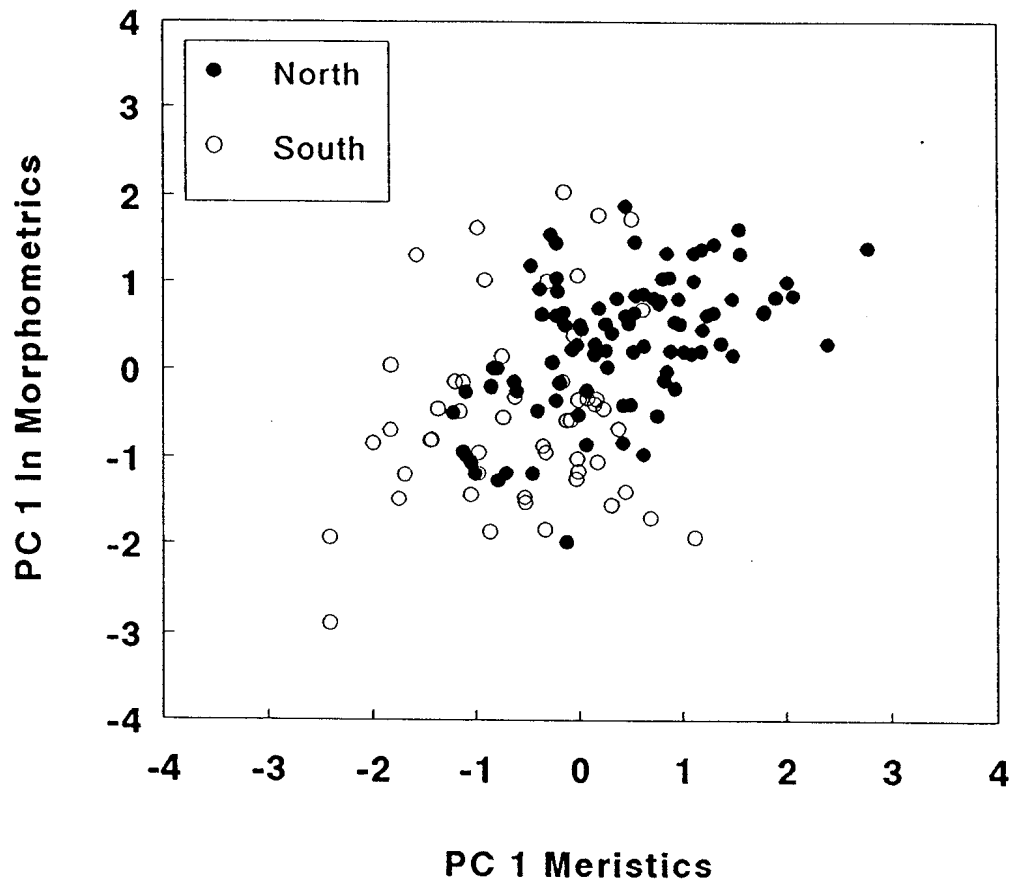
Figure 5.18. PC 1 log transformed morphometrics vs PC 1 meristics for north and south populations, identified by geographic locality. Morphometric PCs were calculated from a covariance matrix and meristic PCs were calculated from a correlation matrix. There appears to be a slight substructuring of the data by north and south sampling areas, but no ability to discriminate between the two areas.
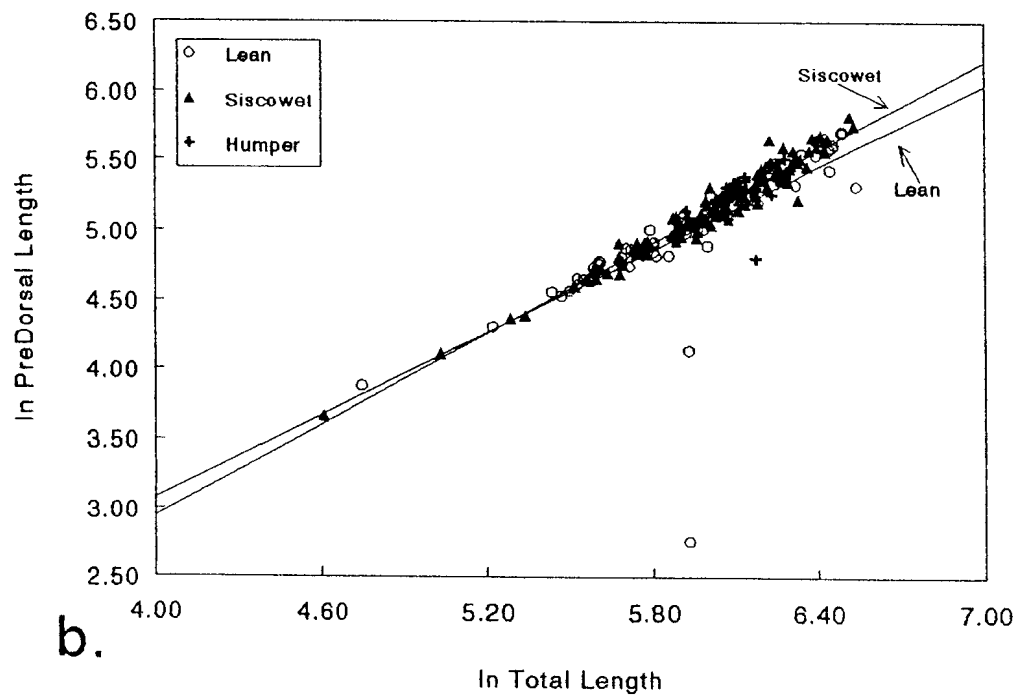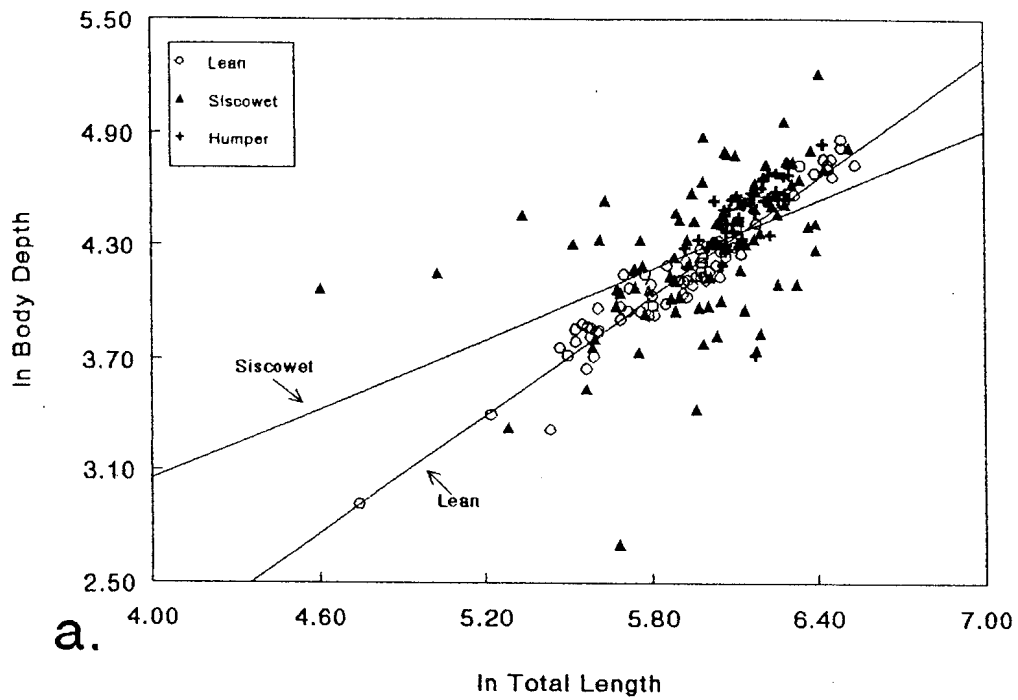
Figure 5.19 a-b. Natural log regression of morphometric variables against total length (ANCOVA).
(a) Body depth vs total length; (b) Predorsal length vs total length. Although regression lines for
lean and siscowet phenotypes are significantly different, there is no discrimination between them.
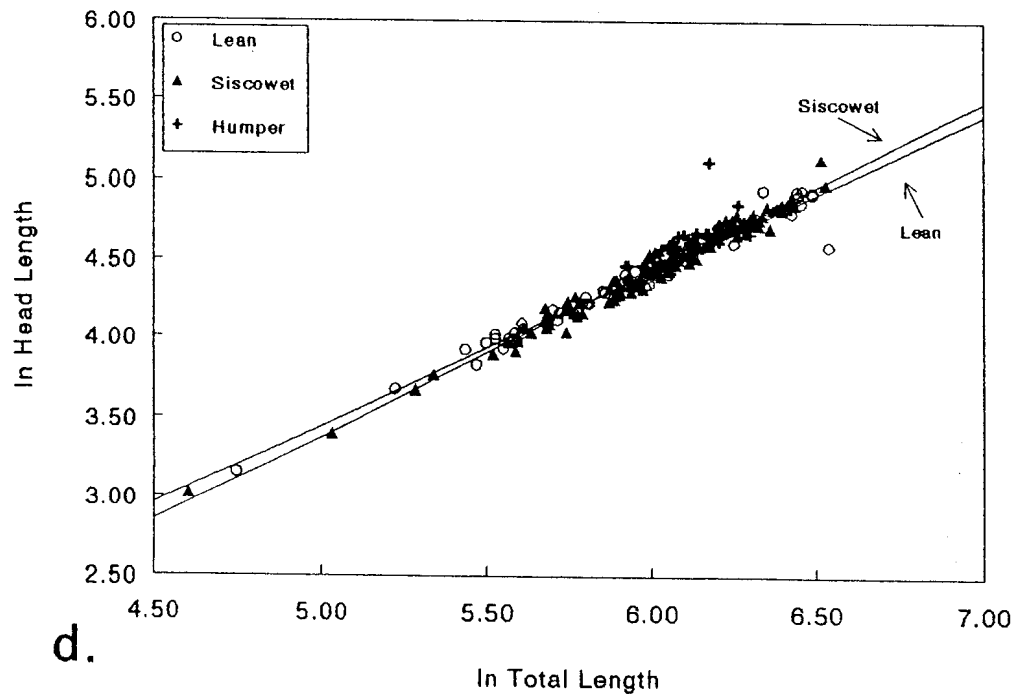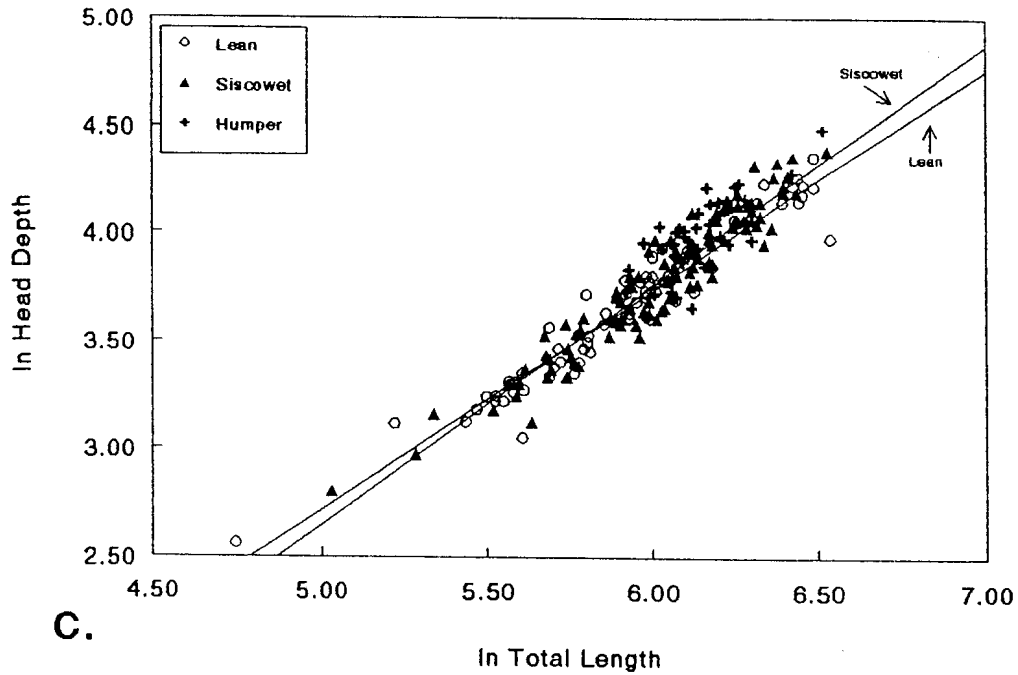
Figure 5.19 c-d. Natural log regression of morphometric variables against total length (ANCOVA). (c) Head depth vs total length; (d) Head length vs total length; (e) Preorbital length vs total length; (f) Premaxillary height vs total length. Although regression lines for lean and siscowet phenotypes are significantly different, there is no discrimination between them.
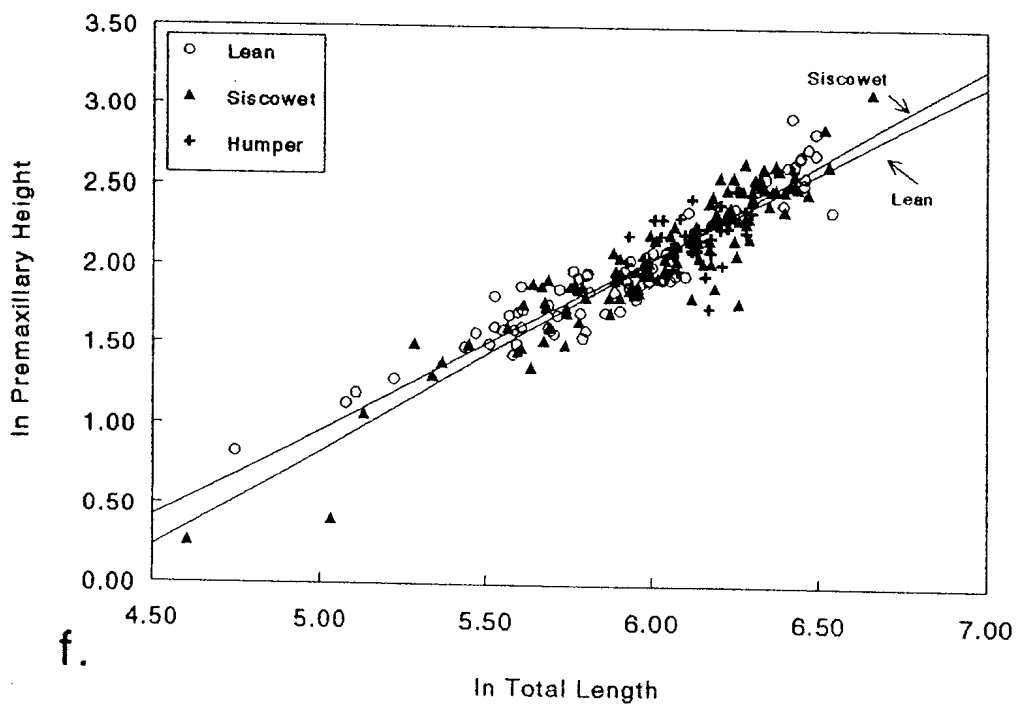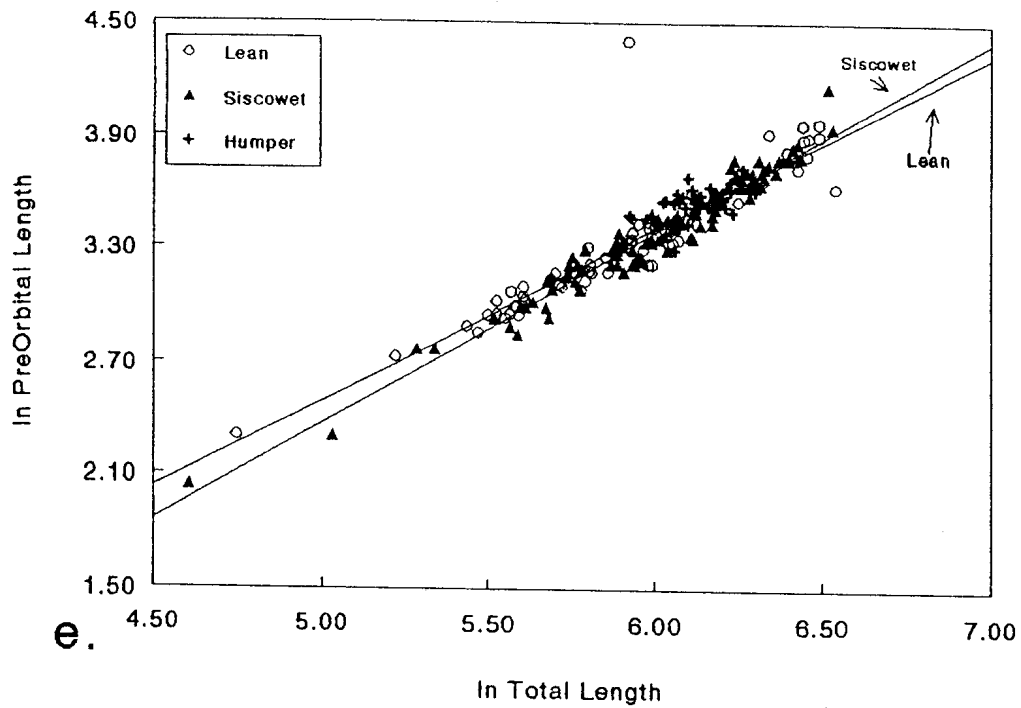
Figure 5.19 e-f. Natural log regression of morphometric variables against total length (ANCOVA). (e) Preorbital length vs total length; (f) Premaxillary height vs total length. Although regression lines for lean and siscowet phenotypes are significantly different, there is no discrimination between them.

LOCATION	Area	Latitude / Longitude	Depth fished	Types Sampled	Gear
1. Apostle Islands, WI	SW	46° 46' N / 90° 47' W	15-100 m	Lean, Siscowet	TR
2. Copper Harbor, MI	S	47° 31' N / 87° 40' W	145-160 m	Siscowet	GN
3. Port Wing, WI	W	46° 54' N / 91° 19' W	145-160 m	Siscowet	GN
4. Duluth, MN	W	46° 47' N / 92° 06' W	145-160 m	Siscowet	GN
5. Silver Bay, MN	W	47° 17' N / 91° 15' W	100-160 m	Siscowet	GN
6. Jacobsville, MI	S	46° 58' N / 88° 23' W	15-65 m	Lean, Siscowet	TR
7. Sand Bay, MI	S	46° 53' N / 88° 19' W	15-138 m	Lean, Siscowet	TR
8. Traverse Island, MI	S	47° 03' N / 88° 16' W	32-90 m	Lean, Siscowet	TR
9. Traverse Bay, MI	S	47° 09' N / 88° 13' W	15-100 m	Lean, Siscowet	TR
10. Michipicoten Island, Ontario	E	47° 46' N / 85° 42' W	15-100 m	Lean, Siscowet	TR
11. Gargantua Bay, Ontario	E	47° 33' N / 84° 57' W	15-110 m	Lean, Siscowet	TR
12. Agawa Bay, Ontario	E	47° 19' N / 84° 38' W	19-85 m	Lean	TR
13. Alona Bay, Ontario	E	47° 09' N / 84° 42' W	20-130 m	Lean, Siscowet	TR

Table 5.1. Sampling localities. Area refers to geographic area classification used in morphometric analysis. Gear used as follows: GN = multifilament nylon gill net; TR = bottom trawl; rod = caught by rod and reel. Collections in all areas except 2, 3, and 4 were made "across-contour" (across all depths indicated). Collections in areas 2, 3, and 4 were commercial sets. (continued)

LOCATION	Area	Latitude / Longitude	Depth fished	Types Sampled	Gear
14. Sawyer Bay, Ontario, Canada	N	48° 22' N / 88° 52' W	15-70 m 60-87 m	Lean Siscowet	GN
15. Thompson Island, Isle Royale, MI	N	47° 53' N / 89° 13' W	48-85 m 76-95 m 76-95 m	Lean Siscowet Humper	GN
16. Isle Royale Light, MI	N	47° 55' N / 88° 45' W	53-77 m 53-116 m 53-116 m	Lean Siscowet Humper	GN
17. Mott Island, Isle Royale, MI	N	48° 08' N / 88° 30' W	69-94 m 69-124 m 69-94 m	Lean Siscowet Humper	GN
Localities outside Laurentian Great Lakes basin :					
Alaska, Kenai Peninsula (river)					rod
Prince Lake, Northwest Territory					GN
Grinnell Lake, Northwest Territory					GN
Cambridge Lake, Northwest Territory					GN

Table 5.1. Continued.

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MORPHOMETRICS:

Total Length : *length from snout to caudal fin*

Weight (grams)

Body Depth : *length from dorsal fin insertion to pelvic fin insertion*

Head Length : *length from snout to farthest point on opercle flap*

Head Depth : *length from top of head at scale origin to anteriormost branchiostegal ray origin*

PreDorsal Length : *length from dorsal fin insertion to tip of snout*

PreOrbital Length : *length from eye to tip of snout*

PostOrbital Length : *length from eye to posterior tip of opercle flap*

SubOrbital Length : *length from eye to posterior tip of maxillary*

PostMaxillary Length : *length from posterior tip of maxillary to posterior tip of opercle flap*

Dentary Length : *length of dentary bone*

Mandible Length : *length of lower jaw from anterior to quadrate articulation*

Premaxillary Length (width) : *length of premaxillary bone anterior to posterior of toothed side*

Premaxillary Height : *length of premaxillary bone from dorsal tip to tooth row (excluding teeth)*

MERISTICS:

Ventral Pores : *number of pores on exterior right side of lower jaw*

Gill Rakers : *number of mature gill rakers on first basibranchial arch of right side*

Branchiostegal Rays : *number of branchiostegal rays on right side*

Dorsal Rays : *number of principal rays in dorsal fin*

Pectoral Rays : *number of principal rays in right pectoral fin*

Pelvic Rays : *number of principal rays in right pelvic fin*

Anal Rays : *number of principal rays in anal fin*

Lateral Line Pores : *number of pores in lateral line on right side*

Diagonal Scale Rows : *number of sclaes in diagonal rows on right side to caudal peduncle*

Vertical Scale Rows : *number of scales from dorsal fin origin to pelvic fin origin on right side*

Caudal Scales : *number of scales around caudal peduncle*

Pyloric Caeca : *number of pyloric caeca extending from stomach*

Table 5.2. List of morphometric and meristic measurements collected from wild Lake Superior *Salvelinus namaycush.*

	Lean					Siscowet					Humper			
	N	Mean	Stand. Dev	95% CI	CV	N	Mean	Stand. Dev	95% CI	CV	N	Mean	Stand. Dev	95% CI	CV
Total Length	91	384.40	137.5	356.2, 412.6	.36	160	447.27	125.2	427.9, 466.7	0.2	48	465.23	48.6	451.4,	0.11
Weight (grams)	62	565.74	677.4	397.1, 734.3	1.2	90	714.59	596.8	591.3, 837.9	0.8	47	981.42	418.2	854.9,	0.43
Body Depth/TL	71	0.18	0.02	0.17, 0.18	0.0	102	0.19	0.02	0.18, 0.19	0.1	47	0.19	0.02	0.18, 0.19	0.12
Head Length/TL	71	0.21	0.01	0.20, 0.21	0.0	102	0.21	0.01	0.20, 0.21	0.0	47	0.22	0.02	0.22, 0.23	0.10
Head Depth/TL	71	0.10	0.01	0.10, 0.11	0.0	102	0.11	0.01	0.10, 0.11	0.1	47	0.12	0.01	0.11, 0.12	0.11
PreDorsal Length/TL	70	0.40	0.06	0.39, 0.41	0.1	102	0.42	0.03	0.42, 0.43	0.0	46	0.43	0.04	0.42, 0.44	0.08
Preorbital Length/TL	71	0.08	0.02	0.07, 0.08	0.2	102	0.07	0.01	0.07, 0.08	0.0	47	0.08	0.01	0.07, 0.08	0.08
Postorbital Length/TL	71	0.14	0.01	0.13, 0.14	0.0	101	0.14	0.02	0.14, 0.15	0.1	47	0.15	0.01	0.14, 0.15	0.07
Suborbital Length/TL	71	0.05	0.01	0.05, 0.06	0.1	101	0.06	0.01	0.05, 0.06	0.1	47	0.06	0.01	0.06, 0.07	0.09
Postmaxillary Length/TL	71	0.11	0.01	0.10, 0.11	0.0	101	0.11	0.01	0.10, 0.11	0.1	47	0.11	0.01	0.10, 0.11	0.10
Dentary Length/TL	87	0.09	0.01	0.09, 0.10	0.0	119	0.10	0.01	0.09, 0.10	0.0	42	0.10	0.01	0.10, 0.11	0.11
Mandible Length/TL	87	0.13	0.01	0.12, 0.13	0.0	119	0.12	0.01	0.12, 0.13	0.0	42	0.13	0.01	0.13, 0.14	0.09
Premaxillary Width/TL	81	0.02	0.004	0.02, 0.03	0.1	115	0.02	0.003	0.02, 0.03	0.1	43	0.02	0.003	0.02, 0.03	0.14
Premaxillary Height/TL	81	0.02	0.003	0.01, 0.02	0.1	115	0.02	0.003	0.01, 0.02	0.1	43	0.02	0.003	0.01, 0.02	0.14
Ventral Pores	78	9.71	1.24	9.4, 9.9	0.1	114	9.37	0.94	9.2, 9.5	0.1	42	8.91	0.88	8.6, 9.2	0.10
Gill Rakers	86	19.81	1.05	19.6, 20.0	0.0	135	19.78	0.93	19.6, 19.9	0.0	48	20.10	1.01	19.8, 20.4	0.05
Branchiostegal Rays	88	12.17	0.24	12.0, 12.3	0.0	160	12.00	0.73	11.9, 12.1	0.0	48	12.27	0.71	12.1, 12.5	0.06
Dorsal Rays	88	10.23	0.67	10.1, 10.4	0.0	160	10.33	0.64	10.2, 10.4	0.0	48	10.13	0.57	9.9, 10.3	0.05
Pectoral Rays	88	12.79	0.76	12.6, 12.9	0.0	160	13.02	0.79	12.9, 13.1	0.0	48	13.06	0.63	12.9, 13.2	0.05
Pelvic Rays	88	9.27	0.64	9.1, 9.4	0.1	160	9.28	0.60	9.2, 9.4	0.0	48	9.38	0.61	9.2, 9.6	0.06
Anal Rays	88	9.78	0.65091X9.6,	9.9	0.0	160	9.96	0.76	9.8, 10.1	0.0	48	9.73	0.68	9.5, 9.9	0.07
Lateral Line Pores	85	124.78	4.12	123.9, 125.7	0.0	122	123.98	5.41	123.0, 124.9	0.0	46	120.94	4.84	119.5,	0.04
Diagonal Scale Rows	85	181.54	13.51	178.7, 184.4	0.0	122	179.43	12.44	177.2, 181.6	0.0	46	182.15	13.23	178.3,	0.07
Vertical Scale Rows	71	76.46	4.96	75.3, 77.6	0.0	101	77.92	5.96	76.8, 79.1	0.0	47	80.17	4.91	78.7, 81.6	0.06
Caudal Scales	71	78.01	5.40	76.8, 79.2	0.0	101	80.88	5.84	79.7, 82.0	0.0	47	82.75	4.53	81.4, 84.0	0.05
Pyloric Caeca	35	150.80	17.94	144.9, 156.7	0.1	85	144.22	16.04	140.8, 147.6	0.1	0	----	----	---	---

Table 5.3. Summary of morphometric measurements for lean, siscowet, and humper lake trout (all lengths in mm). Total length ranges are as follows: Lean: 115-691 mm; Siscowet: 100-780 mm; Humper: 373-616 mm.

	N	Mean	Stand. Dev.	95% CI	CV
Total Length	299	431.0	15432.8	416.9, 451.1	0.29
Weight (grams)	194	724.8	368943.4	639.1, 810.4	0.84
Body Depth/TL	220	0.18	0.02	0.18, 0.19	0.12
Head Length/TL	220	0.21	0.01	0.20, 0.21	0.07
Head Depth/TL	220	0.11	0.01	0.10, 0.11	0.11
PreDorsal Length/TL	218	0.42	0.05	0.41, 0.42	0.11
PreOrbital Length/TL	220	0.08	0.01	0.07, 0.08	0.15
PostOrbital Length/TL	219	0.14	0.01	0.14, 0.15	0.10
SubOrbital Length/TL	219	0.06	0.01	0.05, 0.06	0.13
PostMaxillary Length/TL	219	0.12	0.01	0.10, 0.11	0.11
Dentary Length/TL	248	0.10	0.01	0.09, 0.10	0.10
Mandible Length/TL	248	0.13	0.01	0.12, 0.13	0.10
Premaxillary Length/TL	239	0.02	0.003	0.02, 0.03	0.15
Premaxillary Height/TL	239	0.02	0.003	0.01, 0.02	0.15
Ventral Pores	234	9.94	1.16	9.8, 10.0	0.11
Gill Rakers	269	19.85	0.99	19.7, 19.9	0.05
Branchiostegal Rays	296	12.09	0.74	12.0, 12.2	0.06
Dorsal Rays	296	10.27	0.64	10.2, 10.4	0.06
Pectoral Rays	296	12.96	0.76	12.9, 13.0	0.06
Pelvic Rays	296	9.29	0.62	9.2, 9.4	0.06
Anal Rays	296	9.87	0.72	9.8, 10.0	0.07
Lateral Line Pores	253	123.69	5.07	123.1, 124.3	0.04
Diagonal Scale Rows	253	180.64	12.95	179.0, 182.3	0.07
Vert6ical Scale Rows	219	77.93	5.58	77.2, 78.7	0.07
Caudal Scales	219	80.35	5.70	79.6, 81.1	0.07
Pyloric Caeca	120	146.14	16.81	143.1, 149.2	0.11

Table 5.4. Summary of morphometric and meristic measurements for all samples combined (all lengths in millimeters). Total length ranged from 100-780 mm.

	Lean-Siscowet		Lean-Humper		Humper-Siscowet		P homogeneity of variances	F stat	P
Pairwise Mean Differences									
Body Depth	14.13	***	21.23	***	7.10		0.00	11.05	0.000
Head Length	9.33	*	12.91	***	13.58	***	0.00	12.92	0.000
Head Depth	5.86	**	13.36	***	7.50	**	0.00	13.71	0.000
PreDorsal Length	26.26	**	45.47	***	19.21		0.00	11.02	0.000
PreOrbital Length	1.96		5.39	**	3.43		0.00	4.94	0.008
PostOrbital Length	7.17	**	15.23	***	8.07	**	0.00	12.43	0.000
SubOrbital Length	3.29	**	6.68	***	3.39	**	0.00	11.64	0.000
PostMaxillary Length	5.25	**	10.50	***	5.25	*	0.00	11.02	0.000
Dentary Length	3.49		8.42	**	4.93		0.00	5.98	0.003
Dentary Tooth Row Length	1.84		6.11	**	4.27		0.00	4.38	0.013
Mandible Length	3.92		10.90	**	6.97	*	0.00	5.85	0.003
Premaxillary Width	0.68		2.05	**	1.34		0.00	4.65	0.010
Premaxillary Height	0.81		1.39	*	0.59		0.00	3.06	0.049
Ventral Pores	0.34		0.80	***	0.46	*	0.01	8.09	0.000
Gill Rakers	0.03		0.29		0.32		0.42	1.93	0.147
Branchiostegal Rays	0.17		0.10		0.27		0.74	3.10	0.046
Dorsal Rays	0.10		0.10		0.21		0.44	2.15	0.118
Pectoral Rays	0.22		0.27		0.04		0.20	2.99	0.051
Pelvic Rays	0.00		0.10		0.10		0.83	0.54	0.582
Anal Rays	0.17		0.05		0.23		0.21	2.71	0.068
Lateral Line Pores	0.79		3.84	***	3.05	***	0.03	9.55	0.000
Diagonal Scale Rows	2.11		0.61		2.72		0.70	1.05	0.352
Vertival Scale Rows	1.46		3.70	***	2.25	*	0.15	6.56	0.002
Caudal Scales	2.87	**	4.73	***	1.86		0.15	11.57	0.000

Table 5.5. Pairwise comparison probabilities by phentoype for morphological characteristics. Asterisks indicate level of significance of pairwise mean differences: "*" = p < 0.05; "***" = p < 0.01; "****" = p < 0.001.

	North					West					Southwest			
	N	Mean	Stand.Dev	95%CI	CV	N	Mean	Stand.Dev	95%CI	CV	N	Mean	Stand.D	95% CI	CV
Total Length	12	454.95	85.6	440.1, 469.7	0.19	23	517.65	66.1	490.7, 544.5	0.13	18	309.17	112.4	257.8, 361.6	0.36
Weight (grams)	10	989.96	595.9	877.0, 1102.8	0.60	0	----	-----	----	--	0	---	-----	----	--
Body Depth/TL	12	0.19	0.02	0.18, 0.19	0.12	5	0.21	0.02	0.19, 0.23	0.10	13	0.18	0.02	0.17, 0.20	0.13
Head Length/TL	12	0.22	0.02	0.21, 0.22	0.07	5	0.21	0.01	0.20, 0.19	0.06	13	0.21	0.01	0.21, 0.22	0.03
Head Depth/TL	12	0.11	0.01	0.11, 0.12	0.10	5	0.10	0.01	0.10, 0.11	0.07	13	0.10	0.01	0.09, 0.10	0.08
PreDorsal Lengt/TL	12	0.43	0.04	0.42, 0.43	0.09	5	0.43	0.03	0.40, 0.45	0.06	13	0.43	0.03	0.41, 0.45	0.06
PreOrbital Length/TL	12	0.07	0.01	0.07, 0.08	0.18	5	0.07	0.003	0.07, 0.08	0.04	13	0.08	0.01	0.07, 0.08	0.05
PostOrbital Length/TL	12	0.14	0.01	0.14, 0.15	0.06	5	0.14	0.01	0.13, 0.14	0.03	13	0.14	0.01	0.10, 0.19	0.04
SubOrbitalLength/TL	12	0.06	0.01	0.06, 0.07	0.10	5	0.05	0.01	0.05, 0.06	0.10	13	0.06	0.01	0.05, 0.06	0.08
PostMaxillary Length/TL	12	0.11	0.01	0.11, 0.12	0.08	5	0.11	0.01	0.10, 0.11	0.04	13	0.11	0.01	0.11, 0.12	0.05
Dentary Length/TL	12	0.10	0.01	0.09, 0.10	0.09	10	0.09	0.01	0.09, 0.10	0.13	15	0.11	0.01	0.10, 0.11	0.06
Mandible Length/TL	12	0.13	0.01	0.12 0.13	0.09	10	0.12	0.02	0.11, 0.13	0.13	15	0.13	0.01	0.13, 0.14	0.03
Premaxillary Width/TL	11	0.02	0.004	0.02, 0.03	0.15	8	0.02	0.004	0.02, 0.03	0.20	14	0.02	0.003	0.02, 0.03	0.14
Premaxillary Height/TL	11	0.02	0.003	0.01, 0.02	0.15	8	0.02	0.003	0.01, 0.02	0.16	14	0.02	0.002	0.01, 0.02	0.11
Ventral Pores	11	9.3	0.9	9.09, 9.44	0.10	9	9.7	0.7	9.2, 10.1	0.07	18	10.2	0.9	9.7, 10.6	0.09
Gill Rakers	12	20.1	0.9	19.9, 20.2	0.05	19	19.9	0.7	19.6, 20.2	0.04	18	19.7	0.9	19.3, 20.2	0.05
Branchiostegal Rays Rays	12	12.3	0.7	12.2, 12.4	0.06	24	11.5	0.7	11.2, 11.8	0.06	18	11.6	0.6	11.3, 11.8	0.05
Dorsal Rays	12	10.1	0.6	10.0, 10.3	0.06	24	10.9	0.4	10.7, 11.1	0.04	18	9.9	0.7	9.6, 10.2	0.07
Pectoral Rays	12	13.1	0.8	12.9, 13.2	0.06	24	13.4	0.6	13.1, 13.6	0.04	18	12.7	0.6	12.4, 12.9	0.05
Pelvic Rays	12	9.4	0.4	9.3, 9.5	0.06	24	9.33	0.3	9.1, 9.6	0.06	18	9.3	0.3	9.0, 9.6	0.06
Anal Rays	12	9.7	0.7	9.5, 9.8	0.07	24	10.5	0.6	10.3, 10.8	0.06	18	9.6	0.7	9.2, 9.9	0.07
Lateral Line Pores	12	122.4	5.7	121.4, 123.4	0.05	10	123.9	2.9	122.1, 125.7	0.02	18	125.3	3.5	123.6, 126.9	0.03
Diagonal Scale Rows	12	181.7	11.6	179.3, 184.1	0.08	10	178.4	12.2	170.8, 185.9	0.07	18	177.9	13.5	171.6, 184.2	0.08
Vertical Scale Rows	12	79.7	5.5	78.7, 80.6	0.07	5	75.6	3.6	72.4, 78.8	0.05	13	76.9	4.3	74.6, 79.2	0.06
Caudal Scales	12	82.2	5.3	81.3, 83.2	0.06	5	77.6	5.6	72.7, 82.5	0.07	13	76.5	4.1	74.3, 78.7	0.05
Pyloric Caeca	13	140.9	14.7	132.9, 148.9	0.10	23	146.9	12.6	141.7, 152.0	0.09	14	160.0	15.4	151.9, 168.1	0.10

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Table 5.6. Summary of morphological measurements by locality. All lengths in millimeters. Total length ranges are as follows: North: 265-676 mm; West: 382-631 mm; Southwest: 229-566 mm.

	South					East			
	N	Mean	Stand.Dev.	95% CI	CV	N	Mean	Stand.Dev.	95% CI	CV
Total Length	112	421.3	145.8	394.3, 448.3	0.35	17	325.5	131.5	262.9, 388.0	0.40
Weight (grams)	70	391.8	454.2	285.4, 498.2	1.16	17	426.8	398.1	237.5, 616.1	0.93
Body Depth/TL	66	0.17	0.02	0.17, 0.18	0.11	11	0.17	0.02	0.16, 0.18	0.09
Head Length/TL	66	0.20	0.01	0.20, 0.21	0.05	11	0.20	0.01	0.19, 0.21	0.03
Head Depth/TL	66	0.10	0.01	0.10, 0.11	0.08	11	0.10	0.01	0.10, 0.11	0.09
PreDorsal Lengt/TL	66	0.40	0.06	0.38, 0.41	0.15	11	0.40	0.02	0.39, 0.41	0.04
PreOrbital Length/TL	66	0.07	0.01	0.07, 0.08	0.08	11	0.07	0.01	0.07, 0.08	0.09
PostOrbital Length/TL	65	0.14	0.02	0.13, 0.15	0.17	11	0.13	0.01	0.13, 0.14	0.04
SubOrbitalLength/TL	65	0.05	0.01	0.05, 0.06	0.17	11	0.05	0.01	0.04, 0.05	0.19
PostMaxillary Length/TL	65	0.11	0.02	0.10, 0.11	0.16	11	0.10	0.01	0.10, 0.11	0.08
Dentary Length/TL	88	0.09	0.01	0.09, 0.10	0.09	15	0.09	0.01	0.09, 0.10	0.04
Mandible Length/TL	88	0.12	0.01	0.12, 0.13	0.08	15	0.12	0.01	0.11, 0.12	0.05
Premaxillary Width/TL	84	0.02	0.003	0.02, 0.03	0.14	15	0.02	0.002	0.01, 0.02	0.08
Premaxillary Height/TL	84	0.02	0.003	0.01, 0.02	0.16	15	0.02	0.002	0.01, 0.02	0.11
Ventral Pores	85	9.3	1.2	9.1, 9.6	0.13	12	9.7	1.4	8.9, 10.5	0.15
Gill Rakers	91	19.6	1.0	19.4, 19.8	0.05	13	19.4	1.3	18.7, 20.1	0.07
Branchiostegal Rays	109	12.0	0.7	11.9, 12.2	0.06	17	12.1	0.7	11.8, 12.4	0.06
Dorsal Rays	109	10.3	0.6	10.2, 10.5	0.06	17	10.2	0.4	10.0, 10.3	0.04
Pectoral Rays	109	12.8	0.7	12.6, 12.9	0.06	17	12.8	0.6	12.4, 13.2	0.06
Pelvic Rays	109	9.2	0.6	9.1, 9.3	0.07	17	9.1	0.6	8.8, 9.4	0.07
Anal Rays	109	10.0	0.7	9.9, 10.1	0.07	17	9.9	0.7	9.6, 10.3	0.07
Lateral Line Pores	85	125.6	3.4	124.8, 126.3	0.03	14	121.9	6.3	118.6, 125.2	0.05
Diagonal Scale Rows	85	179.9	11.8	177.5, 182.5	0.06	14	180.2	10.4	174.7, 185.7	0.06
Vertical Scale Rows	67	75.2	5.0	73.9, 76.4	0.07	9	76.8	4.9	73.6, 79.9	0.06
Caudal Scales	67	77.7	5.4	76.4, 79.0	0.07	9	80.8	5.1	77.5, 84.2	0.06
Pyloric Caeca	59	144.4	18.2	139.8, 149.1	0.13	11	142.0	13.3	134.1, 149.9	0.09

Table 5.6 cont. Summary of morphological measurements by locality (all lengths in millimeters). Total length ranges are as follows: South: 115-780 mm; East: 100-517 mm.

	Significance of pairwise mean differences between geographic areas										Prob. of homogeneous variances	F stat prob.
	N-W	N-SW	N-S	N-E	W-SW	W-S	W-E	SW-S	SW-E	S-E		
Body Depth	**	**	***	***	***	***	***	NS	NS	NS	0.468	0.000
Head Length	NS	***	***	***	***	**	***	NS	NS	NS	0.079	0.000
Head Depth	NS	***	***	***	***	**	***	NS	NS	NS	0.673	0.000
PreDorsal Length	NS	**	***	***	**	***	***	NS	NS	NS	0.063	0.000
PreOrbital Length	NS	***	***	***	**	**	***	NS	NS	NS	0.356	0.000
PostOrbital Length	NS	***	***	***	***	***	***	NS	NS	NS	0.033	0.000
SubOrbital Length	NS	***	***	***	***	**	***	NS	NS	NS	0.408	0.000
PostMaxillary Length	NS	***	***	***	***	***	***	NS	NS	NS	0.103	0.000
Dentary Length	NS	**	**	***	**	**	***	NS	NS	NS	0.000	0.000
Dentary Tooth Row	NS	***	**	***	***	*	**	NS	NS	NS	0.000	0.000
Mandible Length	NS	***	***	***	**	*	**	NS	NS	NS	0.000	0.000
Premaxillary Width	NS	**	**	***	NS	NS	*	NS	NS	NS	0.001	0.000
Premaxillary Height	NS	*	NS	**	*	NS	**	NS	NS	NS	0.000	0.000
Ventral Pores	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	0.055	0.010
Gill Rakers	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	0.272	0.003
Branchiostegal Rays	***	***	NS	NS	NS	**	*	*	NS	NS	0.873	0.000
Dorsal Rays	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	0.060	0.000
Pectoral Rays	NS	NS	*	NS	*	**	NS	NS	NS	NS	0.290	0.001
Pelvic Rays	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.959	0.104
Anal Rays	***	NS	***	NS	***	**	*	NS	NS	NS	0.851	0.000
Lateral Line Pores	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	0.000	0.000
Diagonal Scale Rows	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.405	0.708
Vertical Scale Rows	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	0.644	0.000
Caudal Scales	NS	**	***	NS	NS	NS	NS	NS	NS	NS	0.842	0.000

Table 5.7. Significance of mean differences between geographic localities. Probability of homogeneous variances based on Bartlett's test of homogeneity. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = not significant.

	Lean North					Siscowet North					Humper North					Siscowet West					Lean Southwest				
	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV
Total Length	27	437.3	108.4	20.9	0.25	54	454.6	97.9	13.3	0.22	48	465.2	48.6	7.0	0.11	23	517.6	66.1	13.8	0.13	13	259.6	17.9	4.9	0.07
Weight (grams)	21	914.7	814.6	177.7	0.89	44	1034.0	628.7	94.8	0.61	42	981.4	418.2	64.5	0.43	0	---	---	---		0	---	---	---	
Body Depth/TL	27	0.17	0.01	0.003	0.09	51	0.19	0.02	0.003	0.13	47	0.19	0.02	0.003	0.12	5	0.21	0.02	0.01	0.10	10	0.18	0.02	0.01	0.13
Head Length/TL	27	0.21	0.01	0.002	0.05	51	0.22	0.01	0.001	0.04	47	0.22	0.02	0.003	0.10	5	0.21	0.01	0.005	0.06	10	0.21	0.01	0.002	0.04
Head Depth/TL	27	0.11	0.01	0.002	0.09	51	0.11	0.01	0.001	0.09	47	0.12	0.01	0.002	0.11	5	0.10	0.01	0.003	0.07	10	0.10	0.01	0.002	0.08
PreDorsal Lengt/TL	26	0.41	0.04	0.007	0.09	51	0.43	0.04	0.005	0.09	46	0.43	0.04	0.005	0.08	5	0.43	0.03	0.01	0.06	10	0.42	0.02	0.01	0.04
PreOrbital Length/TL	27	0.08	0.03	0.005	0.35	51	0.07	0.01	0.001	0.07	47	0.07	0.01	0.001	0.08	5	0.07	0.01	0.001	0.04	10	0.08	0.003	0.001	0.04
PostOrbital/TL	27	0.14	0.01	0.002	0.06	51	0.14	0.01	0.001	0.05	47	0.15	0.01	0.002	0.07	5	0.14	0.01	0.002	0.04	10	0.14	0.01	0.002	0.05
SubOrbital/TL	27	0.06	0.01	0.001	0.09	51	0.06	0.01	0.001	0.11	47	0.06	0.01	0.001	0.09	5	0.06	0.01	0.003	0.10	10	0.06	0.01	0.001	0.09
PostMaxillary/TL	27	0.11	0.01	0.001	0.06	51	0.11	0.01	0.001	0.05	47	0.11	0.01	0.002	0.10	5	0.11	0.01	0.002	0.04	10	0.11	0.01	0.002	0.06
Dentary/TL	26	0.10	0.01	0.001	0.07	52	0.10	0.01	0.001	0.08	42	0.10	0.01	0.002	0.11	10	0.10	0.01	0.004	0.14	11	0.11	0.01	0.002	0.07
Mandible/TL	26	0.13	0.01	0.002	0.07	52	0.13	0.01	0.002	0.08	42	0.13	0.01	0.002	0.10	10	0.12	0.02	0.005	0.13	11	0.13	0.004	0.001	0.03
Premax Width/TL	25	0.02	0.004	0.001	0.17	50	0.02	0.003	0.000	0.15	43	0.02	0.003	0.001	0.14	8	0.02	0.004	0.002	0.20	10	0.03	0.004	0.001	0.15
Premax Height/TL	25	0.02	0.003	0.001	0.15	50	0.02	0.003	0.000	0.16	43	0.02	0.003	0.000	0.14	8	0.02	0.003	0.001	0.16	10	0.02	0.002	0.001	0.11
Ventral Pores	21	9.6	0.9	0.2	0.09	47	9.5	0.9	0.1	0.10	42	8.9	0.9	0.1	0.10	9	9.7	0.7	0.2	0.07	13	10.2	1.0	0.3	0.10
Gill Rakers	27	20.2	1.1	0.2	0.05	53	20.0	0.8	0.1	0.04	48	20.1	1.0	0.1	0.05	19	19.9	0.7	0.1	0.04	13	20.1	0.6	.02	0.03
Branchiostegal Rays	27	12.3	0.8	0.2	0.07	53	12.3	0.7	0.1	0.05	48	12.3	0.7	0.1	0.06	24	11.5	0.6	0.1	0.06	13	11.5	0.6	0.2	0.06
Dorsal Rays	27	10.3	0.8	0.1	0.07	53	10.1	0.6	0.1	0.06	48	10.1	0.6	0.1	0.06	24	10.9	0.4	0.1	0.04	13	9.7	0.6	0.2	0.06
Pectoral Rays	27	13.0	0.9	0.2	0.07	53	13.1	0.8	0.1	0.06	48	13.1	0.6	0.1	0.05	24	13.4	0.6	0.1	0.04	13	12.5	0.5	0.1	0.04
Pelvic Rays	27	9.4	0.6	0.1	0.07	53	9.4	0.6	0.1	0.06	48	9.4	0.6	0.1	0.07	24	9.3	0.6	0.1	0.06	13	9.2	0.5	0.2	0.06
Anal Rays	27	9.8	0.6	0.1	0.06	53	9.5	0.7	0.1	0.07	48	9.7	0.7	0.1	0.07	24	10.5	0.6	0.1	0.06	13	9.3	0.5	0.1	0.05
Lateral Line Pores	27	123.4	5.3	1.0	0.04	53	123.1	6.5	0.9	0.05	46	120.9	4.8	0.7	0.07	10	123.9	2.9	0.9	0.02	13	126.1	3.0	0.8	0.02
Diagonal Scale Rows	27	180.8	16.3	3.1	0.09	53	181.7	13.5	1.9	0.08	46	182.2	13.2	1.9	0.04	10	178.4	12.2	3.9	0.07	13	179.8	13.7	3.8	0.08
Vertical Scale Rows	27	77.3	5.4	1.0	0.07	51	80.5	5.8	0.8	0.07	47	80.2	4.9	0.7	0.07	5	75.6	3.6	1.6	0.05	10	77.7	3.9	1.2	0.05
Caudal Scales	27	78.2	5.4	1.0	0.07	51	83.9	5.0	0.7	0.06	47	82.7	4.5	0.7	0.06	5	77.6	5.6	2.5	0.07	10	76.7	4.4	1.4	0.06
Pyloric Caeca	1	127.0	---	---	0	12	142.1	4.2	4.2	0.10	0	---	---	---	--	23	146.9	2.6	2.6	0.09	12	162.8	12.3	3.6	0.08

Table 5.8. Summary of univariste statistics for Lake Superior _Salvelinus namaycush_ by population. Total length ranges are as follows: Lean North: 295-658 mm; Siscowet North: 265-676 mm; Humper North: 373-616 mm; Siscowet West: 382-631 mm; Lean Southwest: 229-299 mm.

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	Siscowet Southwest					Lean South					Siscowet South					Lean East					Siscowet East				
	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV	N	Mean	SD	SEM	CV
Total Length	5	438.0	155.0	69.3	0.35	43	403.0	153.1	23.3	0.38	69	432.7	140.9	16.9	0.33	8	309.5	100.1	35.4	0.32	9	339.8	159.3	53.1	0.47
Weight (grams)	0	—	—	—	--	33	406.2	569.2	99.1	1.40	37	378.9	327.1	53.8	0.86	8	307.5	244.7	86.5	0.80	9	532.9	487.5	162.5	0.92
Body Depth/TL	3	0.19	0.02	0.01	0.12	29	0.17	0.01	0.002	0.08	37	0.17	0.02	0.003	0.12	5	0.17	0.01	0.004	0.05	6	0.17	0.02	0.01	0.11
Head Length/TL	3	0.21	0.00	0.00	0.00	29	0.20	0.01	0.002	0.06	37	0.20	0.01	0.001	0.04	5	0.20	0.01	0.004	0.05	6	0.20	0.005	0.002	0.02
Head Depth/TL	3	0.10	0.01	0.01	0.09	29	0.10	0.01	0.001	0.08	37	0.10	0.01	0.001	0.08	5	0.11	0.01	0.004	0.08	6	0.10	0.01	0.003	0.09
PreDorsal Length/TL	3	0.46	0.04	0.02	0.08	29	0.38	0.08	0.02	0.22	37	0.41	0.02	0.003	0.04	5	0.41	0.02	0.01	0.04	6	0.39	0.01	0.004	0.02
PreOrbital Length/TL	3	0.07	0.004	0.002	0.06	29	0.07	0.01	0.001	0.09	37	0.07	0.01	0.001	0.07	5	0.07	0.01	0.003	0.09	6	0.07	0.01	0.002	0.09
PostOrbital/TL	3	0.15	0.003	0.002	0.02	29	0.14	0.01	0.002	0.06	36	0.14	0.03	0.005	0.21	5	0.13	0.005	0.002	0.03	6	0.13	0.005	0.002	0.04
SubOrbital/TL	3	0.06	0.001	0.00	0.01	29	0.05	0.01	0.001	0.13	36	0.06	0.001	0.002	0.19	5	0.05	0.005	0.002	0.10	6	0.05	0.01	0.005	0.26
PostMaxillary/TL	3	0.11	0.002	0.001	0.02	29	0.11	0.01	0.002	0.08	36	0.11	0.02	0.004	0.21	5	0.10	0.01	0.003	0.07	6	0.10	0.01	0.004	0.10
Dentary Length/TL	4	0.11	0.004	0.002	0.04	43	0.09	0.01	0.001	0.09	45	0.09	0.01	0.001	0.09	7	0.09	0.004	0.001	0.04	8	0.09	0.004	0.001	0.04
Mandible Length/TL	4	0.14	0.003	0.001	0.02	43	0.12	0.01	0.002	0.09	45	0.12	0.01	0.001	0.08	7	0.12	0.005	0.002	0.04	8	0.12	0.01	0.002	0.05
Premax Width/TL	4	0.02	0.002	0.001	0.08	39	0.02	0.003	0.00	0.14	45	0.02	0.02	0.00	0.14	7	0.02	0.001	0.001	0.07	8	0.02	0.002	0.001	0.10
Premax Height/TL	4	0.02	0.002	0.001	0.09	39	0.02	0.003	0.00	0.16	45	0.02	0.003	0.00	0.17	7	0.02	0.002	0.001	0.11	8	0.02	0.002	0.001	0.12
Ventral Pores	5	10.0	0.7	0.3	0.07	38	9.5	1.4	0.2	0.15	47	9.2	0.9	0.1	0.10	6	10.3	1.5	0.6	0.15	6	9.2	1.2	0.5	0.13
Gill Rakers	5	18.8	1.1	0.5	0.06	39	19.6	1.0	0.2	0.05	52	19.6	0.9	0.1	0.05	7	19.0	1.2	0.4	0.06	6	19.8	1.5	0.6	0.07
Branchiostegal Rays	5	11.6	0.5	0.2	0.05	40	12.3	0.7	0.1	0.06	69	11.9	0.7	0.1	0.06	8	12.4	0.5	0.2	0.04	9	11.9	0.7	0.3	0.07
Dorsal Rays	5	10.2	0.8	0.4	0.08	40	10.4	0.6	0.1	0.06	69	10.4	0.6	0.1	0.06	8	10.2	0.5	0.2	0.04	9	10.1	0.3	0.1	0.03
Pectoral Rays	5	13.2	0.4	0.2	0.03	40	12.7	0.7	0.1	0.06	69	12.9	0.7	0.1	0.06	8	13.1	0.4	0.1	0.03	9	12.6	1.0	0.3	0.08
Pelvic Rays	5	9.6	0.5	0.2	0.06	40	9.3	0.7	0.1	0.07	69	9.1	0.6	0.1	0.07	8	9.1	0.6	0.2	0.07	9	9.1	0.6	0.2	0.07
Anal Rays	5	10.2	0.8	0.4	0.08	40	9.9	0.7	0.1	0.07	69	10.1	0.7	0.1	0.07	8	10.0	0.8	0.3	0.08	9	9.9	0.6	0.2	0.06
Lateral Line Pores	5	123.0	4.2	1.9	0.03	38	125.6	3.1	0.5	0.02	47	125.5	3.6	0.5	0.03	7	122.7	4.5	1.7	0.04	7	121.1	8.0	3.0	0.06
Diagonal Scale Rows	5	172.8	13.6	6.1	0.08	38	182.5	11.6	1.9	0.06	47	177.9	11.6	1.7	0.07	7	182.0	13.5	5.1	0.07	7	178.4	6.8	2.6	0.04
Vertical Scale Rows	3	74.3	5.1	2.9	0.07	30	75.3	4.8	0.9	0.06	37	75.1	5.2	0.9	0.07	4	76.0	5.4	2.7	0.07	5	77.4	5.0	2.2	0.06
Caudal Scales	3	76.0	3.6	2.1	0.05	30	77.4	5.4	0.9	0.07	37	78.0	5.4	0.9	0.07	4	84.7	4.6	2.3	0.05	5	77.8	3.1	1.4	0.04
Pyloric Caeca	2	143.0	26.9	19.0	0.19	16	147.3	17.8	4.4	0.12	43	143.4	18.5	2.8	0.13	6	14.0	16.4	6.7	0.12	5	144.4	9.8	4.4	0.07

Table 5.8. Continued. Total length ranges are as follows: Siscowet Southwest: 256-566 mm; Lean South: 115-691 mm; Siscowet South: 153-780 mm; Lean East: 179-426 mm; Siscowet East: 100-517 mm.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	9.87	1.71	1.34	1.11	1.07
Percent Variance	47.01	8.14	6.40	5.31	5.09
Total Length	0.97	0.03	0.11	0.02	0.02
Post-Orbital	0.96	-0.05	0.03	0.03	0.05
Head Depth	0.96	-0.03	-0.01	-0.00	0.05
Head Length	0.96	-0.03	0.02	0.01	0.05
Post-Maxillary	0.95	-0.08	0.04	0.03	0.03
Body Depth	0.94	0.07	0.06	0.01	0.05
Sub-Orbital	0.94	-0.04	0.05	0.06	0.07
Pre-Dorsal	0.93	0.00	0.03	0.07	-0.01
Weight	0.90	0.14	0.14	0.05	0.00
Pre-Orbital	0.85	-0.04	0.05	0.07	0.02
Caudal Scales	0.60	0.01	-0.25	-0.17	-0.05
Vertical Scale Rows	0.59	0.11	-0.28	-0.23	-0.16
Diagonal Scale Rows	0.46	-0.09	0.33	0.08	-0.35
Branchiostegal Rays	0.22	0.47	-0.16	-0.18	-0.60
Gill Rakers	0.18	-0.02	0.12	-0.65	0.56
Anal Rays	-0.17	0.53	0.48	0.16	0.16
Dorsal Rays	-0.16	0.63	0.30	-0.11	0.20
Pectoral Rays	0.15	0.66	-0.25	0.13	0.07
Lateral Line Pores	-0.14	-0.06	0.67	0.21	-0.14
Ventral Pores	-0.09	0.17	0.29	-0.64	-0.33
Pelvic Rays	-0.02	0.53	-0.31	0.22	0.16

Table 5.9. Loadings of first five principal components for wild Lake Superior lake trout. Principal component scores were generated from a correlation matrix of untransformed variables.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	9.24	1.88	1.54	1.26	1.12
Percent Variance	44.02	8.97	7.34	6.00	5.32
Total Length	0.98	0.03	0.05	0.02	0.06
Post-Orbital	0.97	-0.07	0.03	0.04	0.05
Head Depth	0.94	-0.05	-0.04	-0.05	0.03
Weight	0.94	0.10	0.07	-0.00	0.01
Post-Maxillary	0.94	-0.12	0.02	0.04	0.03
Pre-Dorsal	0.93	0.03	-0.02	0.00	0.08
Sub-Orbital	0.93	-0.04	0.06	0.04	-0.00
Head Length	0.92	-0.02	0.02	-0.01	0.05
Body Depth	0.92	0.05	0.04	0.05	0.12
Pre-Orbital	0.72	-0.10	0.02	0.19	-0.05
Vertical Scale Rows	0.49	0.16	-0.33	-0.37	-0.29
Diagonal Scale Rows	0.48	-0.10	0.18	-0.06	-0.51
Caudal Scales	0.41	0.05	-0.14	-0.73	0.01
Ventral Pores	-0.24	0.24	0.26	-0.50	0.18
Branchiostegal Rays	0.21	0.46	-0.32	0.37	0.06
Pectoral Rays	0.15	0.64	-0.23	0.27	0.26
Pelvic Rays	-0.06	0.61	-0.17	-0.08	-0.38
Anal Rays	0.04	0.62	0.47	-0.12	0.10
Gill Rakers	0.04	-0.11	0.58	-0.06	0.46
Lateral Line Pores	0.04	-0.09	0.62	0.24	-0.50
Dorsal Rays	-0.03	0.59	0.39	0.04	-0.11

Table 5.10. Loadings of first five principal components for wild Lake Superior lake trout from Isle Royale (north). Principal component scores were generated from a correlation matrix of untransformed variables.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	9.52	2.19	1.66	1.36	1.24
Percent Variance	45.32	10.43	7.89	6.47	5.93
Head Length	0.98	0.04	0.01	0.02	0.06
Total Length	0.97	0.07	0.02	0.06	0.09
Pre-Orbital	0.95	-0.04	0.03	0.01	0.13
Head Depth	0.95	0.06	-0.02	-0.02	0.14
Body Depth	0.94	0.04	0.09	0.07	0.15
Weight	0.92	0.16	0.02	0.05	0.14
Post-Orbital	0.90	0.13	-0.01	-0.13	0.06
Sub-Orbital	0.89	0.14	0.04	-0.10	-0.03
Post-Maxillary	0.88	0.13	-0.01	-0.16	0.03
Pre-Dorsal	0.81	0.21	0.08	0.20	-0.05
Caudal Scales	0.59	-0.50	0.24	0.09	-0.30
Vertical Scale Rows	0.41	-0.73	-0.02	-0.28	-0.10
Lateral Line Scales	0.39	-0.14	-0.46	0.38	-0.05
Anal Rays	-0.33	0.46	-0.04	0.27	0.49
Pelvic Rays	-0.31	0.02	0.57	0.17	0.31
Dorsal Rays	-0.28	-0.36	-0.05	0.16	0.63
Lateral Line Pores	0.24	-0.22	0.17	0.64	-0.22
Gill Rakers	0.09	-0.42	0.11	-0.60	0.32
Ventral Pores	0.08	-0.06	-0.79	-0.04	0.26
Branchiostegal Rays	0.05	-0.67	-0.40	0.29	0.06
Pectoral Rays	0.01	-0.14	-0.46	0.38	-0.05

Table 5.11. Loadings of first five principal components of Lake Superior lake trout from Keweenaw Bay (south). Principal component scores were generated from a correlation matrix of untransformed variables.

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalues	9.97	1.69	1.37	1.11	1.07
Percent Variance	47.46	8.06	6.52	5.30	5.10
Total Length	0.97	0.01	0.09	0.02	0.01
Head Depth	0.96	-0.02	0.00	0.01	0.04
Post-Orbital	0.96	-0.05	0.04	0.04	0.03
Head Length	0.96	-0.02	0.03	0.02	0.04
Post-Maxillary	0.94	-0.08	0.04	0.03	0.00
Body Depth	0.94	0.06	0.06	0.03	0.04
Sub-Orbital	0.94	-0.04	0.07	0.07	0.02
Pre-Dorsal	0.93	0.01	0.03	0.04	-0.03
Weight	0.92	0.10	0.13	0.04	-0.03
Pre-Orbital	0.85	-0.04	0.06	0.06	-0.02
Caudal Scales	0.61	0.02	-0.27	-0.14	0.07
Vertical Scale Rows	0.60	0.15	-0.29	-0.22	-0.07
Diagonal Scale Rows	0.46	-0.06	0.33	-0.18	-0.36
Anal Rays	-0.20	0.47	0.55	0.08	0.15
Gill Rakers	0.19	-0.03	0.15	-0.23	0.76
Branchiostegal Rays	0.18	0.49	-0.22	-0.43	-0.41
Dorsal Rays	-0.17	0.62	0.35	0.01	0.17
Lateral Line Pores	-0.15	-0.05	0.64	0.15	-0.37
Pectoral Rays	0.15	0.67	-0.27	0.21	0.04
Ventral Pores	-0.12	0.10	0.21	-0.79	0.05
Pelvic Rays	-0.01	0.57	-0.19	0.24	-0.02

Table 5.12. Loadings of first five principal components of Lake Superior lake trout from north and south basins. Principal component scores were generated from a correlation matrix of untransformed variables.

		LEAN		SISCOWET		HUMPER	
		PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
C O V A R I A N C E **M A Y R I X**	Eigen Values	1.53	0.09	1.70	0.02	0.14	0.03
	Percent Variance	90.28	5.22	96.33	1.10	58.12	13.33
	ln Total Length	0.33	0.02	0.32	0.01	0.09	0.03
	ln Body Depth	0.35	0.01	0.40	0.04	0.14	-0.08
	ln Head Length	0.33	0.03	0.34	0.02	0.08	0.08
	ln Head Depth	0.34	0.01	0.36	0.04	0.10	0.06
	ln PreDorsal	0.36	-0.28	0.36	0.02	0.09	0.01
	ln PreOrbital	0.32	0.04	0.33	0.00	0.08	0.01
	ln PostOrbital	0.34	0.03	0.35	0.02	0.11	0.05
	ln SubOrbital	0.37	-0.03	0.39	0.00	0.11	0.02
	ln Postmaxillary	0.30	0.02	0.33	0.02	0.12	0.06
	ln Mandible	0.35	0.03	0.36	-0.00	0.10	-0.04
	ln Dentary	0.33	0.03	0.35	0.00	0.09	-0.04
	ln Premax Width	0.36	0.05	0.40	-0.03	0.12	-0.06
	ln Premax Height	0.36	0.04	0.40	-0.12	0.13	-0.05
C O R R E L A T I O N **M A T R I X**	Eigen Values	1.89	1.57	2.16	1.75	2.41	1.48
	Percent Variance	17.22	14.25	19.61	15.94	21.90	13.46
	Ventral Pores	0.32	-0.08	-0.15	0.26	0.31	-0.51
	Gill Rakers	0.20	-0.10	0.27	0.17	-0.21	0.15
	Dorsal Rays	-0.36	0.34	0.01	0.73	0.63	-0.06
	Branchiostegal Rays	0.11	.061	0.56	0.14	0.53	0.02
	Pectoral Rays	-0.19	0.75	0.29	0.46	0.80	-0.02
	Pelvic Rays	-0.48	0.17	0.27	0.61	0.69	0.14
	Anal Rays	-0.50	0.28	-0.41	0.70	0.48	-0.14
	Diagonal Scale Rows	0.66	0.08	0.31	0.07	0.15	0.71
	Vertical Scale Rows	0.67	0.20	0.78	-0.10	0.27	0.68
	Caudal Scales	0.46	0.57	0.83	-0.07	-0.36	0.15
	Lateral Line Pores	-0.07	-0.14	-0.18	-0.08	-0.08	0.40

Table 5.13. Principal component loadings for PC analysis by phenotype.

		LEAN VS SISCOWET		SISCOWET VS HUMPER		LEAN VS HUMPER	
		PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
C O V A R I A N C E M A T R I X	Eigen Values	1.66	0.04	1.29	0.02	1.23	0.06
	Percent Variance	93.79	2.34	94.32	1.50	89.58	4.33
	ln Total Length	0.32	0.01	0.27	0.02	0.28	0.01
	ln Body Depth	0.39	-0.00	0.34	0.03	0.33	0.00
	ln Head Length	0.34	0.01	0.30	0.03	0.30	0.02
	ln Head Depth	0.36	-0.00	0.32	0.04	0.32	0.01
	ln PreDorsal	0.37	-0.18	0.31	0.03	0.33	-0.23
	ln PreOrbital	0.32	0.03	0.28	0.01	0.27	0.03
	ln PostOrbital	0.35	0.01	0.31	0.03	0.31	0.02
	ln SubOrbital	0.38	-0.02	0.34	0.01	0.33	-0.02
	ln Postmaxillary	0.32	0.01	0.29	0.03	0.28	0.02
	ln Mandible	0.35	0.02	0.31	-0.01	0.31	0.03
	ln Dentary	0.35	0.02	0.31	-0.02	0.30	0.03
	ln Premax Width	0.38	0.05	0.35	-0.06	0.32	0.05
	ln Premax Height	0.39	0.05	0.35	-0.10	0.32	0.04
C O R R E L A T I O N M A T R I X	Eigen Values	1.95	1.64	2.06	1.84	1.87	1.73
	Percent Variance	17.75	14.93	18.73	16.72	17.05	15.72
	Ventral Pores	-0.05	-0.02	-0.23	0.34	-0.24	0.02
	Gill Rakers	0.19	0.09	0.21	0.02	0.03	-0.22
	Dorsal Rays	-0.02	0.62	0.03	0.72	0.07	0.56
	Branchiostegal Rays	0.52	0.22	0.50	0.23	0.44	0.36
	Pectoral Rays	0.30	0.58	0.29	0.56	0.47	0.58
	Pelvic Rays	0.09	0.61	0.34	0.56	0.25	0.51
	Anal Rays	-0.37	0.60	-0.32	0.69	-0.13	0.60
	Diagonal Scale Rows	0.33	-0.26	0.39	0.01	0.33	-0.39
	Vertical Scale Rows	0.79	-0.12	0.77	-0.07	0.69	-0.26
	Caudal Scales	0.80	0.02	0.77	-0.19	0.67	-0.24
	Lateral Line Pores	-0.22	-0.20	-0.27	-0.01	-0.53	0.07

Table 5.14. Principal component loadings for pairwise PC analyses of lean, siscowet, and humper phenotypes.

		PC 1	PC 2	PC 3	PC 4	PC 5
C O V A R I A N C E **M O R P H O M E T R I C S**	Eigenvalues	1.44	0.04	0.02	0.01	0.01
	Percent Variance	92.95	2.31	1.30	0.81	0.58
	Ln Total Length	0.30	0.00	0.02	-0.01	0.02
	Ln Body Depth	0.36	-0.01	0.01	-0.10	0.01
	Ln Head Length	0.32	0.01	0.03	0.02	0.00
	Ln Head Depth	0.34	-0.01	0.05	0.01	-0.01
	Ln PreDorsal Length	0.34	-0.17	-0.04	0.01	-0.01
	Ln PreOrbital Length	0.30	0.02	0.02	0.02	-0.03
	Ln PostOrbital Length	0.33	0.01	0.03	0.01	0.01
	Ln SubOrbital Length	0.36	-0.01	0.02	0.04	0.01
	Ln PostMaxillary Length	0.30	0.01	0.03	0.01	0.02
	Ln Mandible Length	0.33	0.02	-0.01	-0.01	-0.02
	Ln Dentary Length	0.32	0.02	-0.01	0.00	-0.02
	Ln Premaxillary Width	0.36	0.05	-0.06	-0.01	-0.05
	Ln Premaxillary Height	0.36	0.05	-0.08	-0.01	-0.02
C O R R E L A T I O N **M E R I S T I C S**	Eigenvalues	1.93	1.73	1.47	1.20	1.01
	Percent Variance	17.51	15.72	13.35	10.91	9.21
	Caudal Scales	0.79	-0.06	-0.26	0.05	0.05
	Vertical Scale Rows	0.71	0.34	-0.16	-0.06	0.14
	Gill Rakers	0.42	-0.68	-0.20	0.09	0.09
	Dorsal Rays	0.41	-0.51	0.47	-0.14	-0.03
	Diagonal Scale Rows	0.40	0.24	-0.11	-0.68	0.14
	Pelvic Rays	0.35	0.13	0.57	0.15	-0.19
	Lateral Line Pores	-0.29	0.04	0.08	-0.79	-0.05
	Ventral Pores	-0.20	-0.07	0.18	0.06	0.91
	Pectoral Rays	0.16	0.55	0.49	0.07	-0.14
	Branchiostegal Rays	0.13	0.56	0.21	0.17	0.28
	Anal Rays	0.01	-0.42	0.67	-0.13	0.06

Table 5.15. Loadings of first five principal components of Lake Superior wild lake trout based on a covariance matrix of natural log-transformed morphometrics and a correlation matrix of meristics.

	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**
C O V A R I A N C E M O R P H O M E T R I C S					
Eigenvalues	0.60	0.02	0.02	0.01	0.01
Percent Variance	87.32	3.55	2.25	1.65	1.23
Ln Total Length	0.18	0.02	0.00	0.01	-0.00
Ln Body Depth	0.24	0.03	-0.09	-0.03	0.02
Ln Head Length	0.19	0.03	0.03	0.02	-0.01
Ln Head Depth	0.22	0.05	0.01	0.02	-0.02
Ln PreDorsal Length	0.21	0.03	-0.04	0.01	-0.06
Ln PreOrbital Length	0.18	0.01	0.04	-0.09	-0.02
Ln PostOrbital Length	0.20	0.03	0.02	0.02	0.02
Ln SubOrbital Length	0.23	0.01	0.03	-0.00	0.03
Ln PostMaxillary Length	0.19	0.03	0.02	0.02	0.03
Ln Mandible Length	0.21	-0.02	-0.01	-0.00	0.01
Ln Dentary Length	0.21	-0.03	0.00	-0.00	0.01
Ln PreMax Width	0.25	-0.08	-0.01	-0.00	0.02
Ln PreMax Height	0.25	-0.09	0.01	0.02	-0.03
C O R R E L A T I O N M E R I S T I C S					
Eigenvalues	1.90	1.65	1.42	1.14	1.03
Percent Variance	17.03	14.97	12.88	10.34	9.37
Ventral Pores	-0.04	0.45	0.02	-0.60	-0.43
Gill Rakers	-0.23	0.26	0.31	-0.25	0.70
Dorsal rays	0.39	0.54	0.29	0.11	0.14
Branchiostegal Rays	0.55	-0.05	-0.31	0.31	0.19
Pectoral Rays	0.61	0.19	-0.37	0.05	0.30
Pelvic Rays	0.58	0.22	-0.11	0.06	-0.38
Anal Rays	0.39	0.58	0.32	-0.13	0.01
Diagonal Scale Rows	0.19	-0.33	0.65	0.22	-0.04
Vertical Scale Rows	0.53	-0.53	0.29	-0.07	-0.12
Caudal Scales	0.39	-0.43	0.33	-0.52	0.11
Lateral Line Pores	-0.23	0.29	0.53	0.51	-0.19

Table 5.16. Principal component loadings for lake trout from northern sampling area (Isle Royale and Sawyer Bay) based on a covariance matrix of natural log-transformed morphometrics and a correlation matrix of meristics.

		PC 1	PC 2	PC 3	PC 4	PC 5
C O V A R I A N C E M O R P H O M E T R I C S	Eigenvalues	1.55	0.10	0.01	0.01	0.01
	Percent Variance	91.05	5.91	0.86	0.62	0.48
	Ln Total Length	0.33	0.02	0.02	0.03	0.01
	Ln Body Depth	0.37	0.01	0.02	0.04	-0.06
	Ln Head Length	0.33	0.03	0.02	0.00	0.02
	Ln Head Depth	0.33	0.02	0.03	0.01	0.01
	Ln PreDorsal Length	0.36	-0.29	-0.01	-0.00	-0.01
	Ln PreOrbital Length	0.32	0.04	-0.01	-0.01	0.01
	Ln PostOrbital Length	0.34	0.03	0.02	0.01	0.02
	Ln SubOrbital Length	0.35	-0.04	-0.01	-0.02	0.05
	Ln PostMaxillary Length	0.30	0.03	0.02	0.02	0.01
	Ln Mandible Length	0.34	0.03	0.01	-0.02	0.00
	Ln Dentary Length	0.34	0.03	0.02	-0.02	-0.00
	Ln PreMax Width	0.37	0.06	-0.02	-0.07	-0.04
	Ln PreMax Height	0.39	0.05	-0.10	0.04	0.00
C O R R E L A T I O N M E R I S T I C S	Eigenvalues	2.31	1.64	1.41	1.26	1.12
	Percent Variance	21.00	14.87	12.85	11.48	10.22
	Ventral Pores	0.17	-0.21	0.79	-0.05	-0.17
	Gill Rakers	0.17	0.45	0.08	-0.44	0.62
	Dorsal rays	-0.17	0.63	0.42	0.03	0.26
	Branchiostegal Rays	0.50	0.19	0.54	0.12	-0.42
	Pectoral Rays	0.23	0.69	-0.08	0.28	-0.22
	Pelvic Rays	-0.33	0.49	-0.20	0.10	-0.41
	Anal Rays	-0.60	0.19	0.32	0.34	0.29
	Diagonal Scale Rows	0.45	-0.36	0.20	0.43	0.32
	Vertical Scale Rows	0.75	0.13	-0.00	-0.43	0.01
	Caudal Scales	0.75	0.19	-0.29	0.12	0.03
	Lateral Line Pores	0.35	0.04	-0.18	0.67	0.26

Table 5.17. Principal component loadings for lake trout from southern sampling area (Keweenaw bay and Copper Harbor) based on a covariance matrix of natural log-transformed morphometrics and a correlation matrix of meristics.

		PC 1	PC 2	PC 3	PC 4	PC 5
C O V A R I A N C E M O R P H O M E T R I C S	Eigenvalues	1.14	0.04	0.02	0.01	0.01
	Percent Variance	91.07	3.22	1.56	0.96	0.72
	Ln Total Length	0.26	0.01	0.02	-0.00	-0.02
	Ln Body Depth	0.32	-0.01	0.01	-0.09	0.00
	Ln Head Length	0.28	0.01	0.03	0.03	-0.01
	Ln Head Depth	0.30	-0.00	0.05	0.01	-0.01
	Ln PreDorsal Length	0.31	-0.18	-0.3	0.01	0.00
	Ln PreOrbital Length	0.27	0.03	0.02	0.03	0.06
	Ln PostOrbital Length	0.29	0.01	0.03	0.01	-0.02
	Ln SubOrbital Length	0.31	-0.01	0.01	0.03	0.00
	Ln PostMaxillary Length	0.26	0.01	0.04	0.01	-0.03
	Ln Mandible Length	0.29	0.02	-0.00	-0.01	0.01
	Ln Dentary Length	0.29	0.02	-0.01	-0.00	0.01
	Ln PreMax Width	0.33	0.05	-0.06	-0.01	0.03
	Ln PreMax Height	0.32	0.05	-0.09	0.02	-0.04
C O R R E L A T I O N M E R I S T I C S	Eigenvalues	2.01	1.65	1.26	1.09	1.05
	Percent Variance	18.29	15.01	11.50	9.95	9.54
	Gill Rakers	0.12	0.06	0.39	-0.68	0.39
	Dorsal rays	-0.09	0.68	0.34	-0.19	-0.06
	Branchiostegal Rays	0.45	0.34	-0.08	0.45	0.09
	Pectoral Rays	0.38	0.59	-0.23	-0.09	-0.15
	Pelvic Rays	0.22	0.53	-0.24	0.07	-0.22
	Anal Rays	-0.36	0.60	0.30	0.02	0.05
	Diagonal Scale Rows	0.34	-0.20	0.64	0.30	-0.19
	Vertical Scale Rows	0.78	-0.09	0.17	0.04	0.02
	Caudal Scales	0.77	-0.10	0.18	-0.14	0.01
	Lateral Line Pores	-0.38	-0.02	0.54	0.21	-0.41

Table 5.18. Principal component loadings for lake trout from northern and southern sampling areas combined based on a covariance matrix of natural log-transformed morphometrics and a correlation matrix of meristics.

MEASURE	MSE	PHENOTYPE	PHENOTYPE x TL	LOCATION	LOCATION x TL
Body Depth	0.01	*** (0.12)	*** (0.13)	*** (0.57)	*** (0.58)
Head Length	0.004	** (0.02)	** (0.02)	NS (0.001)	NS (0.001)
Head Depth	0.01	NS (0.03)	NS (0.03)	* (0.02)	** (0.03)
PreDorsal Length	0.03	NS (0.02)	NS (0.03)	NS (0.01)	NS (0.01)
Weight	0.12	NS (0.01)	NS (0.01)	** (0.06)	** (0.07)
PreOrbital Length	0.01	** (0.06)	** (0.06)	NS (0.004)	NS (0.005)
SubOrbital Length	0.01	NS (0.03)	NS (0.03)	* (0.05)	* (0.05)
PostOrbital Length	0.01	NS (0.01)	NS (0.01)	NS (0.01)	NS (0.01)
PostMaxillary Length	0.01	NS (0.02)	NS (0.02)	** (0.03)	** (0.03)
Mandible Length	0.01	** (0.05)	** (0.05)	NS (0)	NS (0)
Dentary Length	0.01	*** (0.07)	*** (0.07)	NS (0.01)	NS (0.005)
Premaxillary Width	0.02	** (0.09)	** (0.08)	* (0.04)	* (0.04)
Premaxillary Height	0.02	NS (0.06)	NS (0.06)	NS (0.02)	NS (0.02)

Table 5.19. Analysis of the proportion of differences among phenotypes (TYPE) and geographic locations, standardized by total length. Measures that differed significantly for type and locality categories are labeled as follows: "*" = p < 0.05; "**" = p < 0.01; "***" = p < 0.001. Mean squares are listed in parentheses.

CHAPTER VI
INTRALACUSTRINE SPECIATION OF Salvelinus namaycush
IN LAKE SUPERIOR

Abstract

Initial stages of intralacustrine speciation are hypothesized as the model for evolutionary divergence of freshwater fish species which exhibit ecophenotypic differentiation with partial reproductive isolation. Models of speciation hypothesized for morphologically divergent sympatric populations often invoke divergence in allopatry as the process leading to differentiation, and secondary contact as the factor leading to sympatry. Speciation hypotheses are presented here to model patterns of divergence in the complex freshwater environment of a recently glaciated lake. The null hypothesis is allopatric differentiation in glacial refugia. Alternative models include allopatric divergence via lake level fluctuations, or parapatric divergence due to allopatric or allochronic reproduction. Competitive speciation (Rosenzweig 1978) and alternative adaptations (West-Eberhard 1986) are considered plausible models for parapatric speciation in a lacustrine environment. Morphological, ecological, and behavioral differences among populations of *Salvelinus namaycush* in Lake Superior support an hypothesis of intralacustrine speciation mediated by ecophenotypic differentiation in the presence of differences in time and place of reproduction.

Introduction

Large freshwater systems promote the evolution of remarkable biological diversity. Lake Victoria and other African rift lakes have been regarded as prime examples of "explosive evolution," with at least 200 species of cichlids in lakes less than 1 million years old (Greenwood 1974, Mayr 1976). Recently glaciated lakes, especially those in the northern hemisphere such as the Laurentian Great Lakes and Lake Baikal, have diverse arrays of of salmonid, cottid, and coregonid species (Behnke 1972). Vertical variation in lacustrine environments provide especially significant contributions to ecological and morphological divergence. Critical features of lakes such as light, temperature, pressure, density, viscosity, substrate texture, seasonal temperature fluctuations, food, competitors, and predators change with depth. These features set the stage for unique adaptations in aquatic organisms. Lake Baikal, Lake Malawi, Lake Tanganyika, Lake Victoria, Lake Ohrid, and the Laurentian Great Lakes share a number of peculiar characteristics with respect to their fish fauna. All of these systems contain closely related species with wide ranges of morphological diversity (Brooks 1950, Taliev 1955, Stankovic 1960, Fryer and Iles 1972, Greenwood 1974, Smith and Todd 1984) and low levels of genetic differentiation (Hindar et al. 1986, Meyer et al. 1990, Sturmbauer and Meyer 1992). Many of the morphological differences are related to ecological and trophic specializations. My specific interest lies in the processes underlying the development of intraspecific morphological diversity that has the potential to lead to phylogenetic branching and lineage evolution.

The study of the patterns and the processes of speciation are both dependent upon what is considered the unit of speciation or the "species," two entities which may or may not be identical. The definition of the "species" has undergone scrutiny in the philosophical discourse of the biological sciences (see reviews by Mayr 1982, Endler and McLellan 1988, deQueiroz and Donoghue 1988, Kluge 1990). One reviewer even stated, "...there are as many [definitions of the species] as there are naturalists." (Tremaux 1865). In the wake of Neo-

Darwinism, the emphasis shifted from the strict designation of a kind of organism with unique characteristics to something more dynamic - populations of interbreeding individuals (Wright 1978). Still, it must be emphasized that no universal definition of a "species" encompasses all different types of species such as sexual, asexual, hybrid, etc. (Nelson 1989).

In this paper species are defined as monophyletic or paraphyletic groups of organisms within which gene exchange can occur, but among which there is little or no heritable gene exchange. This definition is similar to the biological species concept espoused by Mayr (1963) and Dobzhansky (1951). In addition, an attempt has been made to provide for recognition of ecological and behavioral factors which provide extrinsic rather than intrinsic barriers to reproduction. Students of speciation are becoming increasingly aware of processes such as introgression that reduce the stability of reproductive barriers between species. Molecular biological techniques are documenting more examples of species that are either morphologically distinct or behaviorally, temporally, or spatially reproductively isolated, but not as genetically different as had been formerly supposed to be necessary for species-level differentiation (Utter et al. 1989, Wirgin et al. 1989, Meyer et al. 1990, Shields et al. 1990, Safford and Booke 1992). The frequency of occurrence of these species groups has significant implications for current models of speciation.

Strict allopatric speciation mechanisms involve geological, environmental, or geographic barriers which interrupt gene flow among formerly cohesive populations. During the course of natural selection on different stocks, intrinsic barriers to reproductive isolation develop as genetic and behavioral differences accumulate (Mayr 1963, Wright 1978). These differences are manifest in assortative mating if the populations ultimately come into secondary contact (Paterson 1981). This mechanism of speciation is supported by empirical studies (Muller 1940, Mayr 1954, 1963, Caisse and Antonovics 1978, Porter 1989, Ritchie et al. 1989).

Mechanisms of speciation in which barriers to gene flow develop between stocks exhibiting partial genetic contact are less well understood than the more widely recognized examples of allopatric speciation. Parapatric, or micro-allopatric speciation (Endler 1977) may be invoked for cases of intralacustrine (within lake basin) speciation which fail the predictions of strict allopatric models (Stankovic 1960, Kohzov 1963, Smith and Todd 1984). Examples of non-allopatric divergence involve obvious differences in behavior, morphology, life history, or genetic characteristics. Sympatric geographic distribution may or may not be accompanied by morphological divergence in characters normally used by taxonomists for classification of the different species. Micro-allopatric divergence could occur more frequently if the major avenues open for niche diversification involve physiological specialization for a specific set of environmental variables (Kohn and Orians 1962). The only ecological requirement is that multiple species be able to occupy different niches in the same community on a sustained basis (Van Valen 1988). The evolutionary response to ecological variation forms the basis of a number of hypothesized mechanisms of micro-allopatric speciation (Maynard-Smith 1966, Bush 1969, Rosenzweig 1978, West-Eberhard 1986, Diehl and Bush 1989).

Speciation models were investigated for African cichlids (Rensch 1933, Brooks 1950, Fryer and Iles 1972, Greenwood 1974, Meyer et al. 1990), sculpins in Lake Baikal (Taliev 1955, Kohzov 1963, Smith and Todd 1984), and ciscoes in the Laurentian Great Lakes (Todd et al. 1981, Smith and Todd 1984, Bernatchez et al. 1989, Shields et al. 1990). Evidence supported allopatric speciation via fluctuating water levels as well as multiple colonization from fluvial systems for some of the African cichlids (Fryer and Iles 1972), Lake Baikal sculpins (Taliev 1955, Kohzov 1963), and some of the North American coregonids (Bernatchez et al. 1989). Additional differentiation of cichlids was attributed to sexual selection and competition (Dominey 1984). Given the high probability of isolation from water level fluctuations in the Laurentian Great Lakes during the Pleistocene (Bailey and Smith 1981), some species pairs of ciscoes may have diverged in this

manner, but some diverged by other means (Smith and Todd 1984). In the other cases, invocation of a strict allopatric model seems to be an over-simplification of the problem. Some evidence was not consistent with allopatric origin of morphologically differentiated sympatric populations. In contrast to the hundreds of species pairs found in species flocks in the African rift lakes and 29 species of Lake Baikal sculpins, only two species pairs and one species group were found in the Great Lakes ciscoes: the *Coregonus hoyi - C. kiyi* species pair, the *C. artedii - C. nigripinnis* pair, and the *C. zenithicus - C. alpenae - C. johannae - C. reighardi* group. The absence of large species flocks within the Great Lakes coregonids was likely be due to the geologically young age of the Laurentian Great Lakes relative to the African Great Lakes (20000 years before present (ybp) vs. 0.75-10 million ybp). Among the species hypothesized to have diverged by non-allopatric processes, ecological rather than geological or geographic barriers play a key role in reproductive segregation (Kohzov 1960, Smith and Todd 1984).

It is hypothesized that intralacustrine speciation by ecological divergence is one mechanism leading to lineage evolution in *Salvelinus namaycush*. The speciation process is twofold - one facet involves the establishment of diversity within a species and the other involves the development of isolating mechanisms which preserve the differentiating pattern (Mayr 1942). Divergence may be either phenotypic or genotypic, or both (Simpson 1944), but phenotypic divergence need not be accompanied by genetic changes, nor do genetic changes have to be manifest in phenotypic divergence. The appearance of a different character, be it genetic or phenotypic, upon which natural selection may act is a critical part of the evolutionary process.

Hypotheses

Darwin argued that "descent by modification" gave all aspects of morphology historical significance (Darwin 1859, Paterson 1986). But even Darwin recognized the inherent problem in using only morphology to look at

taxonomic relationships. The use of molecular comparative methods is of critical importance to phylogenetic systematics and taxonomic studies. Molecular techniques can help to illuminate the nature of the bond of correlated phenotypic characters within a genome by allowing comparative examination of patterns of shared genetic characters (protein loci, restriction sites, nuclear sequences) with patterns of shared phenotypic characters. Using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis in combination with morphological analysis, I have documented the relationships among the three morphological forms of lake trout in Lake Superior. The hypothesis of intralacustrine speciation was tested by investigating whether the siscowets are more closely related to native (wild) lake trout in Lake Superior or to the native (lean) lake trout of tributary systems and lake systems not presently connected to Lake Superior. By combining molecular data with morphological and ecological observations, insight can be gained into the genetic contribution to hypothesized models of speciation.

Null Hypothesis : Allopatric Speciation and Immigration

The null model to test hypotheses of lean-siscowet lake trout speciation, given the geologic history of the Laurentian Great Lakes basin, is allopatric speciation in tributary waters or glacial refugia, with subsequent immigration into the main body of water (White 1978, Smith and Todd 1984). Natural selection operating under this model is controlled by local environments in the tributaries or refugia. In the complete absence of gene exchange, and if natural selection operated differentially, diversification in morphological or genetic traits occurs prior to reproductive isolation. Selection on traits in this manner would be independent among the isolated populations, and would show corresponding genetic discontinuities (Wright 1946, White 1978, Endler 1986).

The allopatric model assumes that each localized population experiences random mating, that selection varies geographically, and that repeated colonization occurs as tributary systems periodically come in contact with the main

body of water. Genetic distance and sequence divergence between lean, siscowet, and humper populations would correspond to time since isolation thousands of years ago. Siscowets, humpers, and leans within the Superior basin should have phenotypically similar sister species outside of the Great Lakes basin. The predictions of this model require that the original source of siscowet, humper, and lean lake trout exist in lakes outside of the Laurentian Great Lakes.

The test of this model begins with an attempt to determine the extent of genetic variation among the wild populations of Lake Superior lake trout, and the relationship between Lake Superior populations and populations from outside the Laurentian Basin. If allopatric divergence occurred prior to the invasion of Lake Superior by lake trout, populations of siscowet-like and humper-like phenotypes should exist in lakes outside of the Laurentian Great Lakes. Similar phenotypic divergence among the Great Lakes could be explained by dispersal within the system.

Alternative Hypothesis : Divergence Via Fluctuating Water Levels

Fluctuation of water levels in lake basins provides an alternative mechanism for allopatric speciation, and is favored by theoreticians as the mode of divergence of some lacustrine species (Fryer and Iles 1972, Greenwood 1974, White 1978). This hypothesis is particularly attractive for areas, such as the Great Lakes, which have undergone severe geologic or climatic changes without mass extinction of fauna. Speciation could occur if the geologic nature of the lakes provided physical barriers between major basins at low water levels. Populations would then be effectively allopatric and subject to the independent effects of natural selection. After a period of time, when lake levels rose due to natural climatic fluctuations, the isolated groups would be re-united and would maintain reproductive isolation in sympatry. Some examples from African Great Lake haplochromines supported this hypothesis (Fryer and Iles 1972, Greenwood 1984b).

This model assumes that mating within an isolated population is random, physical barriers within the basin prevent migration or gene flow at low water levels, and divergence in allopatry is sufficient for reproductive isolation in secondary contact. The existence of geologic, climatic, and fossil evidence for fluctuations in water levels in the Laurentian Great Lakes (Prest 1970, Farrand 1969) suggests that isolation could have occurred among populations of lake trout between major Laurentian basins. If allopatric divergence was complete, the lean and siscowet phenotypes would cladistically be terminal species or sister species derived from a single species (White 1978, Smith and Todd 1984) and would not be expected to be derived from multiple colonizations, i.e. hybrid species (Fryer and Iles 1972). Genetic distance and sequence divergence estimates for this model should correspond to time since isolation less than 8000 years ago, and lake trout populations in Lake Superior should share a maternal ancestor within the lake.

A corollary to predictions of this model is that high water levels would allow sympatric existence to occur before enough divergence accumulated to ensure full reproductive isolation. Around 11600 ybp, proglacial lakes had water depths that were greater than current lake depths and provided additional habitat that could have supported a deep-water-adapted form of lake trout. Fish migrations could have occurred between Pleistocene Lake Agassiz, other Canadian basins, and lakes within the Laurentian basin. The populations that were able to recolonize the open waters after the glacial retreat are expected to have had close relatives (sibling species) in waters associated with the refugia (Smith and Todd 1984). Depending upon the extent of divergence between the allopatric populations, the result of secondary contact may range from zygote inviability to differential F1 survival to full hybrid viability and introgression (Mayr 1942, Thoday and Gibson 1962, White 1978, Templeton 1981).

Two scenarios can be postulated to account for allopatric divergence of lean and siscowet lake trout due to water level fluctuations:

(a) Ancestral lake trout colonized the Lake Superior basin during a high water stage (12,900 - 11,600 ybp) (Figure 6.1 a). The form that invaded the upper lake basin (glacial Lake Keweenaw or Lake Duluth) evolved into a siscowet-like form as water levels in the Great Lake basins fluctuated and isolated Lake Keweenaw or Lake Duluth (Figure 6.1 b). This siscowet-like form evolved in the upper basin, isolated from the lean forms which became established in the lower lake basins (Figure 6.1 c). During the final glacial retreat when water levels rose again, the lean form re-invaded the Superior basin and became the dominant competitor, forcing the siscowet form into deep water habitat (Figure 6.1 d). At lowest water levels, some populations of the siscowet form may have hybridized with leans, become isolated over deep shoals, and eventually evolved into the humper form. The current distribution of leans, siscowets, and humpers in the lakes is attributed to post-glacial dispersal (Figure 6.1 e).

(b) Water level fluctuations within Lake Superior may alternatively be responsible for lean-siscowet divergence. During deep water stages, lake trout populations may have been distributed within the lake as local stocks (Figure 6.2 a). As lake levels dropped, local populations would have become isolated into basins formerly in deeper water (Figure 6.2 b). Populations forced into deeper water may have come in close proximity to shoals that were previously geographically distant. As lake levels rose, populations returned to their original habitat, but some fraction of the population may have remained as a founder population near the shoal (Figure 6.2 c). Populations in the original habitat and near the shoal were separated by deepwater habitat. The shoal population exploited the deepwater resources and independent selective forces led to divergence of the two populations. As lake levels lowered again, the differentiated populations were forced into contact (Figure 6.2 d) and could have hybridized because reproductive isolation was incomplete. The return of higher lake levels was accompanied by the return of populations to original

habitats, but hybridization and straying resulted in a breakdown of any former genetic segregation (Figure 6.2 e).

These two scenarios provide mechanisms by which water level fluctuations alone are responsible for the differences between leans and siscowets, but can only be accepted if an ad hoc hypothesis is considered to solve complications. As water levels lower, deep water habitat is not created, rather it is removed. If the siscowet is best adapted to a deep water environment, we assume with this hypothesis that it began in a shallow water environment and was later forced into deep water. The temperature of the waters in the proglacial lakes most likely remained around 0 - 4°C at the ice front. Siscowets live at temperatures of 3 - 5°C in Lake Superior, compared to temperatures of 7 - 15°C in the shallower waters which leans occupy. Temperature differences may have provided a way for siscowets to be best adapted to early Lake Superior. It is possible that temperature differences could have led to such differentiation by excluding siscowets from surviving in the warmer waters of the lower lakes during interglacial periods. However, in other deep freshwater lakes of North America colonized from the same glacial refugia, there are no extant siscowet-like populations.

Alternative Hypothesis : Parapatric (micro-allopatric) Divergence via Reproductive Allochrony or Allopatry

Closely related populations may live in the same area, yet diverge because of differences in behavior or the time or place of reproduction. Fish with low vagility or strong homing tendencies will be more likely to show parapatric divergence than highly mobile and panmictic species (Grant 1977, Lewis 1973, Endler 1977, White 1978). Ecological isolation can also contribute to parapatric divergence if a particular species demonstrates habitat choice (Diehl and Bush 1989). Sympatric populations which diverge this way were called "ecological races" or "biological races" by Mayr (1942), which he considered to be manifestations of allopatry (see also Stebbins 1950).

This model assumes that closely related populations are sympatric but reproductively isolated. The traits under selection are related to differences in ecology and spawning behavior. If habitat choice becomes a character trait, behavioral divergence will lead to allopatric reproduction. If allochrony in reproduction exists, temporal divergence will precede morphological divergence. The two forms may physically share a spawning site and still be reproductively isolated.

This hypothesis could be tested by comparing genetic relatedness among populations within Lake Superior. The allopatric/allochronic reproduction model predicts that the genetic relatedness of diverging populations will be high. Genetic distance and sequence divergence estimates based on mtDNA clonal haplotypes among leans, siscowets, and humpers will correspond to time since differentiation less than 8000 years ago. Populations of leans, siscowets, and humpers in Lake Superior will share more recently-derived mtDNA clones with each other than with populations outside Lake Superior. Sequence divergence estimates from complex restriction phenotypes will be lower between siscowet and lean populations inside of Lake Superior than between siscowet populations from inside and lean populations from outside of Lake Superior. Parapatric patterns of speciation may be similar to the patterns which result from allopatric divergence followed by secondary contact (Endler 1977). The absence of populations outside Lake Superior that are closely related to siscowets and humpers would be evidence that divergence was intralacustrine and not allopatric.

Two somewhat radical hypotheses have been proposed to model ecological divergence. Rosenzweig's model of competitive speciation (1978) proposes divergence through phenotypic selection in a complex adaptive landscape (sensu Wright 1940a). West-Eberhard's model of alternative adaptations (1986) proposes that differences among sympatric populations originate as ecophenotypic alternatives which can pre-adapt sibling species for coexistence in sympatry. Divergence is reinforced as genetic differences accumulate in developmental or behavioral mechanisms.

Competitive speciation, in contrast to "species competition" of MacArthur and Levins (1967), generates new species rather than eliminating an established species (Rosenzweig 1978). Competition occurs among phenotypic variants in terms of fitness value and density. As a phenotype increases in density, intermediates representing the parental form may be less able than the extreme phenotypes to utilize the available environment (Rosenzweig 1978). The increase in density of a phenotype is accompanied by a decrease in its fitness and an increase in the fitness of a similar, but slightly different phenotype. The similar phenotype at low density will be able to exploit a different niche, and will increase its own fitness and density. The fitness of some intermediate phenotypes could be low enough at low density that "gaps" will form in the adaptive landscape, and divergence between competing phenotypes will become more rapid (Rosenzweig 1978). The competitive speciation hypothesis provides a means for genetic divergence to occur in response to environmental pressure on survival of phenotypes. Similar, but not identical phenotypes, and corresponding genotypes, compete for survival by responding to density and fitness of neighboring phenotypes, as well as to resource fluctuations. The success of a population depends upon its response to resource fluctuations.

Parapatric speciation of siscowets and humpers from an ancestral lean population could occur as a result of spatial and temporal differences in spawning time that have developed as a function of habitat choice. The ancestral colonist of Lake Superior would have entered a completely unexploited environment. Shallow water niches were probably occupied first because of their similarity to habitats in glacial refugia. Established populations would compete with additional colonists and populations would expand into deeper water habitat. Homing tendencies of the lake trout would guarantee that most of the local population would return for the next spawning season. In the deep waters of Lake Superior, temperature is stable and cold, and light penetration is low. Thermal and visual cues normally associated with the onset of lake trout spawning behavior are severely reduced or absent, and olfactory, lateral line, or tactile cues may

predominate (N.R. Foster, pers. comm.). If photoperiod contributes to the timing of spawning, the onset of spawning for deepwater-adapted fish may occur at different times than for shallow water fish, or spawning period may be prolonged. Even slight differences in spawning onset and duration may prevent the related populations from interbreeding. Differences in resource fluctuations in shallow and deep water could affect the survival of alternate phenotypes and lead to disjunct distributions in juveniles or adults. Survival of phenotypes will be subject to density dependent selection.

Parapatric divergence can also occur if natural selection acts differentially on polymorphic traits. Alternative adaptations (West-Eberhard 1986) can be thought of as different evolved phenotypes occurring in the same lifestage and population, but not simultaneously in the same individual. A novel trait that arises may be a naturally evoked phenotype that becomes stable. Phenotypic divergence is followed by the accumulation of genetic differences in the form of modifier genes added later. The effects of modifier genes may act in a form of "genetic switch" mechanism to allow alternative adaptations to persist simultaneously as a stable polymorphism. The accumulation of modifier genes is expressed in developmental, behavioral, or metabolic differences which reinforce divergence and ultimately lead to reproductive isolation. The modifier genes are the means by which different phenotypes acquire enhanced survival. In contrast to competitive speciation, the survival of populations modeled by the alternative adaptation hypothesis depends upon the phenotype responses to abiotic environmental cues.

Adaptation of lake trout to deep water may have resulted in selection for traits favoring buoyancy, thermal adaptation, or energy conservation. A fish possessing these traits may outcompete a fish which must expend energy to stay in deep water. The deep water form can exploit shallow water if buoyant lift is provided by lipids rather than active inflation of the swim bladder (Gee 1984). Once in shallow water it must compete with the alternate morphological form. The expression of alternative phenotypes may be governed by a small number of

regulatory genes and different morphotypes may appear to show little, if any, genetic divergence. Random mutations among developmental regulatory genes will evolve and be transmitted to successive generations resulting in genotypic as well as phenotypic divergence. The expected amount of intrinsic genetic divergence in Lake Superior lake trout is further reduced by the geologically young age of this ecosystem.

Speciation is likely to occur among expanding (or colonizing) populations if the overall habitat is diverse enough relative to the natural history of the species to permit more than one efficient means of exploiting the environment (Kohn and Orians 1962). These two models of parapatric speciation assume that divergence occurs according to environmental pressure on phenotype, and genotypic divergence is secondary. Variations in character traits are initially ecological, their expression determined by density dependent fluctuations or facultative regulatory mechanisms. Geographic variation in the ratio of phenotypic alternatives varies according to the geographic variation in the suitability of those alternatives (West-Eberhard 1986). New characters that arise in a population influenced by selection on polymorphic traits originate through strong selection in the absence of reproductive isolation.

The ability to detect evidence of allopatric or parapatric speciation among morphologically divergent populations of lake trout depends upon the ability to detect discontinuities in the distribution of mtDNA genotypes. Allopatric divergence among freshwater fishes is supported by genetic discontinuities corresponding to known geological or geographic barriers (Avise et al. 1981, Bernatchez et al. 1989). Detecting parapatric patterns of speciation will depend upon the ability to detect patterns of dispersal and genetic continuity. Genetic discontinuities may be undetectable if parapatric divergence is occurring. Evidence for reproductive isolation will be found in differences in physiological or behavioral mechanisms which limit contact between populations during reproduction.

Discussion

Species-level divergence of populations has generally been attributed to allopatric mechanisms in preference to proposed parapatric patterns. In some cases, trait divergence is clearly evident in the absence of strict allopatry (*Drosophila* sp., Thoday and Gibson 1962; Lake Baikal sculpins, Kohzov 1963; *Rhagoletis* sp., Bush 1969; Lake Nabugabo cichlid fish, Greenwood 1984b, Great Lakes ciscoes, Smith and Todd 1984). Assortative mating of *Salvelinus namaycush* siscowet and lean forms is documented as far back as 1872 (Milner 1873). There is no doubt that morphological differences are present among lake trout populations in Lake Superior. Restriction fragment length and restriction site analysis of mitochondrial DNA is generally adequate for elucidating historical relationships from sequence variation among closely related taxa (Nei and Li 1979, Nei and Tajima 1985, Avise et al. 1987, Maoris et al. 1987). However, mtDNA differences were insufficient for discriminating among lean, siscowet, and humper lake trout.

The Null Hypothesis

Divergence of lean, siscowet, and humper lake trout could not have occurred as a result of isolation in glacial refugia. There is no evidence that discontinuous geographic distribution among numerous glacial refugia has resulted in genetic discontinuities or reproductive isolation. All evidence indicates that only the lean form now exists outside of the Great Lakes basin. Lean lake trout inhabit some of the Canadian Great Lakes and deeper cold lakes in the northern United States. Morphological diversity among lake trout populations that reflects the morphological diversity present in Lake Superior lake trout should exist in other lakes with appropriate deepwater habitat. There is no known geographic differentiation in morphology among lean lake trout throughout its distribution. The wide distribution of mtDNA genotypes and the lack of fixed differences corresponding to phenotypic or geographic segregation of lake trout supports the

rejection of the null hypothesis of allopatric divergence. If allopatric divergence occurred prior to the invasion of Lake Superior by lake trout, there would be a closer relationship between the siscowet and leans from outside the basin based on distribution of mtDNA clonal haplotypes. In contrast, divergence within the lake after invasion would be supported by a closer relationship between siscowets and leans within the Superior basin than between siscowets and leans from outside the Superior basin. Allopatric divergence in glacial refugia is not an acceptable model for divergence among lake trout populations.

Alternative Hypothesis: Divergence due to Water Level Fluctuations

Water level fluctuations are unlikely to have caused the observed differentiation of siscowets from leans within the Lake Superior basin. There is no evidence that the lake trout that exist outside of Lake Superior are derived from the lake trout within Lake Superior. Fossil evidence (Lindsey 1964) and geological evidence (Prest 1970) combined with known lake trout distribution suggests the contrary--lake trout in Lake Superior were derived from populations in glacial refugia. Differences could have arisen between individual local populations (either lean or siscowet) if they were forced into deep basins, but siscowets would have had to be differentiated from leans prior to water level fluctuation. A pattern of diversity in the mtDNA genome allowing discrimination of adjacent populations should have resulted from vicariant patterns of divergence if reproductive isolation were the result of segregation during low water levels. Lean, siscowet, and humper populations in a particular geographic area according to the water level fluctuation model would have appeared as monophyletic groups whose sister taxa were monophyletic groups of geographic equivalents from another area of the lake. Evidence of divergence due to water level fluctuations was seen among the cichlids of Lake Nabugabo and Lake Victoria (Fryer and Iles 1972), some sculpin species in Lake Baikal (Kohzov 1963, Smith and Todd 1984), and was also implicated in the reduced genome diversity in populations of *Coregonus clupeaformis* in northeastern Canadian lakes (Bernatchez et al. 1989).

Reduced genetic diversity has been documented among populations of lake trout stocked into the Great Lakes from a small number of hatchery brood stock (Dehring et al. 1981, Ihssen et al. 1988, Evans and Willox 1991). In contrast, the genetic diversity and pattern of phenotypic divergence among Lake Superior lake trout was not consistent with the pattern of water level fluctuations during the Pleistocene glaciation.

Alternative Hypothesis: Parapatric Speciation

There is unambiguous evidence that lean and siscowet lake trout in Lake Superior are genetically segregated. In contrast, the mtDNA genomes of lake trout in Lake Superior are highly diverse and show no correspondence to lean, siscowet, or humper phenotypes. Physiological divergence in patterns of fat storage suggest that leans and siscowets have different life history adaptations (Eschmeyer and Phillips 1965), but morphometrics are unable to discriminate lean, siscowet, and humper lake trout in the wild.

Density-dependent survivorship has been estimated for lake trout in some Canadian lakes (Matuszek et al. 1990). Lake trout demonstrated increased growth rates and decreased levels of cannibalism as populations of forage fish, the cisco (*Coregonus artedii*), increased. The density dependent response of lake trout combined with opportunities for colonization in the post-glacial Great Lakes provides the potential for competitive speciation. Colonization of unoccupied habitats in the Laurentian Basin occurred repeatedly as glaciers advanced and receded and lake levels fluctuated. Density-dependent pressures altered the fitness of phenotypes exploiting resources at certain times and localities. Shallow and deepwater populations could competitively depress the fitness of populations which attempt to exploit intermediate habitat. Behavioral and physiological differences related to habitat choice and homing to spawning grounds are especially vulnerable to divergence under the competitive speciation model. Smith and Todd (1984) suggest that some Great Lakes ciscoes may have diverged by competitive speciation. Density-dependent fluctuations in growth rate were

extended to represent density-dependent population pressures which may contribute to divergence in spawning times among cisco populations.

A sample of the mtDNA genome of lake trout phenotypes from Lake Superior indicates that there is no substantial genetic divergence, and that lake trout populations may be sharing the same mtDNA gene pool. While this does not conclusively eliminate a genetic component for observed morphological differences, it does suggest that the differences are not exhibited in the mtDNA genome. The lack of mtDNA divergence could be attributed to limited time and incomplete reproductive isolation. Contiguous spawning grounds, especially those located over steep gradient spawning banks, provide ample opportunities for hybridization to occur in zones of contact (Pratt and King 1980). Wright's Island Model showed that it only requires migration of a small percentage of the effective population size to break down genetic segregation (Wright 1931, 1969).

Genetic divergence among modifier or regulatory genes, as modeled in the alternative adaptation hypothesis (West-Eberhard 1986), is especially favorable for parapatric speciation (Endler 1977) because covariant character sets evolve semi-independently (West-Eberhard 1986). The coordinated expression of a set of genes is governed by regulatory or modifier genes. Different physiological (fat storage) or developmental (growth) characteristics can evolve simultaneously within the same genome, without complete reproductive isolation. Siscowets grow more slowly in the wild than leans. Lean lake trout 5 years of age averaged 14-18 inches (340-410 mm) (Cable 1956), while siscowets 5 years of age averaged only 9-14 inches (220-340 mm) (Pratt and King 1980). In contrast, siscowet offspring raised under identical conditions as lean offspring were 25% larger at the same age (Stauffer and Peck 1981; Chapter 2). This may argue in favor of environmental influence on morphological divergence, but the fat content of the siscowet offspring under controlled conditions was still higher and non-overlapping with the fat content of lean offspring. The phenotype of an organism is the result of genetic and environmental influences as well as the interaction between genes and environment. Growth characteristics may change with environment, but the

physiological differences in fat storage between lean and siscowet lake trout are genetically based.

The effect of environment on phenotype is a familiar topic in organismal biology (see review by West-Eberhard 1989 and references therein). Environmentally-induced modifications are referred to as phenotypic plasticity -- the ability of a single genotype to produce more than one phenotype in response to environmental conditions, i.e. alternative morphologies, physiological states, or behaviors (West-Eberhard 1989, Wimberger 1991). Environmentally-induced traits were often thought to be undesirable elements in organismal biology when evolutionary scientists concentrated their efforts on the more conservative aspects of biological processes such as canalization, developmental constraints, stabilizing selection, and balancing selection (Schmalhausen 1949, Mayr 1963, Waddington 1975). This school of thought argued that selection was more effective in the absence of phenotypic plasticity and that the effects of plasticity slowed the processes of evolutionary change (Grant 1977, Falconer 1981). The concepts of morphological stasis and developmental canalization (Waddington 1975, Wake et al. 1983) supported the idea that non-morphological plasticity can contribute to morphological stasis in the presence of environmental perturbations (West-Eberhard 1989).

One trait common to these examples of trophic polymorphisms in fishes is that the induced morphological differences, while often bimodal, do not necessarily follow strict patterns of inheritance. For example, from Sage and Selander's (1975) seminal study on *Cichlasoma minckleyi*, the differences in pharyngeal jaw morphology were shown to correspond to individual size and food types, not to parental phenotype (Kornfield and Taylor 1983). In Wimberger's (1991) study of jaw morphology of *Geophagus*, different species fed on two food types showed similar patterns of change corresponding to diet in the shape of jaw bones involved in food handling and mastication. Fat content analyses (Eschmeyer and Phillips 1965) and hatchery breeding studies (Eschmeyer and Phillips 1965, Stauffer and Peck 1981) have demonstrated that genetic differences

between lean and siscowet lake trout are heritable and not plastic. Intermediate fat content of lean x siscowet hybrid offspring provide further evidence of genetic transmission of physiological differences.

The family Salmonidae contains representatives in recently glaciated lakes that show a wide array of morphological, behavioral, and ecological adaptations (Behnke 1972). Sympatric populations of members of the subfamilies Salmoninae and Coregoninae are common in freshwater lakes in North America and Eurasia. The most widely recognized system is the arctic charr complex (*Salvelinus alpinus*). Four different forms of arctic charr are recognized, each of which has a unique ecological adaptation resulting in extrinsic reproductive isolation (Svardson 1949-1957, Frost 1965, Johsson and Hindar 1982, Behnke 1984, Skulason et al. 1989) with little or no genetic divergence (Kornfield et al. 1981, Hindar et al. 1986, Magnusson and Ferguson 1987). The ecological adaptation to shallow versus deepwater environments is not limited to salmonid fishes. Sculpins (Cottidae) in Lake Baikal also have shallow/deepwater morphological adaptations (Stankovic 1960, Kohzov 1963, Smith and Todd 1984). Morphological diversity in the absence of genetic diversity is also a characteristic of Atlantic herring (*Clupea harengus*)(Ryman et al. 1984). All of these systems show patterns that have been interpreted as evidence of allopatric divergence followed by secondary contact (Svardson, 1949-1957, Mayr 1963), while others believe that the diversity in sympatry has occurred in post-glacial times in response to water level fluctuations and ecological adaptations (Behnke 1972, Smith and Todd 1984, Hindar et al. 1986). The common feature of all of these systems is the presence of ecological rather than geographic isolation contributing barriers to gene flow. Parapatric divergence must be considered as a model for non-allopatric speciation patterns which are not consistent with strict allopatric models.

The Hybrid Origin of the Humper Phenotype

The distinctness of the humper population was supported by its unique morphological and growth characteristics (Rahrer 1965). Observed morphological

characteristics of the humper phenotype could be the result of ontogenetic intermediacy, retained plesiomorphic intermediacy, hybridization and introgression, or ecophenotypic intermediacy (Smith 1992). Ontogenetic intermediacy can be eliminated because there are no trends corresponding to age structure in mtDNA RFLP or morphological data sets for lean, siscowet, or humper phenotypes. The humper phenotype cannot be the result of retained plesiomorphic intermediacy because there has been no evidence that morphologically divergent or intermediate populations of lake trout existed outside of Lake Superior (Patriarche and Peck 1970, Peck 1975). The "Rush Lake trout," *Salvelinus namaycush huronicus*, of Hubbs and Lagler (1941) has morphological and osteological characteristics of the lean lake trout and does not resemble the humper phenotype. Hybridization combined with ecophenotypic adaptation to offshore shoals could have led to the evolution of self-sustaining stocks of humper lake trout.

Humpers exhibit intermediacy in osteological characters useful in discriminating lean and siscowet lake trout. While all humpers examined possess the opercular notch characteristic of siscowets, the supraethmoid has a high frequency of conflicting characteristics (Chapter 3). Humpers possess the same mtDNA diversity shared by leans and siscowets, evidence that all three populations have experienced some contemporary hybridization (Chapter 4). However, humpers are known to spawn in early August while sympatric lean and siscowet populations are still maturing (personal observations), and humpers are known to mature at smaller sizes and show slower growth (Rahrer 1965). Humpers live over isolated deepwater shoals in Lake Superior, surrounded by water greater than 100 meters deep. They do not stray far from these shoals either to forage or to spawn (Thurston 1962, Rahrer 1965, Dehring et al. 1981).

Hybridization and introgression between historical lean and siscowet populations as water levels fluctuated could have led to the evolution of a separate lake trout stock in Lake Superior. Leans typically inhabit inshore areas, and siscowets typically inhabit offshore areas. Populations of lake trout could

have colonized the various habitats in early Lake Superior. During water level fluctuations after the last glacial retreat, water levels could have dropped enough to bring leans and siscowets into closer proximity by eliminating some deepwater habitat (Prest 1970, Farrand 1969). Deepwater shoals could have been used by siscowet lake trout and became an area of contact between lean and siscowet populations during low water levels. As water levels rose again, residual stocks may have remained over the shoals, and founded new populations.

The lack of intermediate lake trout phenotypes in near-shore areas may be explained with a model of gene flow patterns (Figure 6.3). In near-shore areas, lean populations are in great abundance, although there are some areas of contact with offshore siscowets. Hybridization would likely result in a very low net flow of "siscowet" genes into the lean genome, so intermediates would be rare. Over offshore shoals, lean habitat is restricted, and shoals are surrounded by the deepwater siscowets. If hybridization occurs over these shoals, the net flow of genes would be much greater from the "siscowet" genome, and many more intermediates would be produced than in the near-shore example. Assuming that the hybrids are able to reproduce, a viable population could be sustained over the offshore shoals. Since morphological characters have been shown to be heritable (Stauffer and Peck 1981), the intermediate characteristics of the lean x siscowet hybrid parents would be transmitted to hybrid x hybrid offspring in subsequent generations. Low resource availability combined with the cold temperatures of the deep water in Lake Superior could be contributing factors to the humpers' low growth rate and small size at maturity. Geological evidence and the intermediate morphological, physiological, and genetic characteristics of humper lake trout make the hypothesis of hybrid origin combined with ecophenotypic adaptations a plausible model.

Conclusions

Lack of fixed genetic differences in a hypervariable mtDNA genome among Lake Superior *Salvelinus namaycush* populations is not consistent with an hypothesis of allopatric divergence in glacial refugia. The siscowet phenotype is endemic to Lake Superior and is not found in any other deep cold lakes throughout the lake trout's distribution. Divergence in allopatry as a result of lake level fluctuations is a plausible alternative, and may adequately explain the origin of the humper phenotype. However, there is no evidence of a reduction in the mtDNA diversity that corresponds to isolation in major lake basins. Competitive speciation may have led to divergence of leans and siscowets through density-dependent population responses to resource fluctuations in shallow versus deep water (Rosenzweig 1978). Evolution of modifier or regulatory genes governing the expression of ecophenotypic alternatives is a plausible model for divergence also. Alternative adaptations (West-Eberhard 1986) to temperature or depth differences could lead to divergence among genes controlling physiological or developmental processes.

The hybrid origin of the humper phenotype is hypothesized as the result of introgression of the siscowet genome into the genome of lean populations isolated over deepwater shoals during glacial periods of water level fluctuations. The intermediate morphology, osteology, physiology, and genetic profile of humper lake trout support a hybrid origin. Unique features of the humper phenotype such as the thin abdominal wall, smaller size at maturity, and August spawning suggest that the humper populations have been reproductively segregated from lean and siscowet populations since water levels stabilized.

The lack of differentiated lake trout phenotypes in other deep cold lakes in North America with suitable habitat suggests that divergence of leans, siscowets, and humpers has occurred within the Lake Superior basin. Similar hypotheses of intralacustrine divergence through ecophenotypic differentiation have been postulated for Lake Baikal sculpins and Great Lakes ciscoes. Most of the

morphological differentiation has apparently been retained despite gene flow. Differences develop in time and place of spawning as a result of populations' different responses to resource fluctuations. Physiological and behavioral differences evolve through adaptations to different depths, temperatures, and climatic changes. Homing characteristics of salmonids further enhance divergence leading to extrinsic reproductive isolation. Populations of *S. namaycush* in Lake Superior are isolated by spatial and temporal differences in spawning, but genetic divergence is subtle and does not represent species level divergence.

Figure 6.1. Hypothesis of divergence of lean and siscowet *Salvelinus namaycush* by differentiation in allopatry. T1-T5 represent relative time periods. (a) Original lake trout colonists invade the Great Lakes basin from various glacial refugia and disperse via proglacial lakes; (b) A cold water-adapted form evolves in glacial Lake Duluth (S) and original colonist form evolves in lower basins (L); (c) Lower lake levels enhance divergence in isolation; (d) Rising lake levels bring S and L phenotypes into secondary contact; (e) Current pattern of lean (L) and siscowet (S) distribution. Since the 1960's, native lake trout have been extinct in all of the Great Lakes except Lake Superior, which also contains populations of siscowet lake trout.
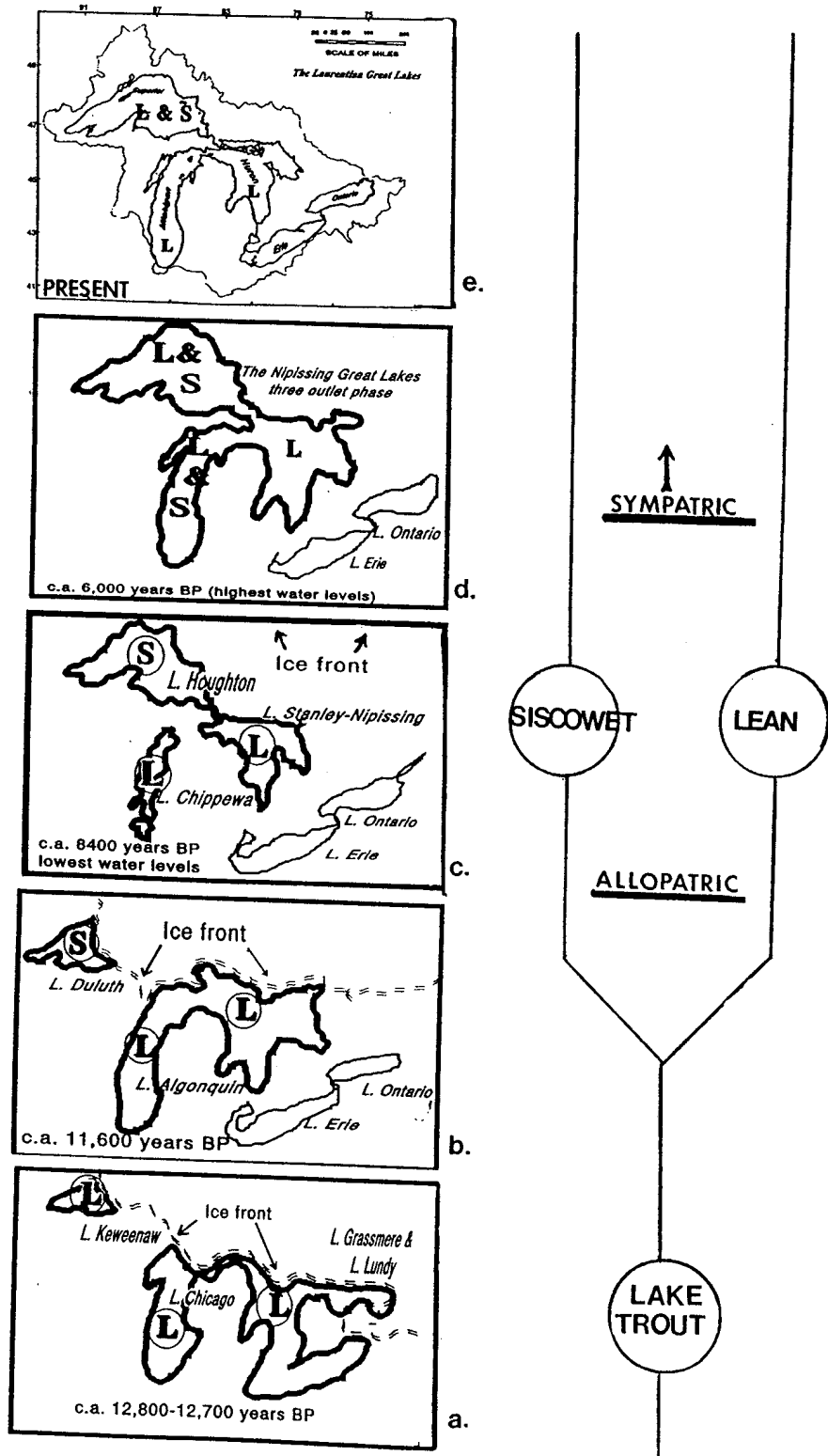
Figure 6.1.

Figure 6.2. Hypothesis of divergence of lean and siscowet *S. namaycush* as a result of lake level fluctuations in Lake Superior. T1-T5 represent relative time periods. (a) Original lake trout colonists occupy near-shore habitat; (b) Lowering lake levels force lake trout populations into formerly deeper water and expose previously submerged shoals; (c) Lake levels rise and original lean populations (L) move back to inshore habitat, siscowets (S) begin to diverge in populations which have dispersed over shoals and into deep water; (d) Lake levels lower slightly and bring differentiated populations into secondary contact; (e) Leans and siscowets occupy contiguous or overlapping habitat and are segregated by time and place of spawning but not by intrinsic genetic differences.

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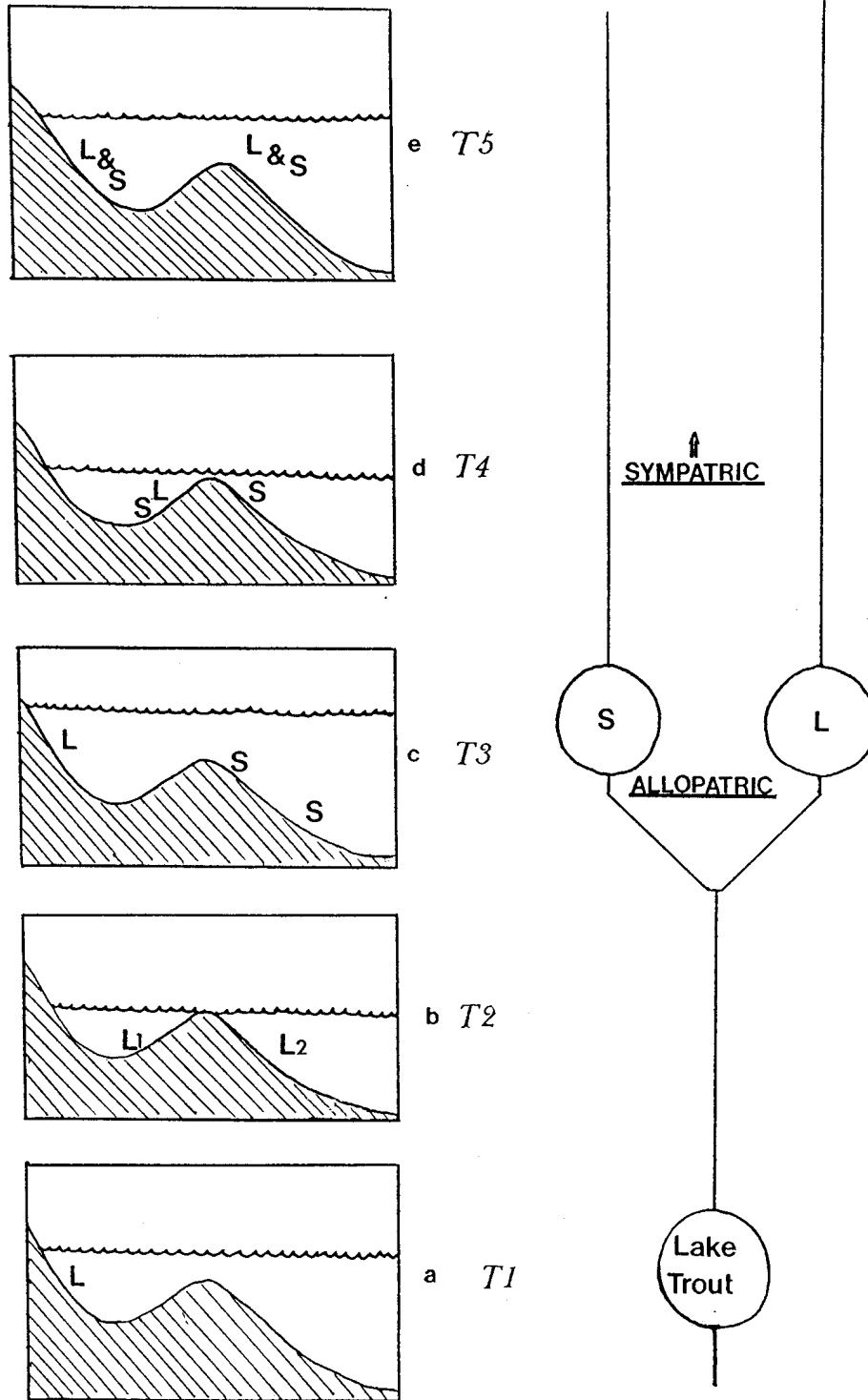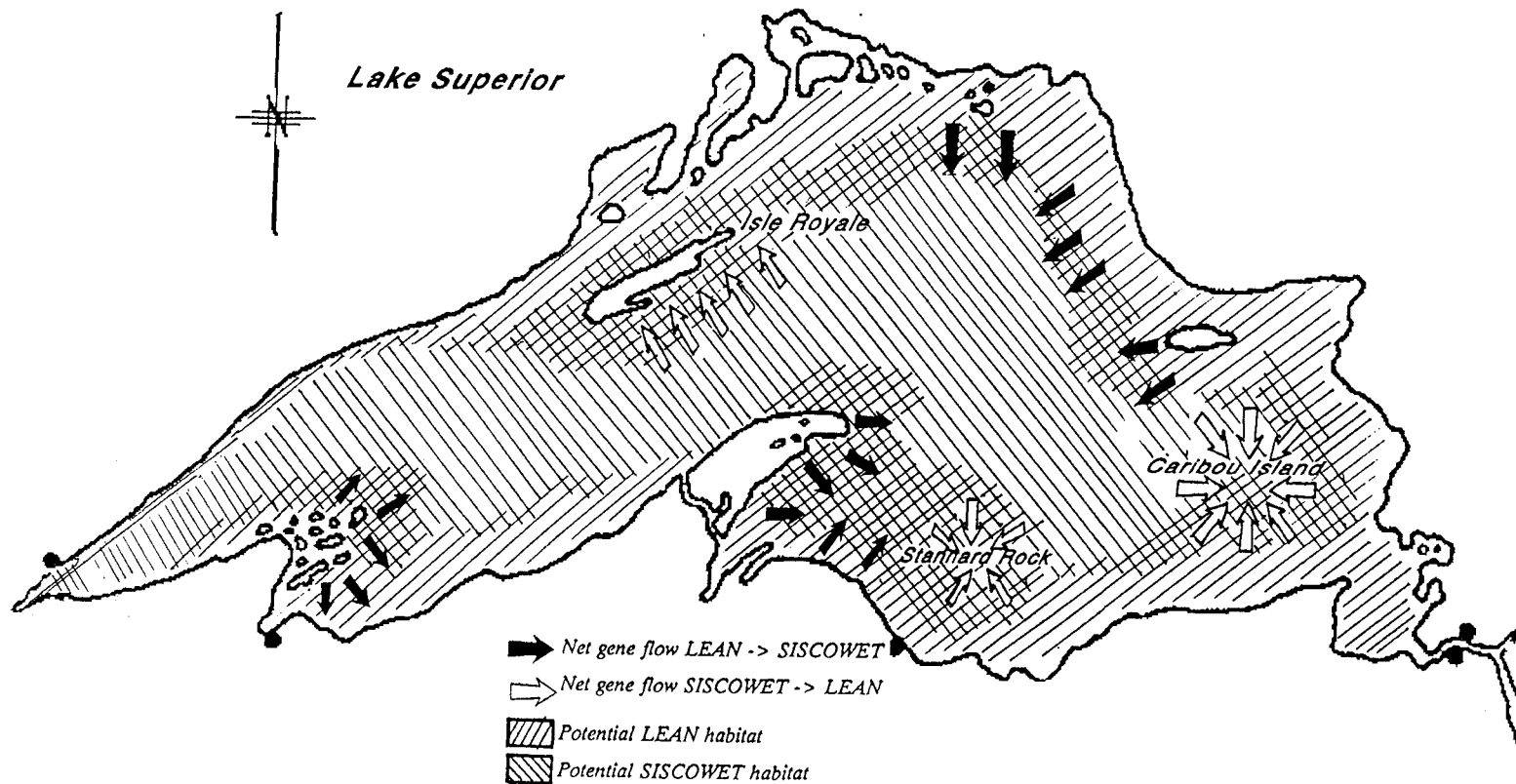
Figure 6.2.

Figure 6.3. Hypothesis of origin of hybrid populations from which the humper lake trout phenotype may be descended. Areas with cross-hatching indicate probable contact zones between lean and siscowet populations. Dark arrows in inshore areas indicate locations where gene flow from lean populations would be great enough to prevent establishment of intermediate populations. Open arrows indicate locations where siscowet gene flow may overwhelm lean gene flow and allow establishment of intermediates. Suitable habitat for humper lake trout populations is found in the areas around Isle Royale, Caribou Island, and Stannard Rock Reef.

APPENDICES

APPENDIX A: Protocol for Restriction Enzyme Length Polymorphism Analysis of Vertebrate Mitochondrial DNA With [32]P End-Labelling

Preparation of Mitochondrial DNA from Animal Tissue

Preparation of a mitochondrially-enriched fraction of a tissue homogenate was conducted using a modified procedure of Lansman et al. (1981) and Maniatis et al. (1982). Animals were sacrificed in the field and liver tissue was removed and placed on wet ice for no more than 10 days or flash frozen with liquid nitrogen when available. Greater yields of mitochondria were achieved by keeping tissues on wet ice for at least 2 days before processing or freezing at - 70°C. This seemed to weaken the cell membranes and enhance release of mitochondria during homogenization. Tissues which were flash frozen in liquid nitrogen provided the cleanest mtDNA fractions. In most siscowet samples the lipid content of the homogenate appeared to be much greater than comparable volumes of lean or humper tissue. Samples with high lipid content in the homogenate yielded less purified mitochondrial DNA after ultracentrifugation and were often contaminated with nuclear background when analyzed with ^{32}P end-labeling and electrophoresis.

Between 0.5 and 1.0-grams of tissue per sample were homogenized in 20-ml of cold homogenizing buffer (200 mM EDTA, 10 mM Tris, 10 mM NaCl in a 5:1 ratio with 1.5 M sucrose in 100 mM EDTA, 10 mM tris (TE)). Tissues were ground with a Tissuemizer in 10-ml of cold buffer for two 10-second bursts. The grinding unit and grinding vessel were then rinsed in the remaining 10-ml of buffer solution which was added to the homogenate to a balanced volume of about 25 ml. Homogenates were then centrifuged in a Beckman JA-17 high speed centrifuge at 1200 x G (3000 rpm) for 5 minutes to pellet nuclei and large debris. High concentration of nuclei and lipids in lake trout liver tissue required that two of these slow speed spins be performed in order to remove a significant amount of nuclear material and cellular debris. The supernatant was saved and

centrifuged at 23,000 X G (14,000 rpm) in a Beckman JA-17 rotor at 4°C for 20 minutes to pellet mitochondria and small debris.

The supernatant from the high speed spin was discarded and the pellet resuspended in 1-ml of buffer (100 mM EDTA, 10 mM tris, 10 mM NaCl) at room temperature. To this 0.125-ml of 20% SDS (20% sodium dodecyl sulfate in distilled water) was added to lyse the mitochondrial membrane and release DNA. The sample was mixed gently and let stand for 15-20 minutes at room temperature (about 23° C). Finally, 0.187-ml of cesium chloride-saturated distilled water was added to each sample to weight down remaining cellular debris. The samples were placed at 4°C overnight then centrifuged in a Beckman JA-17 rotor at 4°C at 17,000 X G (12,000 rpm) for 20 minutes. The supernatant was transferred to a sterile culture tube. To reduce significant nuclear background after end-labelling, samples were run through a 23-gauge hypodermic needle to break up large pieces of nuclear DNA. The samples were then prepared for ultracentrifugation.

Equilibrium Density Centrifugation

The density of all samples was adjusted to 1.4 gram/ml by adding a predetermined amount of solid cesium chloride (CsCl) based on the volume of the solution after the addition of 0.23-ml of propidium iodide (PI) intercalating dye (5.0 g/ml). The density-adjusted samples were transferred to labelled 3.8-ml Seton ultracentrifuge tubes and underlayered with the appropriate volume of 1.7 g/ml density PI in TE. The appropriate volume was calculated to result in a final density of 1.55 g/ml after ultracentrifugation by multiplying the final volume of density adjusted sample by 1.33. Samples were then overlayered with light mineral oil and balanced to within 0.02 g.

Samples were centrifuged in an SW60Ti rotor at 36,000 rpm for 24 - 36 hours at 21°C in a Beckman ultracentrifuge. Samples were viewed under long-wave ultraviolet light to fluoresce the dye in the nuclear and mitochondrial

fractions. The mitochondrial band was collected with about 100 microliters of the nuclear band.

The best purification results were obtained by performing a velocitization on the mtDNA sample collected from the first banding. Velocitization results in a concentration of mitochondrial and nuclear DNA at the bottom of the sample and the exclusion of most of the buoyant proteins. Each sample volume was measured and an equivalent volume of TE was added to each sample. A volume of 1.4 g/ml CsCl and PI in TE was added to each ultracentrifuge tube according to the following calculation:

v 1.4 g/ml CsCl = 3.8 ml - ((2 x sample volume) + 0.7 ml)

where 3.8 ml is the maximum volume of the ultracentrifuge tube. A 0.7-ml sample of 1.7 g/ml CsCl and PI in TE was carefully underlayered in each ultracentrifuge tube containing the predetermined volume of 1.4 g/ml CsCL and PI in TE. The diluted mtDNA sample from the first banding was carefully overlayered into each ultracentrifuge tube and the tubes were balanced with light mineral oil. The velocitization samples were centrifuged in the SW60Ti rotor at 45000 rpm (175K x G) for 3.5 hours, 21°C, with no brake during deceleration.

After velocitization, the bottom 1.5-ml of each ultracentrifuge tube was collected by puncturing the bottom of the tube with a hypodermic needle. This step does not have to be performed under UV illumination. The sample should be at a density of 1.5 g/ml after the velocitization step.

The mitochondrial fraction was rebanded after addition of the sample to a labelled Seton ultracentrifuge tube containing 1.0-ml of 1.5 g/ml PI in TE. The samples were balanced with light mineral oil and centrifuged in an SW60Ti rotor for 24-36 hours at 36,000 rpm (145K x G) The purified mitochondrial band was collected under ultraviolet light and stored at 4°C in the dark until dialysis.

Propidium Iodide Extraction and Dialysis

Sample tubes were filled with isopropyl alcohol (prepared with CsCl-saturated distilled water) to extract the intercalating dye and free lipids. The tubes were mixed and allowed to stand. The upper layer of isopropyl alcohol containing some of the dye was removed. This was repeated 3-5 times or until all of the PI appeared to have been removed.

Sterile dialysis tubing (stored in 50% EtOH) was placed in 1 X TE (100 mM EDTA, 10 mM Tris) until needed. Individual samples were transferred to dialysis tubing and sealed with dialysis clips. Samples were dialyzed against a 1 X TE buffer at room temperature for about an hour, 1/2 X TE at room temperature for about an hour (2 changes), and 1/2 X TE at 4°C overnight. The dialysis buffer was changed 2 - 3 additional times and samples were kept in the dark until they were transferred to sterile cryogenic tubes and stored at -20°C.

Restriction Endonuclease Digestion

Mitochondrial DNA samples were digested and stored as soon after dialysis as possible. Thawing and re-freezing samples was avoided, especially for siscowet samples, because degradation of the sample DNA prior to electrophoresis appeared to be rapid. Manufacturer's buffer stock solutions (New England BioLabs, Beverly, MA) were used for specific restriction endonucleases. Sample volume for digestion varied from 5 - 15 microliters, depending upon DNA concentration. About 5 microliters of restriction enzyme mix was added to each sample. Digest buffer volume was calculated to provide a final 1 X concentration. Enzyme volume was calculated to allow about 2-4 units of enzyme per sample based on previous results (some enzymes required a slightly higher concentration for complete digestion). Sterile water was added to dilute the reaction mix to the desired volume and concentration. Samples were incubated according to manufacturer's instructions, most being at 37°C for 4-6 hours. Some enzymes (Taq I, Sal I) were incubated at 65°C for optimal activity.

Samples were removed from the incubator at the appropriate time and stored at -20°C until end-labelling and electrophoresis was performed.

^{32}P End-Labelling of Restriction Endonuclease Fragments

Radioactive end-labelling mix was prepared similar to the digest mix. About 5 microliters of prepared label mix were added to each sample. The label buffer stock (6 mM KCl, 10 mM Tris, 10 mM MgCl$_2$, 7 mM beta-mercaptoethanol) was added to the labelling mix to a final 1 X concentration. For each gel set, about 1-2 units of the large fragment (Klenow) DNA polymerase I was used. The volume of ^{32}P labelled deoxyribonucleotides (dATP, dTTP, dGTP, dCTP) was adjusted according to activity of nucleotides, averaging 0.5 microliters per gel. Fragments produced with 5' overhanging ends were labelled at room temperature for 25-30 minutes. Fragments produced with 3' overhangs or blunt ends were labelled at 37°C for 25-30 minutes.

About 1/5 volume of blucose dye (blue glucose dye in 5 X TBE) was added to each sample to prevent DNA polymerase I from chewing back the ends of fragments. The samples were then vortexed and centrifuged. Half of sample was applied to an agarose gel and half to an acrylamide gel of chosen concentration. Concentrations varied according to the products of the restriction enzyme digest. Hexameric and pentameric enzymes often produced fragments with lengths greater than 1000 bp which could be accurately resolved on 0.8-1.2 % agarose gels. Products of tetrameric enzyme digests were often less than 1000 bp and had to be resolved on 3.5-4.0 % polyacrylamide gels. The higher concentrations of each gel matrix allowed DNA fragments to migrate more slowly so that smaller pieces could be clearly resolved and measured. Lower gel matrix concentrations allowed fragments to migrate more freely so that larger fragments could be easily resolved. Labelled size standards were run with samples in each gel. The size standards used were labelled Hind III cut Lambda DNA and Hae III cut Phi-X DNA. A 1 X concentration of TBE pH 8.3 (8.9 mM tris, 10 mM boric acid, 1.1 mM EDTA) was used as the running buffer for electrophoresis.

Agarose gels were run at about 5 mAmps per gel until blue dye was at bottom. Acrylamide gels were run at about 7.5 mAmps per gel until blue dye was 25-27-mm from the origin. Running time varied from 10-24 hours. Agarose gels were not run greater than 40 mAmps and polyacrylamide gels were not run greater than 100 mAmps to prevent degradation within the gel matrix. It was discovered that allowing agarose gels to run at amperage greater than 40 caused blurring of bands as they passed too quickly through the matrix.

After electrophoresis was complete, gels were adhered to 3-mm Whatman chromatography filter paper and dried with heat and vacuum. The dried gel was inserted into an autoradiography cassette with a sheet of Fuji X-ray film and allowed to expose. Labelled fragments were sometimes enhanced by using one or two reflecting screens in cassette. Cassettes with screens were exposed at -70°C for 2 days to 2 weeks, depending upon strength of samples and activity of the radioactive nucleotides. Cassettes without screens were left at room temperature for 4 days to 3 weeks. Because of the tendency of screens to blur adjacent fragments on the autoradiographs, acrylamide gels from tetrameric enzyme digests were exposed at room temperature without screens.

APPENDIX B: Pairwise Sequence Divergence Matrices

Pairwise Sequence Divergence Estimates for Restriction Enzyme Fragment Products:

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
A1	0															
A2	0.001	0														
A3	0.0024	0.0034	0													
A4	0.0014	0.0024	0.0039	0												
A5	0.0034	0.0024	0.0059	0.0048	0											
A6	0.0014	0.0024	0.0039	0.0029	0.0029	0										
A7	0.0014	0.0005	0.0039	0.0029	0.0019	0.0019	0									
A8	0.0029	0.0039	0.0005	0.0044	0.0054	0.0035	0.0035	0								
A9	0.0038	0.0048	0.0063	0.0053	0.0073	0.0053	0.0053	0.0069	0							
A10	0.0048	0.0038	0.0073	0.0062	0.0062	0.0063	0.0043	0.0079	0.0009	0						
A11	0.0053	0.0062	0.0078	0.0038	0.0088	0.0068	0.0068	0.0084	0.0014	0.0023	0					
A12	0.0062	0.0073	0.0089	0.0077	0.0047	0.0058	0.0068	0.0084	0.0023	0.0032	0.0037	0				
A13	0.0053	0.0043	0.0079	0.0068	0.0058	0.0058	0.0038	0.0074	0.0014	0.0005	0.0028	0.0028	0			
A14	0.0014	0.0024	0.0038	0.0028	0.0048	0.0029	0.0029	0.0044	0.0033	0.0042	0.0047	0.0057	0.0047	0		
A15	0.0038	0.0048	0.0063	0.0053	0.0023	0.0033	0.0043	0.0058	0.0057	0.0066	0.0071	0.0032	0.0062	0.0023	0	
A16	0.0048	0.0038	0.0073	0.0062	0.0014	0.0043	0.0033	0.0069	0.0066	0.0057	0.0081	0.0042	0.0052	0.0033	0.0009	0
A17	0.0044	0.0034	0.007	0.0059	0.0039	0.0029	0.0029	0.0066	0.0043	0.0033	0.0058	0.0048	0.0029	0.0048	0.0053	0.0043
A18	0.0024	0.0034	0.0049	0.0038	0.0058	0.0039	0.0039	0.0054	0.0062	0.0073	0.0077	0.0088	0.0078	0.0038	0.0062	0.0073
A19	0.0049	0.0059	0.0024	0.0064	0.0085	0.0065	0.0065	0.0029	0.0089	0.0099	0.0104	0.0115	0.0105	0.0063	0.0089	0.0099
A20	0.0058	0.0048	0.0085	0.0073	0.0024	0.0054	0.0044	0.008	0.0098	0.0088	0.0113	0.0072	0.0083	0.0073	0.0047	0.0038
A21	0.0074	0.0085	0.0049	0.009	0.0058	0.007	0.008	0.0044	0.0115	0.0125	0.013	0.0088	0.0121	0.0089	0.0062	0.0073
A22	0.0039	0.0029	0.0065	0.0054	0.0044	0.0044	0.0024	0.006	0.0078	0.0068	0.0093	0.0093	0.0063	0.0053	0.0068	0.0058
A23	0.0044	0.0054	0.007	0.0029	0.0069	0.0049	0.0049	0.0065	0.0083	0.0093	0.0067	0.0098	0.0089	0.0058	0.0073	0.0083
A24	0.0062	0.0073	0.0089	0.0077	0.0098	0.0078	0.0078	0.0094	0.0023	0.0032	0.0037	0.0046	0.0037	0.0057	0.0081	0.0091
A25	0.0088	0.0098	0.0115	0.0103	0.0072	0.0083	0.0093	0.011	0.0046	0.0056	0.006	0.0023	0.0051	0.0081	0.0056	0.0066
A26	0.0098	0.0088	0.0125	0.0113	0.0062	0.0093	0.0083	0.0121	0.0056	0.0046	0.007	0.0032	0.0042	0.0091	0.0066	0.0056
A27	0.0078	0.0089	0.0105	0.0093	0.0093	0.0063	0.0084	0.01	0.0037	0.0047	0.0051	0.0042	0.0042	0.0072	0.0076	0.0087
A28	0.0078	0.0068	0.0105	0.0093	0.0083	0.0084	0.0063	0.01	0.0037	0.0028	0.0051	0.0051	0.0023	0.0072	0.0087	0.0076
A29	0.0038	0.0048	0.0063	0.0053	0.0073	0.0053	0.0053	0.0069	0.0057	0.0066	0.0071	0.0081	0.0072	0.0023	0.0047	0.0057
A30	0.0048	0.0038	0.0073	0.0062	0.0062	0.0063	0.0043	0.0079	0.0066	0.0057	0.0081	0.0091	0.0062	0.0033	0.0057	0.0047
A31	0.0063	0.0073	0.0038	0.0078	0.0099	0.0079	0.0079	0.0044	0.0082	0.0092	0.0097	0.0107	0.0098	0.0047	0.0072	0.0082
A32	0.0053	0.0062	0.0078	0.0038	0.0088	0.0068	0.0068	0.0084	0.0071	0.0081	0.0056	0.0096	0.0087	0.0037	0.0061	0.0071
A33	0.0073	0.0062	0.0099	0.0088	0.0038	0.0068	0.0058	0.0094	0.0091	0.0081	0.0106	0.0066	0.0076	0.0057	0.0032	0.0023
A34	0.0024	0.0034	0.005	0.0039	0.0059	0.0039	0.0039	0.0055	0.0063	0.0073	0.0078	0.0089	0.0079	0.0038	0.0063	0.0073
A35	0.0034	0.0024	0.006	0.0049	0.0049	0.005	0.0029	0.0066	0.0073	0.0063	0.0089	0.0099	0.0069	0.0048	0.0073	0.0063
A36	0.005	0.006	0.0024	0.0065	0.0086	0.0066	0.0066	0.003	0.009	0.01	0.0105	0.0116	0.0106	0.0064	0.009	0.01
A37	0.0039	0.0049	0.0065	0.0024	0.0074	0.0054	0.0054	0.007	0.0078	0.0089	0.0062	0.0104	0.0094	0.0053	0.0078	0.0089
A38	0.005	0.0039	0.0076	0.0065	0.0044	0.0035	0.0035	0.0071	0.009	0.0079	0.0105	0.0094	0.0074	0.0064	0.0069	0.0058
A39	0.0066	0.0076	0.004	0.0081	0.0081	0.005	0.0071	0.0035	0.0106	0.0117	0.0122	0.0111	0.0112	0.008	0.0085	0.0096
A40	0.0039	0.0029	0.0066	0.0054	0.0044	0.0045	0.0024	0.0061	0.0079	0.0069	0.0094	0.0094	0.0064	0.0054	0.0069	0.0058
A41	0.0073	0.0063	0.01	0.0089	0.0089	0.009	0.0069	0.0106	0.0033	0.0023	0.0047	0.0057	0.0028	0.0067	0.0092	0.0082
A42	0.009	0.01	0.0064	0.0105	0.0127	0.0106	0.0106	0.007	0.0047	0.0057	0.0062	0.0072	0.0062	0.0083	0.0108	0.0119
A43	0.0089	0.0099	0.0116	0.0104	0.0073	0.0084	0.0094	0.0111	0.0047	0.0057	0.0061	0.0023	0.0052	0.0082	0.0057	0.0066
A44	0.0099	0.0089	0.0127	0.0115	0.0062	0.0094	0.0084	0.0122	0.0057	0.0047	0.0071	0.0032	0.0042	0.0092	0.0066	0.0057
A45	0.0069	0.0079	0.0096	0.0084	0.0094	0.0074	0.0074	0.0091	0.0028	0.0038	0.0042	0.0042	0.0033	0.0062	0.0077	0.0088
A46	0.0038	0.0048	0.0064	0.0053	0.0073	0.0054	0.0054	0.007	0.0057	0.0067	0.0072	0.0082	0.0073	0.0023	0.0047	0.0057
A47	0.0073	0.0063	0.01	0.0089	0.0038	0.0069	0.0058	0.0096	0.0092	0.0082	0.0107	0.0066	0.0077	0.0057	0.0033	0.0023
A48	0.007	0.0081	0.0045	0.0086	0.0108	0.0087	0.0087	0.005	0.0069	0.0079	0.0084	0.0094	0.0085	0.0074	0.01	0.0111
A49	0.007	0.006	0.0098	0.0086	0.0065	0.0055	0.0055	0.0093	0.0069	0.0058	0.0084	0.0073	0.0054	0.0074	0.0079	0.0069
A50	0.0044	0.0054	0.007	0.0058	0.0079	0.0059	0.0059	0.0075	0.0083	0.0093	0.0098	0.0108	0.0099	0.0058	0.0083	0.0093
A51	0.0054	0.0044	0.008	0.0069	0.0069	0.007	0.0049	0.0086	0.0093	0.0083	0.0108	0.0119	0.0089	0.0068	0.0093	0.0083
A52	0.007	0.008	0.0044	0.0085	0.0106	0.0086	0.0086	0.005	0.011	0.0121	0.0125	0.0136	0.0127	0.0084	0.011	0.0121
A53	0.0058	0.0069	0.0085	0.0043	0.0094	0.0074	0.0074	0.0091	0.0098	0.0108	0.0082	0.0124	0.0115	0.0073	0.0098	0.0108
A54	0.0079	0.0069	0.0106	0.0094	0.0043	0.0074	0.0064	0.0102	0.0119	0.0108	0.0135	0.0092	0.0104	0.0093	0.0067	0.0057
A55	0.0096	0.0106	0.007	0.0111	0.0079	0.0091	0.0102	0.0065	0.0136	0.0148	0.0152	0.0108	0.0143	0.011	0.0083	0.0093
A56	0.007	0.0059	0.0097	0.0085	0.0064	0.0054	0.0054	0.0092	0.011	0.0099	0.0125	0.0115	0.0094	0.0084	0.0089	0.0078
A57	0.0059	0.0049	0.0086	0.0074	0.0064	0.0065	0.0044	0.0081	0.0099	0.0089	0.0115	0.0115	0.0084	0.0073	0.0089	0.0078
A58	0.0093	0.0083	0.0121	0.0108	0.0108	0.011	0.0089	0.0127	0.0051	0.0042	0.0066	0.0076	0.0047	0.0087	0.0112	0.0101
A59	0.011	0.0121	0.0084	0.0125	0.0148	0.0127	0.0127	0.009	0.0066	0.0076	0.0081	0.0091	0.0082	0.0103	0.0129	0.0139
A60	0.0098	0.0108	0.0125	0.0082	0.0135	0.0115	0.0115	0.0132	0.0056	0.0066	0.0041	0.008	0.0071	0.0091	0.0116	0.0127
A61	0.0108	0.0119	0.0136	0.0124	0.0092	0.0104	0.0115	0.0132	0.0066	0.0076	0.008	0.0041	0.0071	0.0101	0.0076	0.0086
A62	0.0119	0.0108	0.0148	0.0135	0.0082	0.0115	0.0104	0.0143	0.0076	0.0066	0.009	0.0051	0.0061	0.0112	0.0086	0.0076
A63	0.011	0.0099	0.0138	0.0125	0.0104	0.0094	0.0094	0.0133	0.0066	0.0057	0.0081	0.0071	0.0052	0.0103	0.0107	0.0097
A64	0.0127	0.0138	0.01	0.0143	0.0143	0.0111	0.0133	0.0096	0.0082	0.0092	0.0097	0.0087	0.0088	0.0119	0.0124	0.0135

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
A65	0.0089	0.0099	0.0116	0.0104	0.0115	0.0094	0.0094	0.0111	0.0047	0.0057	0.0061	0.0061	0.0052	0.0082	0.0097	0.0107
A66	0.0099	0.0089	0.0127	0.0115	0.0104	0.0105	0.0084	0.0122	0.0057	0.0047	0.0071	0.0071	0.0042	0.0092	0.0107	0.0097
A67	0.0058	0.0068	0.0084	0.0073	0.0093	0.0073	0.0073	0.009	0.0076	0.0087	0.0091	0.0101	0.0092	0.0042	0.0066	0.0076
A68	0.0068	0.0058	0.0094	0.0083	0.0083	0.0084	0.0063	0.01	0.0087	0.0076	0.0101	0.0112	0.0082	0.0052	0.0076	0.0066
A69	0.0084	0.0094	0.0058	0.0099	0.0121	0.01	0.01	0.0064	0.0103	0.0113	0.0118	0.0129	0.0119	0.0067	0.0092	0.0103
A70	0.0084	0.0073	0.0111	0.0099	0.0078	0.0069	0.0069	0.0106	0.0103	0.0092	0.0118	0.0107	0.0088	0.0067	0.0072	0.0062
A71	0.0074	0.0064	0.0102	0.009	0.009	0.0091	0.007	0.0108	0.0073	0.0062	0.0088	0.0098	0.0068	0.0078	0.0104	0.0093
A72	0.0091	0.008	0.0119	0.0106	0.0085	0.0075	0.0075	0.0114	0.0089	0.0078	0.0104	0.0093	0.0073	0.0094	0.0099	0.0089
A73	0.0119	0.0108	0.0148	0.0135	0.0082	0.0115	0.0104	0.0143	0.0076	0.0066	0.009	0.0051	0.0061	0.0112	0.0086	0.0076
A74	0	0.001	0.0024	0.0014	0.0034	0.0014	9.0014	0.0029	0.0038	0.0048	0.0053	0.0062	0.0053	0.0014	0.0038	0.0048
A75	0.001	0	0.0034	0.0024	0.0024	0.0024	0.0005	0.0039	0.0048	0.0038	0.0062	0.0073	0.0043	0.0024	0.0048	0.0038
B76	0.0005	0.0014	0.0029	0.0019	0.0029	0.001	0.001	0.0024	0.0043	0.0053	0.0058	0.0058	0.0048	0.0019	0.0033	0.0043
B77	0.0048	0.0038	0.0073	0.0062	0.0062	0.0063	0.0043	0.0079	0.0009	0	0.0023	0.0032	0.0005	0.0042	0.0066	0.0057
B78	0.0063	0.0073	0.0038	0.0078	0.0099	0.0079	0.0079	0.0044	0.0023	0.0033	0.0037	0.0047	0.0038	0.0057	0.0082	0.0092
B79	0.0073	0.0062	0.0099	0.0088	0.0038	0.0068	0.0058	0.0094	0.0032	0.0023	0.0046	0.0009	0.0018	0.0066	0.0042	0.0032
B80	0.0089	0.0099	0.0063	0.0104	0.0073	0.0084	0.0094	0.0058	0.0047	0.0057	0.0061	0.0023	0.0052	0.0082	0.0057	0.0066
B81	0.0014	0.0024	0.0038	0.0028	0.0048	0.0029	0.0029	0.0044	0.0033	0.0042	0.0047	0.0057	0.0047	0	0.0023	0.0033
B82	0.0024	0.0014	0.0048	0.0038	0.0038	0.0038	0.0019	0.0054	0.0042	0.0033	0.0057	0.0066	0.0038	0.0009	0.0033	0.0023
B83	0.0044	0.0054	0.0019	0.0059	0.008	0.006	0.006	0.0024	0.0043	0.0053	0.0058	0.0068	0.0058	0.0048	0.0073	0.0084
B84	0.0039	0.0049	0.0065	0.0024	0.0064	0.0044	0.0044	0.006	0.0038	0.0048	0.0023	0.0053	0.0043	0.0043	0.0058	0.0068
B85	0.0058	0.0048	0.0085	0.0073	0.0024	0.0054	0.0044	0.008	0.0098	0.0088	0.0113	0.0072	0.0083	0.0073	0.0047	0.0038
B86	0.0074	0.0085	0.0049	0.009	0.0058	0.007	0.008	0.0044	0.0115	0.0125	0.013	0.0088	0.0121	0.0089	0.0062	0.0073
B87	0.0062	0.0073	0.0089	0.0077	0.0098	0.0078	0.0078	0.0094	0.0023	0.0032	0.0037	0.0046	0.0037	0.0057	0.0081	0.0091
B88	0.0089	0.0099	0.0063	0.0104	0.0125	0.0105	0.0105	0.0069	0.0047	0.0057	0.0061	0.0071	0.0062	0.0082	0.0107	0.0118
B89	0.0115	0.0125	0.0089	0.013	0.0098	0.011	0.0121	0.0084	0.0071	0.0081	0.0086	0.0046	0.0076	0.0107	0.0081	0.0091
B90	0.0078	0.0068	0.0105	0.0093	0.0083	0.0084	0.0063	0.01	0.0037	0.0028	0.0051	0.0051	0.0023	0.0072	0.0087	0.0076
B91	0.0038	0.0048	0.0063	0.0053	0.0073	0.0053	0.0053	0.0069	0.0057	0.0066	0.0071	0.0081	0.0072	0.0023	0.0047	0.0057
B92	0.0048	0.0038	0.0073	0.0062	0.0062	0.0063	0.0043	0.0079	0.0066	0.0057	0.0081	0.0091	0.0062	0.0033	0.0057	0.0047
B93	0.0053	0.0063	0.0079	0.0068	0.0068	0.0038	0.0058	0.0074	0.0072	0.0082	0.0087	0.0076	0.0077	0.0038	0.0042	0.0052
B94	0.0024	0.0034	0.005	0.0039	0.0059	0.0039	0.0039	0.0055	0.0063	0.0073	0.0078	0.0089	0.0079	0.0038	0.0063	0.0073
B95	0.0034	0.0024	0.006	0.0049	0.0049	0.005	0.0029	0.0066	0.0073	0.0063	0.0089	0.0099	0.0069	0.0048	0.0073	0.0063
B96	0.0059	0.0049	0.0086	0.0074	0.0024	0.0054	0.0044	0.0081	0.0099	0.0089	0.0115	0.0073	0.0084	0.0073	0.0048	0.0038
B97	0.005	0.0039	0.0076	0.0065	0.0044	0.0035	0.0035	0.0071	0.009	0.0079	0.0105	0.0094	0.0074	0.0064	0.0069	0.0058
B98	0.0063	0.0073	0.009	0.0078	0.0099	0.0079	0.0079	0.0096	0.0023	0.0033	0.0037	0.0047	0.0038	0.0057	0.0082	0.0092
B99	0.0089	0.0099	0.0116	0.0104	0.0073	0.0084	0.0094	0.0111	0.0047	0.0057	0.0061	0.0023	0.0052	0.0082	0.0082	0.0092
B100	0.0099	0.0089	0.0127	0.0115	0.0062	0.0094	0.0084	0.0122	0.0057	0.0047	0.0071	0.0032	0.0042	0.0092	0.0057	0.0066
B101	0.0116	0.0127	0.009	0.0132	0.0099	0.0111	0.0122	0.0085	0.0072	0.0082	0.0087	0.0047	0.0077	0.0108	0.0082	0.0092
B102	0.009	0.0079	0.0117	0.0105	0.0084	0.0074	0.0074	0.0112	0.0047	0.0038	0.0062	0.0052	0.0033	0.0083	0.0088	0.0077
B103	0.0106	0.0117	0.008	0.0122	0.0122	0.0091	0.0112	0.0075	0.0062	0.0073	0.0077	0.0067	0.0068	0.0099	0.0104	0.0115
B104	0.0079	0.0069	0.0106	0.0094	0.0084	0.0085	0.0064	0.0102	0.0038	0.0028	0.0052	0.0052	0.0023	0.0073	0.0088	0.0077
B105	0.0096	0.0106	0.007	0.0111	0.0122	0.0102	0.0102	0.0065	0.0053	0.0062	0.0067	0.0067	0.0058	0.0089	0.0104	0.0115
B106	0.0048	0.0038	0.0074	0.0063	0.0063	0.0064	0.0044	0.008	0.0067	0.0057	0.0082	0.0092	0.0062	0.0033	0.0057	0.0047
B107	0.0063	0.0073	0.009	0.0078	0.0048	0.0058	0.0069	0.0085	0.0082	0.0092	0.0097	0.0057	0.0088	0.0047	0.0023	0.0033
B108	0.007	0.0081	0.0045	0.0086	0.0108	0.0087	0.0087	0.005	0.0069	0.0079	0.0084	0.0094	0.0085	0.0074	0.01	0.0111
B109	0.008	0.007	0.0108	0.0096	0.0044	0.0075	0.0065	0.0103	0.0078	0.0068	0.0093	0.0053	0.0063	0.0084	0.0057	0.0048
B110	0.0044	0.0054	0.007	0.0058	0.0079	0.0059	0.0059	0.0075	0.0083	0.0093	0.0098	0.0108	0.0099	0.0058	0.0083	0.0093
B111	0.0054	0.0044	0.008	0.0069	0.0069	0.007	0.0049	0.0086	0.0093	0.0083	0.0108	0.0119	0.0089	0.0068	0.0093	0.0083
B112	0.0148	0.0068	0.0058	0.0102	0.0096	0.0043	0.0079	0.0067	0.0093	0.0119	0.0062	0.0043	0.0033	0.0058	0.0039	0.0029
B113	0.007	0.008	0.0044	0.0085	0.0106	0.0086	0.0086	0.005	0.011	0.0121	0.0125	0.0136	0.0127	0.0084	0.011	0.0121
B114	0.0079	0.0069	0.0106	0.0094	0.0043	0.0074	0.0064	0.0102	0.0119	0.0108	0.0135	0.0092	0.0104	0.0093	0.0067	0.0057
B115	0.0096	0.0106	0.007	0.0111	0.0079	0.0091	0.0102	0.0065	0.0136	0.0148	0.0152	0.0108	0.0143	0.011	0.0083	0.0093
B116	0.007	0.0059	0.0097	0.0085	0.0064	0.0054	0.0054	0.0092	0.011	0.0099	0.0125	0.0115	0.0094	0.0084	0.0089	0.0078
B117	0.0059	0.0049	0.0086	0.0074	0.0064	0.0065	0.0044	0.0081	0.0099	0.0089	0.0115	0.0115	0.0084	0.0073	0.0089	0.0078
B118	0.0075	0.0086	0.005	0.0091	0.0102	0.0081	0.0081	0.0045	0.0116	0.0127	0.0132	0.0132	0.0122	0.009	0.0105	0.0116
B119	0.0093	0.0083	0.0121	0.0108	0.0108	0.011	0.0089	0.0127	0.0051	0.0042	0.0066	0.0076	0.0047	0.0087	0.0112	0.0101
B120	0.0108	0.0119	0.0136	0.0124	0.0092	0.0104	0.0115	0.0132	0.0066	0.0076	0.008	0.0041	0.0071	0.0101	0.0076	0.0086
B121	0.0119	0.0108	0.0148	0.0135	0.0082	0.0115	0.0104	0.0143	0.0076	0.0066	0.009	0.0051	0.0061	0.0112	0.0086	0.0076
B122	0.0099	0.011	0.0127	0.0115	0.0115	0.0084	0.0105	0.0122	0.0057	0.0066	0.0071	0.0061	0.0062	0.0092	0.0097	0.0107
B123	0.0068	0.0058	0.0094	0.0083	0.0083	0.0084	0.0063	0.01	0.0087	0.0076	0.0101	0.0112	0.0082	0.0052	0.0076	0.0066
B124	0.0084	0.0073	0.0111	0.0099	0.0078	0.0069	0.0069	0.0105	0.0103	0.0092	0.0118	0.0107	0.0088	0.0067	0.0072	0.0062
B125	0.01	0.0111	0.0074	0.0116	0.0116	0.0085	0.0106	0.007	0.0119	0.013	0.0135	0.0124	0.0125	0.0083	0.0088	0.0098
B126	0.0024	0.0014	0.005	0.0039	0.0019	0.001	0.001	0.0045	0.0063	0.0053	0.0078	0.0068	0.0048	0.0038	0.0043	0.0033
D127	0.0014	0.0005	0.0039	0.0029	0.0019	0.0019	0	0.0035	0.0053	0.0043	0.0068	0.0068	0.0038	0.0029	0.0043	0.0033
D128	0.0073	0.0062	0.0099	0.0088	0.0038	0.0068	ა.0058	0.0094	0.0032	0.0023	0.0046	0.0009	0.0018	0.0066	0.0042	0.0032
D129	0.0063	0.0053	0.009	0.0078	0.0058	0.0048	0.0048	0.0085	0.0023	0.0014	0.0037	0.0028	0.0009	0.0057	0.0062	0.0052

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
D130	0.0024	0.0014	0.0048	0.0038	0.0038	0.0038	0.0019	0.0054	0.0042	0.0033	0.0057	0.0066	0.0038	0.0009	0.0033	0.0023
D131	0.0044	0.0034	0.007	0.0059	0.0039	0.0029	0.0029	0.0066	0.0043	0.0033	0.0058	0.0048	0.0029	0.0048	0.0053	0.0043
D132	0.0049	0.0059	0.0024	0.0064	0.0085	0.0065	0.0065	0.0029	0.0089	0.0099	0.0104	0.0115	0.0105	0.0063	0.0089	0.0099
D133	0.0058	0.0048	0.0085	0.0073	0.0024	0.0054	0.0044	0.008	0.0098	0.0088	0.0113	0.0072	0.0083	0.0073	0.0047	0.0038
D134	0.0049	0.0039	0.0075	0.0064	0.0044	0.0034	0.0034	0.007	0.0089	0.0078	0.0104	0.0093	0.0073	0.0063	0.0068	0.0058
D135	0.0062	0.0073	0.0089	0.0077	0.0098	0.0078	0.0078	0.0094	0.0023	0.0032	0.0037	0.0046	0.0037	0.0057	0.0081	0.0091
D136	0.0098	0.0088	0.0125	0.0113	0.0062	0.0093	0.0083	0.0121	0.0056	0.0046	0.007	0.0032	0.0042	0.0091	0.0066	0.0056
D137	0.0089	0.0078	0.0116	0.0104	0.0083	0.0073	0.0073	0.0111	0.0047	0.0037	0.0061	0.0051	0.0033	0.0082	0.0087	0.0076
D138	0.0105	0.0116	0.0079	0.0121	0.0121	0.009	0.0111	0.0074	9.0062	0.0072	0.0076	0.0066	0.0067	0.0098	0.0103	0.0113
D139	0.0093	0.0104	0.0121	0.0077	0.0108	0.0078	0.0099	0.0116	0.0051	0.0061	0.0037	0.0056	0.0057	0.0087	0.0091	0.0101
D140	0.0048	0.0038	0.0073	0.0062	0.0062	0.0063	0.0043	0.0079	0.0066	0.0057	0.0081	0.0091	0.0062	0.0033	0.0057	0.0047
D141	0.0079	0.009	0.0054	0.0094	0.0094	0.0064	0.0085	0.0049	0.0098	0.0108	0.0113	0.0103	0.0104	0.0062	0.0067	0.0077
D142	0.0054	0.0044	0.008	0.0069	0.0069	0.007	0.0049	0.0086	0.0053	0.0043	0.0067	0.0077	0.0048	0.0058	0.0083	0.0073
D143	0.0069	0.0079	0.0096	0.0084	0.0053	0.0064	0.0074	0.0091	0.0067	0.0077	0.0082	0.0042	0.0073	0.0073	0.0047	0.0057
D144	0.005	0.006	0.0024	0.0065	0.0086	0.0066	0.0066	0.003	0.009	0.01	0.0105	0.0116	0.0106	0.0064	0.009	0.01
D145	0.0059	0.0049	0.0086	0.0074	0.0024	0.0054	0.0044	0.0081	0.0099	0.0089	0.0115	0.0073	0.0084	0.0073	0.0048	0.0038
D146	0.0066	0.0076	0.004	0.0081	0.0081	0.005	0.0071	0.0035	0.0106	0.0117	0.0122	0.0111	0.0112	0.008	0.0085	0.0096
D147	0.0054	0.0065	0.0081	0.0039	0.007	0.0039	0.006	0.0076	0.0094	0.0105	0.0078	0.0099	0.01	0.0069	0.0073	0.0084
D148	0.0055	0.0066	0.003	0.007	0.0081	0.0061	0.0061	0.0025	0.0096	0.0106	0.0111	0.0111	0.0102	0.007	0.0085	0.0096
D149	0.0099	0.0089	0.0127	0.0115	0.0062	0.0094	0.0084	0.0122	0.0057	0.0047	0.0071	0.0032	0.0042	0.0092	0.0066	0.0057
D150	0.0116	0.0127	0.009	0.0132	0.0099	0.0111	0.0122	0.0085	0.0072	0.0082	0.0087	0.0047	0.0077	0.0108	0.0082	0.0092
D151	0.0038	0.0048	0.0064	0.0053	0.0073	0.0054	0.0054	0.007	0.0057	0.0067	0.0072	0.0082	0.0073	0.0023	0.0047	0.0057
D152	0.0048	0.0038	0.0074	0.0063	0.0063	0.0064	0.0044	0.008	0.0067	0.0057	0.0082	0.0092	0.0062	0.0033	0.0057	0.0047
D153	0.0073	0.0063	0.01	0.0089	0.0038	0.0069	0.0058	0.0096	0.0092	0.0082	0.0107	0.0066	0.0077	0.0057	0.0033	0.0023
D154	0.008	0.0091	0.0054	0.0096	0.0096	0.0065	0.00863	0.005	0.0099	0.011	0.0115	0.0104	0.0105	0.0063	0.0068	0.0078
D155	0.0087	0.0098	0.0061	0.0103	0.0103	0.0071	0.0093	0.0056	0.0085	0.0096	0.01	0.009	0.0091	0.0091	0.0096	0.0106
D156	0.0044	0.0054	0.007	0.0058	0.0079	0.0059	0.0059	0.0075	0.0083	0.0093	0.0098	0.0108	0.0099	0.0058	0.0083	0.0093
D157	0.0054	0.0044	0.008	0.0069	0.0069	0.007	0.0049	0.0086	0.0093	0.0083	0.0108	0.0119	0.0089	0.0068	0.0093	0.0083
D158	0.007	0.008	0.0044	0.0085	0.0106	0.0086	0.0086	0.005	0.011	0.0121	0.0125	0.0136	0.0127	0.0084	0.011	0.0121
D159	0.0069	0.0079	0.0096	0.0084	0.0053	0.0064	0.0074	0.0091	0.0108	0.0119	0.0124	0.0082	0.0115	0.0083	0.0057	0.0067
D160	0.0079	0.0069	0.0106	0.0094	0.0043	0.0074	0.0064	0.0102	0.0119	0.0108	0.0135	0.0092	0.0104	0.0093	0.0067	0.0057
D161	0.0059	0.007	0.0086	0.0074	0.0074	0.0044	0.0065	0.0081	0.0099	0.011	0.0115	0.0104	0.0105	0.0073	0.0078	0.0089
D162	0.007	0.0059	0.0097	0.0085	0.0064	0.0054	0.0054	0.0092	0.011	0.0099	0.0125	0.0115	0.0094	0.0084	0.0089	0.0078
D163	0.0086	0.0097	0.006	0.0102	0.0102	0.007	0.0092	0.0055	0.0127	0.0138	0.0143	0.0132	0.0133	0.01	0.0105	0.0116
D164	0.0093	0.0083	0.0121	0.0108	0.0108	0.011	0.0089	0.0127	0.0051	0.0042	0.0066	0.0076	0.0047	0.0087	0.0112	0.0101
D165	0.011	0.0121	0.0084	0.0125	0.0148	0.0127	0.0127	0.009	0.0066	0.0076	0.0081	0.0091	0.0082	0.0103	0.0129	0.0139
D166	0.0108	0.0119	0.0136	0.0124	0.0092	0.0104	0.0115	0.0132	0.0066	0.0076	0.008	0.0041	0.0071	0.0101	0.0076	0.0086
D167	0.0119	0.0108	0.0148	0.0135	0.0082	0.0115	0.0104	0.0143	0.0076	0.0066	0.009	0.0051	0.0061	0.0112	0.0086	0.0076
D168	0.0136	0.0148	0.011	0.0152	0.0119	0.0132	0.0143	0.0105	0.0091	0.0101	0.0106	0.0066	0.0097	0.0129	0.0101	0.0112
D169	0.0099	0.011	0.0127	0.0115	0.0115	0.0084	0.0105	0.0122	0.0057	0.0066	0.0071	0.0061	0.0062	0.0092	0.0097	0.0107
D170	0.011	0.0099	0.0138	0.0125	0.0104	0.0094	0.0094	0.0133	0.0066	0.0057	0.0081	0.0071	0.0052	0.0103	0.0107	0.0097
D171	0.011	0.0099	0.0138	0.0125	0.0104	0.0094	0.0094	0.0133	0.0066	0.0057	0.0081	0.0071	0.0052	0.0103	0.0107	0.0097
D172	0.0127	0.0138	0.01	0.0143	0.0143	0.0111	0.0133	0.0096	0.0082	0.0092	0.0097	0.0087	0.0088	0.0119	0.0124	0.0135
D173	0.0115	0.0125	0.0143	0.0098	0.013	0.0099	0.0121	0.0138	0.0071	0.0081	0.0056	0.0076	0.0076	0.0107	0.0112	0.0122
D174	0.0116	0.0127	0.009	0.0132	0.0143	0.0122	0.0122	0.0085	0.0072	0.0082	0.0087	0.0087	0.0077	0.0108	0.0124	0.0135
D175	0.0073	0.0084	0.01	0.0089	0.0089	0.0058	0.0079	0.0096	0.0092	0.0103	0.0107	0.0097	0.0098	0.0057	0.0062	0.0072
D176	0.01	0.0111	0.0074	0.0116	0.0116	0.0085	0.0106	0.007	0.0119	0.013	0.0135	0.0124	0.0125	0.0083	0.0088	0.0098
D177	0.0073	0.0063	0.01	0.0089	0.0078	0.0079	0.0058	0.0096	0.0092	0.0082	0.0107	0.0107	0.0077	0.0057	0.0072	0.0062
D178	0.008	0.0091	0.0108	0.0096	0.0096	0.0065	0.0086	0.0103	0.0078	0.0089	0.0093	0.0083	0.0084	0.0084	0.0089	0.0099
D179	0.0108	0.0119	0.0081	0.0124	0.0124	0.0092	0.0114	0.0076	0.0105	0.0116	0.0121	0.011	0.0111	0.0111	0.0116	0.0127
D180	0.0089	0.0078	0.0116	0.0104	0.0083	0.0073	0.0073	0.0111	0.0047	0.0037	0.0061	0.0051	0.0033	0.0082	0.0087	0.0076
D181	0.0089	0.0078	0.0116	0.0104	0.0083	0.0073	0.0073	0.0111	0.0047	0.0037	0.0061	0.0051	0.0033	0.0082	0.0087	0.0076
D182	0.0089	0.0078	0.0116	0.0104	0.0083	0.0073	0.0073	0.0111	0.0047	0.0037	0.0061	0.0051	0.0033	0.0082	0.0087	0.0076
D183	0.0378	0.0363	0.0358	0.0398	0.0339	0.0358	0.0358	0.0354	0.0368	0.0353	0.0387	0.0344	0.0349	0.0377	0.0353	0.034

	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30	A31	A32
A17	0															
A18	0.007	0														
A19	0.0097	0.0024	0													
A20	0.0064	0.0033	0.0058	0												
A21	0.0102	0.0048	0.0024	0.0033	0											
A22	0.0054	0.0014	0.0039	0.0019	0.0054	0										
A23	0.008	0.0019	0.0044	0.0043	0.0058	0.0024	0									
A24	0.0068	0.0038	0.0062	0.0072	0.0088	0.0053	0.0057	0								
A25	0.0073	0.0062	0.0088	0.0047	0.0062	0.0067	0.0072	0.0023	0							
A26	0.0062	0.0072	0.0098	0.0037	0.0072	0.0057	0.0082	0.0032	0.0009	0						
A27	0.0053	0.0053	0.0078	0.0067	0.0083	0.0058	0.0062	0.0014	0.0018	0.0027	0					
A28	0.0053	0.0053	0.0078	0.0057	0.0093	0.0038	0.0062	0.0014	0.0027	0.0018	0.0018	0				
A29	0.0073	0.0014	0.0038	0.0047	0.0062	0.0028	0.0033	0.0032	0.0056	0.0066	0.0047	0.0047	0			
A30	0.0063	0.0023	0.0048	0.0038	0.0073	0.0019	0.0043	0.0042	0.0066	0.0056	0.0057	0.0037	0.0009	0		
A31	0.01	0.0038	0.0014	0.0073	0.0038	0.0053	0.0058	0.0057	0.0081	0.0091	0.0072	0.0072	0.0023	0.0033	0	
A32	0.0089	0.0028	0.0053	0.0062	0.0077	0.0043	0.0019	0.0046	0.007	0.008	0.0061	0.0061	0.0014	0.0023	0.0037	0
A33	0.0068	0.0047	0.0073	0.0014	0.0047	0.0033	0.0057	0.0066	0.0041	0.0032	0.0061	0.0051	0.0032	0.0023	0.0057	0.0046
A34	0.007	0.0049	0.0075	0.0085	0.0102	0.0065	0.007	0.0089	0.0115	0.0125	0.0105	0.0105	0.0063	0.0073	0.009	0.0078
A35	0.006	0.0059	0.0086	0.0074	0.0112	0.0054	0.008	0.0099	0.0125	0.0115	0.0116	0.0094	0.0073	0.0063	0.01	0.0089
A36	0.0098	0.0075	0.005	0.0112	0.0075	0.0092	0.0097	0.0116	0.0143	0.0154	0.0133	0.0133	0.009	0.01	0.0064	0.0105
A37	0.0086	0.0064	0.0091	0.01	0.0117	0.008	0.0054	0.0104	0.013	0.0141	0.0121	0.0121	0.0078	0.0089	0.0105	0.0062
A38	0.0045	0.0075	0.0103	0.007	0.0108	0.006	0.0086	0.0116	0.0121	0.011	0.01	0.01	0.009	0.0079	0.0117	0.0105
A39	0.0082	0.0092	0.0066	0.0108	0.007	0.0098	0.0103	0.0133	0.0138	0.0149	0.0117	0.014	0.0106	0.0117	0.008	0.0122
A40	0.0055	0.0065	0.0092	0.007	0.0108	0.005	0.0075	0.0105	0.0121	0.011	0.0111	0.009	0.0079	0.0069	0.0106	0.0094
A41	0.0058	0.0099	0.0127	0.0115	0.0154	0.0094	0.0121	0.0057	0.0081	0.0071	0.0072	0.0052	0.0092	0.0082	0.0119	0.0107
A42	0.0096	0.0116	0.009	0.0154	0.0116	0.0133	0.0138	0.0072	0.0097	0.0107	0.0088	0.0088	0.0108	0.0119	0.0083	0.0124
A43	0.0073	0.0115	0.0143	0.0098	0.0115	0.0121	0.0125	0.0071	0.0046	0.0056	0.0066	0.0076	0.0107	0.0118	0.0135	0.0122
A44	0.0063	0.0125	0.0154	0.0088	0.0125	0.011	0.0136	0.0081	0.0056	0.0046	0.0076	0.0066	0.0118	0.0107	0.0146	0.0133
A45	0.0064	0.0094	0.0122	0.0121	0.0138	0.01	0.0105	0.0052	0.0066	0.0076	0.0057	0.0057	0.0088	0.0098	0.0115	0.0103
A46	0.0074	0.0063	0.009	0.0099	0.0116	0.0079	0.0084	0.0082	0.0107	0.0118	0.0098	0.0098	0.0047	0.0057	0.0073	0.0062
A47	0.0069	0.0099	0.0127	0.0062	0.0099	0.0084	0.011	0.0118	0.0091	0.0081	0.0113	0.01063	0.0082	0.0072	0.0108	0.0097
A48	0.0076	0.0097	0.007	0.0135	0.0097	0.0114	0.0119	0.0094	0.0121	0.0132	0.0111	0.0111	0.01	0.0111	0.0074	0.0116
A49	0.0054	0.0096	0.0124	0.0079	0.0096	0.0102	0.0106	0.0093	0.0067	0.0077	0.0089	0.0099	0.0099	0.011	0.0127	0.0115
A50	0.007	0.008	0.0097	0.0085	0.0054	0.0065	0.0075	0.0092	0.0068	0.0078	0.0083	0.0043	0.0073	0.0073	0.0048	0.0058
A51	0.0024	0.0097	0.0125	0.0091	0.013	0.0081	0.0108	0.0094	0.0099	0.0089	0.0079	0.0079	0.01	0.009	0.0128	0.0116
A52	0.0091	0.0019	0.0044	0.0053	0.0069	0.0034	0.0038	0.0057	0.0082	0.0092	0.0073	0.0073	0.0033	0.0043	0.0058	0.0047
A53	0.008	0.0029	0.0054	0.0043	0.0079	0.0024	0.0048	0.0067	0.0092	0.0082	0.0083	0.0062	0.0043	0.0033	0.0068	0.0057
A54	0.0119	0.0044	0.0019	0.0079	0.0044	0.0059	0.0064	0.0083	0.0108	0.0119	0.0099	0.0099	0.0058	0.0068	0.0033	0.0073
A55	0.0106	0.0053	0.0058	0.0068	0.0084	0.0048	0.0024	0.0072	0.0097	0.0107	0.0088	0.0088	0.0047	0.0057	0.0073	0.0033
A56	0.0085	0.0053	0.0079	0.0019	0.0053	0.0038	0.0063	0.0092	0.0066	0.0057	0.0088	0.0077	0.0067	0.0057	0.0093	0.0082
A57	0.0124	0.0069	0.0044	0.0053	0.0019	0.0074	0.0079	0.0108	0.0082	0.0092	0.0104	0.0115	0.0083	0.0093	0.0058	0.0098
A58	0.0065	0.0044	0.007	0.0038	0.0074	0.0029	0.0054	0.0083	0.0088	0.0077	0.0068	0.0068	0.0058	0.0048	0.0084	0.0073
A59	0.0075	0.0034	0.0059	0.0038	0.0074	0.0019	0.0044	0.0073	0.0088	0.0077	0.0078	0.0058	0.0048	0.0038	0.0073	0.0062
A60	0.0078	0.0067	0.0093	0.0082	0.0119	0.0062	0.0088	0.0027	0.0051	0.0041	0.0042	0.0023	0.0061	0.0051	0.0087	0.0076
A61	0.0116	0.0083	0.0058	0.0119	0.0083	0.0099	0.0104	0.0042	0.0066	0.0076	0.0057	0.0057	0.0076	0.0087	0.0052	0.0091
A62	0.0104	0.0072	0.0098	0.0107	0.0124	0.0088	0.0062	0.0032	0.0055	0.0065	0.0046	0.0046	0.0066	0.0076	0.0091	0.0051
A63	0.0093	0.0082	0.0108	0.0066	0.0082	0.0088	0.0092	0.0041	0.0018	0.0027	0.0037	0.0046	0.0076	0.0086	0.0101	0.009
A64	0.0083	0.0092	0.0119	0.0057	0.0092	0.0077	0.0103	0.0051	0.0027	0.0018	0.0046	0.0037	0.0086	0.0076	0.0112	0.01
A65	0.0063	0.0083	0.011	0.0077	0.0115	0.0068	0.0093	0.0042	0.0046	0.0037	0.0028	0.0028	0.0076	0.0066	0.0103	0.0091
A66	0.01	0.0099	0.0073	0.0115	0.0078	0.0105	0.011	0.0057	0.0061	0.0071	0.0042	0.0062	0.0092	0.0103	0.0067	0.0107
A67	0.0084	0.0062	0.0089	0.0088	0.0104	0.0068	0.0073	0.0023	0.0037	0.0046	0.0028	0.0028	0.0057	0.0066	0.0082	0.0071
A68	0.0073	0.0073	0.0099	0.0077	0.0115	0.0058	0.0083	0.0032	0.0046	0.0037	0.0037	0.0018	0.0066	0.0057	0.0092	0.0081
A69	0.0094	0.0033	0.0058	0.0067	0.0083	0.0048	0.0053	0.0051	0.0076	0.0086	0.0066	0.0066	0.0018	0.0028	0.0042	0.0032
A70	0.0084	0.0043	0.0068	0.0057	0.0093	0.0038	0.0062	0.0061	0.0086	0.0076	0.0076	0.0057	0.0028	0.0018	0.0052	0.0042
A71	0.0122	0.0058	0.0033	0.0093	0.0058	0.0073	0.0078	0.0076	0.0101	0.0112	0.0092	0.0092	0.0042	0.0052	0.0019	0.0057
A72	0.0069	0.0058	0.0084	0.0053	0.0089	0.0043	0.0068	0.0076	0.0081	0.0071	0.0062	0.0062	0.0042	0.0033	0.0067	0.0057
A73	0.0059	0.0048	0.0074	0.0063	0.01	0.0044	0.0069	0.0047	0.0072	0.0062	0.0062	0.0043	0.0053	0.0043	0.0078	0.0067
A74	0.0044	0.0064	0.0091	0.0058	0.0096	0.0049	0.0074	0.0062	0.0067	0.0057	0.0048	0.0048	0.0068	0.0058	0.0094	0.0083
A75	0.0083	0.0092	0.0119	0.0057	0.0092	0.0077	0.0103	0.0051	0.0027	0.0018	0.0046	0.0037	0.0086	0.0076	0.0112	0.01
B76	0.0044	0.0024	0.0049	0.0058	0.0074	0.0039	0.0044	0.0062	0.0088	0.0098	0.0078	0.0078	0.0038	0.0048	0.0063	0.0053
B77	0.0034	0.0034	0.0054	0.0048	0.0085	0.0029	0.0054	0.0073	0.0098	0.0088	0.0089	0.0068	0.0048	0.0038	0.0073	0.0062

	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30	A31	A32
B78	0.0039	0.0029	0.0059	0.0054	0.007	0.0034	0.0039	0.0068	0.0083	0.0093	0.0073	0.0073	0.0043	0.0053	0.0069	0.0058
B79	0.0033	0.0073	0.0099	0.0088	0.0125	0.0068	0.0093	0.0032	0.0056	0.0046	0.0047	0.0028	0.0066	0.0057	0.0092	0.0081
B80	0.0069	0.0089	0.0063	0.0125	0.0089	0.0105	0.011	0.0047	0.0071	0.0081	0.0062	0.0062	0.0082	0.0092	0.0057	0.0097
B81	0.0038	0.0098	0.0125	0.0062	0.0098	0.0083	0.0108	0.0056	0.0032	0.0023	0.0051	0.0042	0.0091	0.0081	0.0118	0.0106
B82	0.0073	0.0115	0.0089	0.0098	0.0062	0.0121	0.0125	0.0071	0.0046	0.0056	0.0066	0.0076	0.0107	0.0118	0.0082	0.0122
B83	0.0048	0.0038	0.0063	0.0073	0.0089	0.0053	0.0058	0.0057	0.0081	0.0091	0.0072	0.0072	0.0023	0.0033	0.0047	0.0037
B84	0.0038	0.0048	0.0073	0.0062	0.0099	0.0043	0.0068	0.0066	0.0091	0.0081	0.0082	0.0062	0.0033	0.0023	0.0057	0.0047
B85	0.005	0.007	0.0044	0.0106	0.007	0.0086	0.0091	0.0068	0.0093	0.0104	0.0084	0.0084	0.0073	0.0084	0.0048	0.0089
B86	0.0034	0.0064	0.0091	0.009	0.0106	0.007	0.0044	0.0062	0.0077	0.0088	0.0068	0.0068	0.0068	0.0078	0.0094	0.0053
B87	0.0064	0.0033	0.0058	0	0.0033	0.0019	0.0043	0.0072	0.0047	0.0037	0.0067	0.0057	0.0047	0.0038	0.0073	0.0062
B88	0.0102	0.0048	0.0024	0.0033	0	0.0054	0.0058	0.0088	0.0062	0.0072	0.0083	0.0093	0.0062	0.0073	0.0038	0.0077
B89	0.0068	0.0038	0.0062	0.0072	0.0088	0.0053	0.0057	0	0.0023	0.0032	0.0014	0.0014	0.0032	0.0042	0.0057	0.0046
B90	0.0094	0.0062	0.0038	0.0098	0.0062	0.0078	0.0083	0.0023	0.0046	0.0056	0.0037	0.0037	0.0057	0.0066	0.0033	0.0071
B91	0.0099	0.0088	0.0062	0.0072	0.0038	0.0093	0.0098	0.0046	0.0023	0.0032	0.0042	0.0051	0.0081	0.0091	0.0057	0.0096
B92	0.0053	0.0053	0.0078	0.0057	0.0093	0.0038	0.0062	0.0014	0.0027	0.0018	0.0018	0	0.0047	0.0037	0.0072	0.0061
B93	0.0073	0.0014	0.0038	0.0047	0.0062	0.0028	0.0033	0.0032	0.0056	0.0066	0.0047	0.0047	0	0.0009	0.0023	0.0014
B94	0.0063	0.0023	0.0048	0.0038	0.0073	0.0019	0.0043	0.0042	0.0066	0.0056	0.0057	0.0037	0.0009	0	0.0033	0.0023
B95	0.0058	0.0028	0.0053	0.0043	0.0058	0.0033	0.0038	0.0047	0.0051	0.0061	0.0033	0.0052	0.0014	0.0023	0.0038	0.0028
B96	0.007	0.0049	0.0075	0.0085	0.0102	0.0065	0.007	0.0089	0.0115	0.0125	0.0105	0.0105	0.0063	0.0073	0.009	0.0078
B97	0.006	0.0059	0.0086	0.0074	0.0112	0.0054	0.008	0.0099	0.0125	0.0115	0.0116	0.0094	0.0073	0.0063	0.01	0.0089
B98	0.0065	0.0085	0.0112	0.0048	0.0085	0.007	0.0096	0.0125	0.0098	0.0088	0.0121	0.011	0.0099	0.0089	0.0127	0.0115
B99	0.0045	0.0075	0.103	0.007	0.0108	0.006	0.0086	0.0116	0.0121	0.011	0.01	0.01	0.009	0.0079	0.0117	0.0105
B100	0.0069	0.0089	0.0116	0.0125	0.0143	0.0105	0.011	0.0047	0.0071	0.0081	0.0062	0.0062	0.0082	0.0092	0.0108	0.0097
B101	0.0073	0.0115	0.0143	0.0098	0.0115	0.0121	0.0125	0.0071	0.0046	0.0056	0.0066	0.0076	0.0107	0.0118	0.0135	0.0122
B102	0.0063	0.0125	0.0154	0.0088	0.0125	0.011	0.0136	0.0081	0.0056	0.0046	0.0076	0.0066	0.0118	0.0107	0.0146	0.0133
B103	0.01	0.0143	0.0116	0.0125	0.0089	0.0149	0.0154	0.0097	0.0071	0.0081	0.0092	0.0103	0.0135	0.0146	0.0108	0.015
B104	0.0044	0.0116	0.0145	0.011	0.0149	0.01	0.0127	0.0072	0.0076	0.0066	0.0057	0.0057	0.0108	0.0098	0.0136	0.0124
B105	0.008	0.0133	0.0106	0.0149	0.0111	0.014	0.0145	0.0088	0.0092	0.0103	0.0073	0.0093	0.0125	0.0136	0.0099	0.0141
B106	0.0054	0.0105	0.0133	0.011	0.0149	0.009	0.0116	0.0062	0.0076	0.0066	0.0067	0.0047	0.0098	0.0088	0.0125	0.0113
B107	0.0091	0.0122	0.0096	0.0149	0.0111	0.0128	0.0133	0.0077	0.0092	0.0103	0.0083	0.0083	0.0115	0.0125	0.0089	0.013
B108	0.0064	0.0073	0.01	0.0089	0.0127	0.0069	0.0094	0.0092	0.0118	0.0107	0.0108	0.0088	0.0057	0.0047	0.0083	0.0072
B109	0.0079	0.0089	0.0116	0.0073	0.0089	0.0094	0.0099	0.0107	0.0081	0.0091	0.0103	0.0113	0.0072	0.0082	0.0098	0.0087
B110	0.0076	0.0097	0.007	0.0135	0.0097	0.0114	0.0119	0.0094	0.0121	0.0132	0.0111	0.0111	0.01	0.0111	0.0074	0.0116
B111	0.0044	0.0106	0.0135	0.0069	0.0106	0.0091	0.0117	0.0104	0.0077	0.0067	0.0099	0.0089	0.011	0.0099	0.0138	0.0125
B112	0.0091	0.0019	0.0044	0.0053	0.0069	0.0034	0.0038	0.0057	0.0082	0.0092	0.0073	0.0073	0.0033	0.0043	0.0058	0.0047
B113	0.008	0.0029	0.0054	0.0043	0.0079	0.0024	0.0048	0.0067	0.0092	0.0082	0.0083	0.0062	0.0043	0.0033	0.0068	0.0057
B114	0.0119	0.0044	0.0019	0.0079	0.0044	0.0059	0.0064	0.0083	0.0108	0.0119	0.0099	0.0099	0.0058	0.0068	0.0033	0.0073
B115	0.0085	0.0053	0.0079	0.0019	0.0053	0.0038	0.0063	0.0092	0.0066	0.0057	0.0088	0.0077	0.0067	0.0057	0.0093	0.0082
B116	0.0124	0.0069	0.0044	0.0053	0.0019	0.0074	0.0079	0.0108	0.0082	0.0092	0.0104	0.0115	0.0083	0.0093	0.0058	0.0098
B117	0.0065	0.0044	0.007	0.0038	0.0074	0.0029	0.0054	0.0083	0.0088	0.0077	0.0068	0.0068	0.0058	0.0048	0.0084	0.0073
B118	0.0075	0.0034	0.0059	0.0038	0.0074	0.0019	0.0044	0.0073	0.0088	0.0077	0.0078	0.0058	0.0048	0.0038	0.0073	0.0062
B119	0.0114	0.0049	0.0024	0.0074	0.0039	0.0054	0.0059	0.0089	0.0104	0.0115	0.0094	0.0094	0.0063	0.0073	0.0038	0.0078
B120	0.0078	0.0067	0.0093	0.0082	0.0119	0.0062	0.0088	0.0027	0.0051	0.0041	0.0042	0.0023	0.0061	0.0051	0.0087	0.0076
B121	0.0093	0.0082	0.0108	0.0066	0.0082	0.0088	0.0092	0.0041	0.0018	0.0027	0.0037	0.0046	0.0076	0.0086	0.0101	0.009
B122	0.0083	0.0092	0.0119	0.0057	0.0092	0.0077	0.0103	0.0051	0.0027	0.0018	0.0046	0.0037	0.0086	0.0076	0.0112	0.01
B123	0.0073	0.0073	0.0099	0.0088	0.0104	0.0078	0.0083	0.0032	0.0037	0.0046	0.0018	0.0037	0.0066	0.0076	0.0092	0.0081
B124	0.0084	0.0043	0.0068	0.0057	0.0093	0.0038	0.0062	0.0061	0.0086	0.0076	0.0076	0.0057	0.0028	0.0018	0.0052	0.0042
D125	0.0069	0.0058	0.0084	0.0053	0.0089	0.0043	0.0068	0.0076	0.0081	0.0071	0.0062	0.0062	0.0042	0.0033	0.0067	0.0057
D126	0.0106	0.0073	0.0048	0.0089	0.0053	0.0079	0.0084	0.0092	0.0097	0.0107	0.0077	0.0098	0.0057	0.0067	0.0033	0.0072
D127	0.0019	0.0049	0.0075	0.0044	0.008	0.0034	0.0059	0.0089	0.0093	0.0083	0.0073	0.0073	0.0063	0.0053	0.009	0.0078
D128	0.0029	0.0039	0.0065	0.0044	0.008	0.0024	0.0049	0.0078	0.0093	0.0083	0.0084	0.0063	0.0053	0.0043	0.0079	0.0068
D129	0.0038	0.0098	0.0125	0.0062	0.0098	0.0083	0.0108	0.0056	0.0032	0.0023	0.0051	0.0042	0.0091	0.0081	0.0118	0.0106
D130	0.0019	0.0089	0.0116	0.0083	0.0121	0.0073	0.0099	0.0047	0.0051	0.0042	0.0033	0.0033	0.0082	0.0072	0.0108	0.0097
D131	0.0038	0.0048	0.0073	0.0062	0.0099	0.0043	0.0068	0.0066	0.0091	0.0081	0.0082	0.0062	0.0033	0.0023	0.0057	0.0047
D132	0	0.007	0.0097	0.0064	0.0102	0.0054	0.008	0.0068	0.0073	0.0062	0.0053	0.0053	0.0073	0.0063	0.01	0.0089
D133	0.0097	0.0024	0	0.0058	0.0024	0.0039	0.0044	0.0062	0.0088	0.0098	0.0078	0.0078	0.0038	0.0048	0.0014	0.0053
D134	0.0064	0.0033	0.0058	0	0.0033	0.0019	0.0043	0.0072	0.0047	0.0037	0.0067	0.0057	0.0047	0.0038	0.0073	0.0062
D135	0.0044	0.0024	0.0049	0.0019	0.0054	0.001	0.0034	0.0048	0.0067	0.0057	0.0048	0.0048	0.0038	0.0028	0.0063	0.0053
D136	0.0068	0.0038	0.0062	0.0072	0.0088	0.0053	0.0057	0	0.0023	0.0032	0.0014	0.0014	0.0032	0.0042	0.0057	0.0046
D137	0.0062	0.0072	0.0098	0.0037	0.0072	0.0057	0.0082	0.0032	0.0009	0	0.0027	0.0018	0.0066	0.0056	0.0091	0.008
D138	0.0043	0.0062	0.0089	0.0057	0.0093	0.0048	0.0073	0.0023	0.0027	0.0018	0.0009	0.0009	0.0057	0.0047	0.0082	0.0071
D139	0.0079	0.0078	0.0053	0.0093	0.0058	0.0084	0.0089	0.0037	0.0042	0.0051	0.0023	0.0042	0.0072	0.0082	0.0047	0.0087

	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30	A31	A32
D140	0.0068	0.0067	0.0093	0.0082	0.0098	0.0073	0.0047	0.0027	0.0032	0.0041	0.0014	0.0032	0.0061	0.0071	0.0087	0.0046
D141	0.0063	0.0023	0.0048	0.0038	0.0073	0.0019	0.0043	0.0042	0.0066	0.0056	0.0057	0.0037	0.0009	0	0.0033	0.0023
D142	0.0085	0.0053	0.0029	0.0068	0.0033	0.0058	0.0063	0.0072	0.0076	0.0087	0.0057	0.0077	0.0038	0.0047	0.0014	0.0052
D143	0.0039	0.0029	0.0054	0.0043	0.0079	0.0024	0.0048	0.0028	0.0052	0.0042	0.0043	0.0023	0.0033	0.0023	0.0058	0.0047
D144	0.0054	0.0043	0.0069	0.0028	0.0043	0.0048	0.0053	0.0042	0.0018	0.0028	0.0038	0.0047	0.0047	0.0057	0.0073	0.0062
D145	0.0098	0.0075	0.005	0.0112	0.0075	0.00921	0.0097	0.0116	0.0143	0.0154	0.0133	0.0133	0.009	0.01	0.0064	0.0105
D146	0.0065	0.0085	0.0112	0.0048	0.0085	0.007	0.0096	0.0125	0.0098	0.0088	0.0121	0.011	0.0099	0.0089	0.0127	0.0115
D147	0.0082	0.0092	0.0066	0.0108	0.007	0.0098	0.0105	0.0133	0.0138	0.0149	0.0117	0.014	0.0106	0.0117	0.008	0.0122
D148	0.007	0.008	0.0108	0.0096	0.0112	0.0086	0.0059	0.0121	0.0125	0.0136	0.0105	0.0127	0.0094	0.0105	0.0122	0.0078
D149	0.0093	0.0081	0.0055	0.0108	0.007	0.0087	0.0092	0.0122	0.0138	0.0149	0.0128	0.0128	0.0096	0.0106	0.007	0.0111
D150	0.0063	0.0125	0.0154	0.0088	0.0125	0.011	0.0136	0.0081	0.0056	0.0046	0.0076	0.0066	0.0118	0.0107	0.0146	0.0133
D151	0.01	0.0143	0.0116	0.0125	0.0089	0.0149	0.0154	0.0097	0.0071	0.0081	0.0092	0.0103	0.0135	0.0146	0.0108	0.015
D152	0.0074	0.0063	0.009	0.0099	0.0116	0.0079	0.0084	0.0082	0.0107	0.0118	0.0098	0.0098	0.0047	0.0057	0.0073	0.0062
D153	0.0064	0.0073	0.01	0.0089	0.0127	0.0069	0.0094	0.0092	0.0118	0.0107	0.0108	0.0088	0.0057	0.0047	0.0083	0.0072
D154	0.0069	0.0099	0.0127	0.0062	0.0099	0.0084	0.011	0.0118	0.0091	0.0081	0.0113	0.0103	0.0082	0.0072	0.0108	0.0097
D155	0.0086	0.0106	0.008	0.0122	0.0085	0.0112	0.0117	0.0125	0.013	0.0141	0.011	0.0132	0.0089	0.0099	0.0063	0.0104
D156	0.0061	0.0114	0.0087	0.013	0.0092	0.012	0.0125	0.0111	0.0116	0.0127	0.0096	0.0117	0.0117	0.0128	0.0091	0.0133
D157	0.0091	0.0019	0.0044	0.0053	0.0069	0.0034	0.0038	0.0057	0.0082	0.0092	0.0073	0.0073	0.0033	0.0043	0.0058	0.0047
D158	0.008	0.0029	0.0054	0.0043	0.0079	0.0024	0.0048	0.0067	0.0092	0.0082	0.0083	0.0062	0.0043	0.0033	0.0068	0.0057
D159	0.0119	0.0044	0.0019	0.0079	0.0044	0.0059	0.0064	0.0083	0.0108	0.0119	0.0099	0.0099	0.0058	0.0068	0.0033	0.0073
D160	0.0096	0.0043	0.0069	0.0028	0.0043	0.0048	0.0053	0.0082	0.0057	0.0066	0.0077	0.0088	0.0057	0.0067	0.0083	0.00721
D161	0.0085	0.0053	0.0079	0.0019	0.0053	0.0038	0.0063	0.0092	0.0066	0.0057	0.0088	0.0077	0.0067	0.00574	0.0093	0.0082
D162	0.0075	0.0034	0.0059	0.0048	0.0064	0.0039	0.0044	0.0073	0.0077	0.0088	0.0058	0.0078	0.0048	0.0058	0.0073	0.0062
D163	0.0065	0.0044	0.007	0.0038	0.0074	0.0029	0.0054	0.0083	0.0088	0.0077	0.0068	0.0068	0.0058	0.0048	0.0084	0.0073
D164	0.0103	0.0059	0.0034	0.0074	0.0039	0.0065	0.007	0.0099	0.0104	0.0115	0.0084	0.0105	0.0073	0.0084	0.0048	0.0089
D165	0.0078	0.0067	0.0093	0.0082	0.0119	0.0062	0.0088	0.0027	0.0051	0.0041	0.0042	0.0023	0.0061	0.0051	0.0087	0.0076
D166	0.0116	0.0083	0.0058	0.0119	0.0083	0.0099	0.0104	0.0042	0.0066	0.0076	0.0057	0.0057	0.0076	0.0087	0.0052	0.0091
D167	0.0093	0.0082	0.0108	0.0066	0.0082	0.0088	0.0092	0.0041	0.0018	0.0027	0.0037	0.0046	0.0076	0.0086	0.0101	0.009
D168	0.0083	0.0092	0.0119	0.0057	0.0092	0.0077	0.0103	0.0051	0.0027	0.0018	0.0046	0.0037	0.0086	0.0076	0.0112	0.01
D169	0.0121	0.0108	0.0083	0.0092	0.0057	0.0115	0.0119	0.0066	0.0041	0.0051	0.0061	0.0071	0.0101	0.0112	0.0076	0.0116
D170	0.0073	0.0073	0.0099	0.0088	0.0104	0.0078	0.0083	0.0032	0.0037	0.0046	0.0018	0.0037	0.0066	0.0076	0.0092	0.0081
D171	0.0063	0.0083	0.011	0.0077	0.0115	0.0068	0.0093	0.0042	0.0046	0.0037	0.0028	0.0028	0.0076	0.0066	0.0103	0.0091
D172	0.01	0.0099	0.0073	0.0115	0.0078	0.0105	0.011	0.0057	0.0061	0.0071	0.0042	0.0062	0.0092	0.0103	0.0067	0.0107
D173	0.0089	0.0088	0.0115	0.0103	0.0119	0.0093	0.0067	0.0046	0.0051	0.006	0.0032	0.0051	0.0081	0.0091	0.0107	0.0066
D174	0.0111	0.0089	0.0063	0.0115	0.0078	0.0094	0.0099	0.0047	0.0061	0.0071	0.0052	0.0052	0.0082	0.0092	0.0057	0.0097
D175	0.0079	0.0048	0.0073	0.0062	0.0078	0.0053	0.0058	0.0066	0.0071	0.0081	0.0052	0.0072	0.0033	0.0042	0.0057	0.0047
D176	0.0106	0.0073	0.0048	0.0089	0.0053	0.0079	0.0084	0.0092	0.0097	0.0107	0.0077	0.0098	0.0057	0.0067	0.0033	0.0072
D177	0.0079	0.0048	0.0073	0.0053	0.0089	0.0033	0.0058	0.0066	0.0081	0.0071	0.0072	0.0052	0.0033	0.0023	0.0057	0.0047
D178	0.0054	0.0054	0.008	0.0069	0.0085	0.0059	0.0064	0.0053	0.0057	0.0067	0.0038	0.0058	0.0058	0.0068	0.0084	0.0073
D179	0.0081	0.008	0.0054	0.0096	0.0059	0.0086	0.0091	0.0078	0.0083	0.0093	0.0063	0.0084	0.0084	0.0094	0.0058	0.0099
D180	0.0043	0.0062	0.0089	0.0057	0.0093	0.0048	0.0073	0.0023	0.0027	0.0018	0.0009	0.0009	0.0057	0.0047	0.0082	0.0071
D181	0.0043	0.0062	0.0089	0.0057	0.0093	0.0048	0.0073	0.0023	0.0027	0.0018	0.0009	0.0009	0.0057	0.0047	0.0082	0.0071
D182	0.0043	0.0062	0.0089	0.0057	0.0093	0.0048	0.0073	0.0023	0.0027	0.0018	0.0009	0.0009	0.0057	0.0047	0.0082	0.0071
D183	0.0358	0.0413	0.0393	0.0373	0.0368	0.0393	0.0429	0.0402	0.0377	0.0363	0.0382	0.0382	0.0412	0.0397	0.0392	0.0432

	A33	A34	A35	A36	A37	A38	A39	A40	A41	A42	A43	A44	A45	A46	A47	A48	A49
A33	0																
A34	0.0099	0															
A35	0.0089	0.001	0														
A36	0.0127	0.0024	0.0035	0													
A37	0.0115	0.0014	0.0024	0.0039	0												
A38	0.0084	0.0024	0.0015	0.005	0.0039	0											
A39	0.0122	0.004	0.005	0.0015	0.0055	0.0035	0										
A40	0.0084	0.0015	0.0005	0.004	0.0029	0.001	0.0045	0									
A41	0.0107	0.0048	0.0038	0.0074	0.0063	0.0054	0.0091	0.0044	0								
A42	0.0146	0.0064	0.0074	0.0039	0.0079	0.0091	0.0054	0.008	0.0033	0							
A43	0.0091	0.0063	0.0073	0.009	0.0078	0.0069	0.0085	0.0069	0.0033	0.0047	0						
A44	0.0081	0.0073	0.0063	0.01	0.0089	0.0058	0.0096	0.0058	0.0023	0.0057	0.0009	0					
A45	0.0113	0.0044	0.0054	0.007	0.0058	0.0059	0.0075	0.0049	0.0014	0.0028	0.0019	0.0028	0				
A46	0.0082	0.0014	0.0024	0.0039	0.0029	0.0039	0.0054	0.0029	0.0043	0.0058	0.0057	0.0067	0.0038	0			
A47	0.0047	0.0048	0.0038	0.0074	0.0063	0.0034	0.007	0.0034	0.0057	0.0093	0.0042	0.0033	0.0062	0.0033	0		
A48	0.0138	0.0045	0.0055	0.002	0.006	0.0071	0.0035	0.0061	0.0054	0.0019	0.0069	0.0079	0.0049	0.0049	0.0085	0	
A49	0.0083	0.0044	0.0054	0.007	0.0059	0.005	0.0066	0.0058	0.0053	0.0069	0.0019	0.0028	0.0038	0.0048	0.0049	0.0085	0
A50	0.0094	0.0045	0.0035	0.0071	0.006	0.002	0.0056	0.003	0.0034	0.007	0.0048	0.0038	0.0039	0.0049	0.0044	0.005	
A51	0.0067	0.0029	0.0039	0.0054	0.0044	0.0054	0.007	0.0044	0.0078	0.0094	0.0093	0.0104	0.0073	0.0043	0.0078	0.0075	
A52	0.0057	0.0039	0.0029	0.0065	0.0054	0.0044	0.0081	0.0034	0.0068	0.0105	0.0104	0.0093	0.0084	0.0053	0.0068	0.0086	
A53	0.0093	0.0054	0.0065	0.0029	0.007	0.0081	0.0045	0.007	0.0105	0.0069	0.0121	0.0132	0.01	0.0069	0.0105	0.005	
A54	0.0082	0.0044	0.0054	0.007	0.0029	0.007	0.0086	0.0059	0.0093	0.011	0.0108	0.0119	0.0089	0.0058	0.0093	0.0091	
A55	0.0033	0.0064	0.0054	0.0091	0.0079	0.0049	0.0086	0.0049	0.0093	0.0132	0.0077	0.0067	0.0099	0.0078	0.0043	0.0112	
A56	0.0067	0.008	0.0091	0.0054	0.0096	0.0086	0.005	0.0086	0.0132	0.0094	0.0093	0.0104	0.0116	0.0094	0.0078	0.0075	
A57	0.0053	0.0054	0.0044	0.0081	0.007	0.0029	0.0066	0.0039	0.0084	0.0122	0.0099	0.0089	0.009	0.0069	0.0078	0.0075	
A58	0.0053	0.0044	0.0034	0.007	0.0059	0.0039	0.0076	0.0029	0.0073	0.0111	0.0099	0.0089	0.0079	0.0058	0.0063	0.0103	
A59	0.0076	0.0078	0.0068	0.0105	0.0093	0.0084	0.0122	0.0073	0.0028	0.0062	0.0061	0.0051	0.0042	0.0072	0.0063	0.0092	
A60	0.0112	0.0094	0.0105	0.0069	0.011	0.0122	0.0085	0.0111	0.0062	0.0028	0.0076	0.0087	0.0057	0.0088	0.0087	0.0084	
A61	0.01	0.0083	0.0093	0.011	0.0067	0.011	0.0127	0.0099	0.0051	0.0066	0.0066	0.0076	0.0047	0.0088	0.0124	0.0048	
A62	0.006	0.0093	0.0104	0.0121	0.0108	0.0099	0.0116	0.0099	0.0061	0.0076	0.0027	0.0037	0.0047	0.0076	0.0112	0.0089	
A63	0.0051	0.0104	0.0093	0.0132	0.0119	0.0089	0.0127	0.0089	0.0051	0.0087	0.0037	0.0027	0.0057	0.0087	0.0071	0.0099	
A64	0.0071	0.0094	0.0084	0.0122	0.011	0.0069	0.0106	0.0079	0.0042	0.0077	0.0057	0.0027	0.0057	0.0097	0.0061	0.011	
A65	0.0107	0.0111	0.0122	0.0085	0.0127	0.0106	0.007	0.0117	0.0077	0.0043	0.0072	0.0047	0.0047	0.0088	0.0082	0.01	
A66	0.0081	0.0073	0.0084	0.01	0.0089	0.009	0.0106	0.0079	0.0042	0.0057	0.0047	0.0082	0.0062	0.0104	0.0119	0.0064	
A67	0.0071	0.0084	0.0073	0.0111	0.0099	0.0079	0.0117	0.0069	0.0033	0.0067	0.0057	0.0057	0.0028	0.0067	0.0092	0.0079	
A68	0.0051	0.0043	0.0053	0.0069	0.0058	0.0069	0.0085	0.0058	0.0072	0.0088	0.0087	0.0047	0.0038	0.0077	0.0082	0.009	
A69	0.0042	0.0053	0.0043	0.0079	0.0068	0.0058	0.0096	0.0048	0.0062	0.0098	0.0097	0.0097	0.0067	0.0028	0.0062	0.0079	
A70	0.0076	0.0069	0.0079	0.0044	0.0084	0.0096	0.0059	0.0085	0.0098	0.0062	0.0113	0.0087	0.0077	0.0038	0.0052	0.009	
A71	0.0037	0.0069	0.0058	0.0096	0.0084	0.0044	0.008	0.0054	0.0077	0.0115	0.0092	0.0124	0.0093	0.0053	0.0088	0.0054	
A72	0.0067	0.0059	0.0049	0.0086	0.0074	0.0065	0.0103	0.0054	0.0048	0.0084	0.0083	0.0082	0.0083	0.0053	0.0047	0.0106	
A73	0.0062	0.0075	0.0065	0.0103	0.0091	0.005	0.0087	0.006	0.0063	0.01	0.0078	0.0073	0.0063	0.0063	0.0078	0.0065	
A74	0.0051	0.0104	0.0093	0.0132	0.0119	0.0089	0.0127	0.0089	0.0051	0.0087	0.0037	0.0068	0.0069	0.0079	0.0073	0.0081	
A75	0.0073	0.0024	0.0034	0.005	0.0039	0.005	0.0066	0.0039	0.0073	0.009	0.0089	0.0027	0.0057	0.0097	0.0061	0.011	
B76	0.0062	0.0034	0.0024	0.006	0.0049	0.0039	0.0076	0.0029	0.0063	0.01	0.0089	0.0099	0.0069	0.0038	0.0073	0.007	
B77	0.0068	0.0029	0.0039	0.0055	0.0044	0.0045	0.0061	0.0035	0.0079	0.0096	0.0084	0.0099	0.0089	0.0079	0.0048	0.0063	
B78	0.0081	0.0073	0.0063	0.01	0.0089	0.0079	0.0117	0.0069	0.0023	0.0057	0.0057	0.004	0.0064	0.0044	0.0069	0.0076	
B79	0.0118	0.009	0.01	0.0064	0.0105	0.0117	0.008	0.0106	0.0057	0.0023	0.0072	0.0047	0.0038	0.0067	0.0082	0.0079	
B80	0.0056	0.0099	0.0089	0.0127	0.0115	0.0084	0.0122	0.0084	0.0047	0.0082	0.0032	0.0082	0.0053	0.0083	0.0119	0.0044	
B81	0.0091	0.0116	0.0127	0.009	0.0132	0.0122	0.0085	0.0122	0.0082	0.0047	0.0047	0.0023	0.0052	0.0092	0.0057	0.0105	
B82	0.0057	0.0038	0.0048	0.0064	0.0053	0.0064	0.008	0.00584	0.0067	0.0083	0.0082	0.0057	0.0067	0.0108	0.0092	0.0069	
B83	0.0047	0.0048	0.0038	0.0074	0.0063	0.0054	0.0091	0.0044	0.0057	0.0093	0.0092	0.0092	0.0062	0.0023	0.0057	0.0074	
B84	0.011	0.007	0.0081	0.0045	0.0086	0.0098	0.0061	0.0087	0.0079	0.0044	0.0094	0.0062	0.0073	0.0033	0.0047	0.0085	
B85	0.0093	0.0065	0.0075	0.0092	0.0049	0.0081	0.0098	0.007	0.0073	0.009	0.0078	0.0105	0.0074	0.0074	0.0111	0.0024	
B86	0.0014	0.0085	0.0074	0.0112	0.01	0.007	0.0108	0.007	0.0115	0.0154	0.0098	0.0089	0.0058	0.0069	0.0094	0.007	
B87	0.0047	0.0102	0.0112	0.0075	0.0117	0.0108	0.007	0.0108	0.0154	0.0116	0.0115	0.0088	0.0121	0.0099	0.0062	0.0135	
B88	0.0066	0.0089	0.0099	0.0116	0.0104	0.0116	0.0133	0.0105	0.0057	0.0072	0.0071	0.0125	0.0138	0.0116	0.0099	0.0097	
B89	0.0091	0.0116	0.0127	0.009	0.0132	0.0145	0.0106	0.0133	0.0082	0.0047	0.0097	0.0081	0.0052	0.0082	0.0118	0.0094	
B90	0.0066	0.0143	0.0154	0.0116	0.0159	0.0149	0.0111	0.0149	0.0107	0.0072	0.0071	0.0107	0.0077	0.0108	0.0146	0.0069	
B91	0.0051	0.0105	0.0094	0.0133	0.0121	0.01	0.014	0.009	0.0052	0.0088	0.0076	0.0081	0.0092	0.0135	0.0118	0.0094	
B92	0.0032	0.0063	0.0073	0.009	0.0078	0.009	0.0106	0.0079	0.0092	0.0108	0.0107	0.0066	0.0057	0.0098	0.0103	0.0111	
B93	0.0023	0.0073	0.0063	0.01	0.0089	0.0079	0.0117	0.0069	0.0082	0.0119	0.0118	0.0118	0.0088	0.0047	0.0082	0.01	

	A33	A34	A35	A36	A37	A38	A39	A40	A41	A42	A43	A44	A45	A46	A47	A48
B94	0.0028	0.0079	0.009	0.0106	0.0094	0.0074	0.0091	0.0085	0.0108	0.0125	0.0103	0.0113	0.0093	0.0062	0.0077	0.0117
B95	0.0099	0	0.001	0.0024	0.0014	0.0024	0.004	0.0015	0.0048	0.0064	0.0063	0.0073	0.0044	0.0014	0.0048	0.0045
B96	0.0089	0.001	0	0.0035	0.0024	0.0015	0.005	0.0005	0.0038	0.0074	0.0073	0.0063	0.0054	0.0024	0.0038	0.0055
B97	0.0062	0.0034	0.0024	0.006	0.0049	0.0019	0.0055	0.0019	0.0063	0.01	0.0048	0.0038	0.0069	0.0048	0.0014	0.0081
B98	0.0084	0.0024	0.0015	0.005	0.0039	0	0.0035	0.001	0.0054	0.0091	0.0069	0.0058	0.0059	0.0039	0.0034	0.0071
B99	0.0118	0.0038	0.0048	0.0064	0.0053	0.0064	0.008	0.0054	0.0009	0.0023	0.0023	0.0033	0.0005	0.0033	0.0067	0.0044
B100	0.0091	0.0063	0.0073	0.009	0.0078	0.0069	0.0085	0.0069	0.0033	0.0047	0	0.0009	0.0019	0.0057	0.0042	0.0069
B101	0.0081	0.0073	0.0063	0.01	0.0089	0.0058	0.0096	0.0058	0.0023	0.0057	0.0009	0	0.0028	0.0067	0.0033	0.0079
B102	0.0118	0.009	0.01	0.0064	0.0105	0.0096	0.0059	0.0096	0.0057	0.0023	0.0023	0.0033	0.0043	0.0083	0.0067	0.0044
B103	0.0103	0.0064	0.0054	0.0091	0.0079	0.0039	0.0075	0.0049	0.0014	0.0048	0.0028	0.0019	0.0019	0.0058	0.0053	0.007
B104	0.0141	0.008	0.0091	0.0054	0.0096	0.0075	0.0039	0.0086	0.0048	0.0014	0.0043	0.0053	0.0033	0.0073	0.0089	0.0034
B105	0.0103	0.0054	0.0044	0.008	0.0069	0.0049	0.0086	0.0039	0.0005	0.0038	0.0028	0.0019	0.0009	0.0048	0.0053	0.0059
B106	0.0141	0.007	0.008	0.0044	0.0085	0.0086	0.005	0.0075	0.0038	0.0005	0.0043	0.0053	0.0024	0.0063	0.0089	0.0024
B107	0.0072	0.0024	0.0014	0.0049	0.0038	0.0029	0.0065	0.0019	0.0033	0.0068	0.0067	0.0057	0.0048	0.0009	0.0023	0.0059
B108	0.0057	0.0038	0.0048	0.0064	0.0053	0.0044	0.0059	0.0044	0.0067	0.0083	0.0033	0.0042	0.0053	0.0023	0.0009	0.0074
B109	0.0138	0.0045	0.0055	0.002	0.006	0.0071	0.0035	0.0061	0.0054	0.0019	0.0069	0.0079	0.0049	0.0049	0.0085	0.0074
B110	0.0073	0.0054	0.0044	0.0081	0.007	0.0069	0.0076	0.0039	0.0043	0.0079	0.0028	0.0019	0.0048	0.0058	0.0024	0.006
B111	0.0067	0.0029	0.0039	0.0054	0.0044	0.0054	0.007	0.0044	0.0078	0.0094	0.0093	0.0104	0.0073	0.0043	0.0078	0.0075
B112	0.0057	0.0039	0.0029	0.0065	0.0054	0.0044	0.0081	0.0034	0.0068	0.0105	0.0104	0.0093	0.0084	0.0053	0.0068	0.0086
B113	0.0093	0.0054	0.0055	0.0029	0.007	0.0081	0.0045	0.007	0.0105	0.0069	0.0121	0.0132	0.01	0.0069	0.0105	0.005
B114	0.0033	0.0064	0.0054	0.0091	0.0079	0.0049	0.0086	0.0049	0.0093	0.0132	0.0077	0.0067	0.0099	0.0078	0.0043	0.0112
B115	0.0067	0.008	0.0091	0.0054	0.0096	0.0086	0.005	0.0086	0.0132	0.0094	0.0093	0.0104	0.0116	0.0094	0.0078	0.0075
B116	0.0053	0.0054	0.0044	0.0081	0.007	0.0029	0.0066	0.0039	0.0084	0.0122	0.0099	0.0089	0.009	0.0069	0.0063	0.0103
B117	0.0053	0.0044	0.0034	0.007	0.0059	0.0039	0.0076	0.0029	0.0073	0.0111	0.0099	0.0089	0.0079	0.0058	0.0063	0.0092
B118	0.0089	0.006	0.007	0.0035	0.0075	0.0076	0.004	0.0066	0.0111	0.0074	0.0116	0.0127	0.0096	0.0074	0.01	0.0055
B119	0.0076	0.0078	0.0068	0.0105	0.0093	0.0084	0.0122	0.0073	0.0028	0.0062	0.0061	0.00514	0.0042	0.0072	0.0087	0.0084
B120	0.006	0.0093	0.0104	0.0121	0.0108	0.0099	0.0116	0.0099	0.0061	0.0076	0.0027	0.0037	0.0047	0.0087	0.0071	0.0099
B121	0.0051	0.0104	0.0093	0.0132	0.0119	0.0089	0.0127	0.0089	0.0051	0.0087	0.0037	0.0027	0.0057	0.0097	0.0061	0.011
B122	0.0081	0.0084	0.0094	0.0111	0.0099	0.0079	0.0096	0.009	0.0052	0.0067	0.0047	0.0057	0.0038	0.0077	0.0092	0.009
B123	0.0042	0.0053	0.0043	0.0079	0.0068	0.0058	0.0096	0.0048	0.0062	0.0098	0.0097	0.0087	0.0077	0.0038	0.0052	0.009
B124	0.0037	0.0069	0.0058	0.0096	0.0084	0.0044	0.008	0.0054	0.0077	0.0115	0.0092	0.0082	0.0083	0.0053	0.0047	0.0106
D125	0.0072	0.0085	0.0096	0.0059	0.01	0.008	0.0044	0.0091	0.0115	0.0078	0.0108	0.0119	0.0099	0.0068	0.0083	0.007
D126	0.0058	0.005	0.0039	0.0076	0.0065	0.0024	0.0061	0.0035	0.0079	0.0117	0.0094	0.0084	0.0085	0.0064	0.0058	0.0098
D127	0.0058	0.0039	0.0029	0.0066	0.0054	0.0035	0.0071	0.0024	0.0069	0.0106	0.0094	0.0084	0.0074	0.0054	0.0058	0.0087
D128	0.0056	0.0099	0.0089	0.0127	0.0115	0.0084	0.0122	0.0084	0.0047	0.0082	0.0032	0.0023	0.0052	0.0092	0.0057	0.0105
D129	0.0076	0.009	0.0079	0.0117	0.0105	0.0064	0.0102	0.0074	0.0038	0.0073	0.0052	0.0042	0.0043	0.0083	0.0077	0.0096
D130	0.0047	0.0048	0.0038	0.0074	0.0063	0.0054	0.0091	0.0044	0.0057	0.0093	0.0092	0.0082	0.0073	0.0033	0.0047	0.0085
D131	0.0068	0.007	0.006	0.0098	0.0086	0.0045	0.0082	0.0055	0.0058	0.0096	0.0073	0.0063	0.0064	0.0074	0.0069	0.0076
D132	0.0073	0.0075	0.0086	0.005	0.0091	0.0103	0.0066	0.0092	0.0127	0.009	0.0143	0.0154	0.0122	0.009	0.0127	0.007
D133	0.0014	0.0085	0.0074	0.0112	0.01	0.007	0.0108	0.007	0.0115	0.0154	0.0098	0.0088	0.0121	0.0099	0.0062	0.0135
D134	0.0033	0.0075	0.0065	0.0103	0.0091	0.005	0.0087	0.006	0.0105	0.0145	0.0121	0.011	0.0111	0.009	0.0084	0.0125
D135	0.0066	0.0089	0.0099	0.0116	0.0104	0.0116	0.0133	0.0105	0.0057	0.0072	0.0071	0.0081	0.0052	0.0082	0.0118	0.0094
D136	0.0032	0.0125	0.0115	0.0154	0.0141	0.011	0.0149	0.011	0.0071	0.0107	0.0056	0.0046	0.0076	0.0118	0.0081	0.0132
D137	0.0051	0.0116	0.0105	0.0145	0.0132	0.009	0.0128	0.01	0.0062	0.0098	0.0076	0.0066	0.0067	0.0108	0.0103	0.0122
D138	0.0087	0.0133	0.0145	0.0106	0.0149	0.0128	0.0094	0.014	0.0098	0.0062	0.0092	0.0103	0.0083	0.0125	0.0141	0.0085
D139	0.0076	0.0121	0.0132	0.0149	0.0104	0.0116	0.0133	0.0087	0.0103	0.0081	0.0091	0.0072	0.0113	0.0129	0.0127	
D140	0.0023	0.0073	0.0063	0.01	0.0089	0.0079	0.0117	0.0069	0.0082	0.0119	0.0118	0.0107	0.0098	0.0057	0.0072	0.0111
D141	0.0052	0.0106	0.0117	0.008	0.0122	0.0102	0.0065	0.0112	0.0136	0.0099	0.013	0.0141	0.0121	0.0089	0.0104	0.0091
D142	0.0047	0.008	0.007	0.0108	0.0096	0.0086	0.0125	0.0075	0.0068	0.0105	0.0104	0.0053	0.0084	0.0084	0.0099	0.0086
D143	0.0033	0.0096	0.0106	0.0124	0.0111	0.0102	0.0119	0.0102	0.0104	0.0121	0.0067	0.0077	0.0089	0.0099	0.0083	0.0102
D144	0.0127	0.0024	0.0035	0	0.0039	0.005	0.0115	0.004	0.0074	0.0039	0.009	0.01	0.007	0.0039	0.0074	0.002
D145	0.0062	0.0034	0.0024	0.006	0.0049	0.0019	0.0055	0.0019	0.0063	0.01	0.0048	0.0038	0.0069	0.0048	0.0014	0.0081
D146	0.0122	0.004	0.005	0.0015	0.0055	0.0035	0	0.0045	0.0091	0.0054	0.0085	0.0096	0.0075	0.0054	0.0014	0.0081
D147	0.011	0.0029	0.0039	0.0055	0.0014	0.0024	0.004	0.0035	0.0079	0.0096	0.0073	0.0084	0.0064	0.0044	0.007	0.0035
D148	0.0122	0.003	0.004	0.0005	0.0045	0.0045	0.001	0.0035	0.008	0.0044	0.0085	0.0096	0.0065	0.0044	0.007	0.0025
D149	0.0081	0.0073	0.0063	0.01	0.0089	0.0058	0.0096	0.0058	0.0023	0.0057	0.0009	0	0.0028	0.0067	0.0033	0.0079
D150	0.0118	0.009	0.01	0.0064	0.0105	0.0096	0.0059	0.0096	0.0057	0.0023	0.0023	0.0033	0.0043	0.0083	0.0067	0.0044
D151	0.0082	0.0014	0.0024	0.0039	0.0029	0.0039	0.0054	0.0029	0.0043	0.0058	0.0057	0.0067	0.0038	0	0.0033	0.0049
D152	0.0072	0.0024	0.0014	0.0049	0.0038	0.0029	0.0065	0.0019	0.0033	0.0068	0.0067	0.0057	0.0048	0	0.0023	0.0059
D153	0.0047	0.0048	0.0038	0.0074	0.0063	0.0034	0.007	0.0034	0.0057	0.0093	0.0042	0.0033	0.0062	0.0033	0	0.0085
D154	0.0104	0.0054	0.0065	0.0029	0.007	0.005	0.0015	0.006	0.0084	0.0048	0.0078	0.0089	0.0069	0.0038	0.0053	0.0039
D155	0.0133	0.0061	0.0071	0.0035	0.0076	0.0056	0.002	0.0066	0.007	0.0034	0.0064	0.0074	0.0054	0.0065	0.008	0.0015

	A33	A34	A35	A36	A37	A38	A39	A40	A41	A42	A43	A44	A45	A46	A47	A48
D156	0.0067	0.0029	0.0039	0.0054	0.0044	0.0054	0.007	0.0044	0.0078	0.0094	0.0093	0.0104	0.0073	0.0043	0.0078	0.0075
D157	0.0057	0.0039	0.0029	0.0065	0.0054	0.0044	0.0081	0.0034	0.0068	0.0105	0.0104	0.0093	0.0084	0.0053	0.0068	0.0086
D158	0.0093	0.0054	0.0065	0.0029	0.007	0.0081	0.0045	0.007	0.0105	0.0069	0.0121	0.0132	0.01	0.0069	0.0105	0.005
D159	0.0042	0.0054	0.0064	0.008	0.0069	0.0059	0.0075	0.0059	0.0104	0.0121	0.0067	0.0077	0.0089	0.0068	0.0053	0.0102
D160	0.0033	0.0064	0.0054	0.0091	0.0079	0.0049	0.0086	0.0049	0.0093	0.0132	0.0077	0.0067	0.0099	0.0078	0.0043	0.0112
D161	0.0062	0.0044	0.0054	0.007	0.0059	0.0039	0.0055	0.005	0.0094	0.0111	0.0089	0.0099	0.0079	0.0058	0.0073	0.0092
D162	0.0053	0.0054	0.0044	0.0081	0.007	0.0029	0.0066	0.0039	0.0084	0.0122	0.0099	0.0089	0.009	0.0069	0.0063	0.0103
D163	0.0089	0.007	0.0081	0.0045	0.0086	0.0066	0.003	0.0076	0.0122	0.0085	0.0116	0.0127	0.0106	0.0085	0.01	0.0066
D164	0.0076	0.0078	0.0068	0.0105	0.0093	0.0084	0.0122	0.0073	0.0028	0.0062	0.0061	0.0051	0.0042	0.0072	0.0087	0.0084
D165	0.0112	0.0094	0.0105	0.0069	0.011	0.0122	0.0085	0.0111	0.0062	0.0028	0.0076	0.0087	0.0057	0.0088	0.0124	0.0048
D166	0.006	0.0093	0.0104	0.0121	0.0108	0.0099	0.0116	0.0099	0.0061	0.0076	0.0027	0.0037	0.0047	0.0087	0.0071	0.0099
D167	0.0051	0.0104	0.0093	0.0132	0.0119	0.0089	0.0127	0.0089	0.0051	0.0087	0.0037	0.0027	0.0057	0.0097	0.0061	0.011
D168	0.0086	0.0121	0.0132	0.0094	0.0136	0.0127	0.009	0.0127	0.0087	0.0052	0.0051	0.0061	0.0072	0.0113	0.0097	0.0073
D169	0.0081	0.0084	0.0094	0.0111	0.0099	0.0079	0.0096	0.009	0.0052	0.0067	0.0047	0.0057	0.0038	0.0077	0.0092	0.009
D170	0.0071	0.0094	0.0084	0.0122	0.011	0.0069	0.0106	0.0079	0.0042	0.0077	0.0057	0.0047	0.0047	0.0088	0.0082	0.01
D171	0.0071	0.0094	0.0084	0.0122	0.011	0.0069	0.0106	0.0079	0.0042	0.0077	0.0057	0.0047	0.0047	0.0088	0.0082	0.01
D172	0.0107	0.0111	0.0122	0.0085	0.0127	0.0106	0.007	0.0117	0.0077	0.0043	0.0072	0.0082	0.0062	0.0104	0.0119	0.0064
D173	0.0096	0.0099	0.011	0.0127	0.0083	0.0094	0.0111	0.0105	0.0066	0.0082	0.0061	0.0071	0.0052	0.0092	0.0107	0.0105
D174	0.0107	0.01	0.0111	0.0074	0.0116	0.0117	0.008	0.0106	0.0067	0.0033	0.0072	0.0082	0.0053	0.0093	0.0119	0.0054
D175	0.0047	0.0058	0.0059	0.0085	0.0073	0.0054	0.007	0.0064	0.0088	0.0104	0.0082	0.0092	0.0073	0.0043	0.0057	0.0096
D176	0.0072	0.0085	0.9096	0.0059	0.01	0.008	0.0044	0.0091	0.0115	0.0078	0.0108	0.0119	0.0099	0.0068	0.0083	0.007
D177	0.0037	0.0058	0.0048	0.0085	0.0073	0.0054	0.0091	0.0044	0.0067	0.0104	0.0092	0.0082	0.0073	0.0043	0.0087	0.0096
D178	0.0073	0.0065	0.0075	0.0092	0.008	0.006	0.0076	0.007	0.0073	0.009	0.0068	0.0078	0.0058	0.0069	0.0084	0.007
D179	0.0099	0.0092	0.0103	0.0066	0.0108	0.0087	0.005	0.0098	0.01	0.0064	0.0094	0.0105	0.0085	0.0096	0.0111	0.0045
D180	0.0051	0.0116	0.0105	0.0145	0.0132	0.009	0.0128	0.01	0.0062	0.0098	0.0076	0.0066	0.0067	0.0108	0.0103	0.0122
D181	0.0051	0.0116	0.0105	0.0145	0.0132	0.009	0.0128	0.01	0.0062	0.0098	0.0076	0.0066	0.0067	0.0108	0.0103	0.0122
D182	0.0051	0.0116	0.0105	0.0145	0.0132	0.009	0.0128	0.01	0.0062	0.0098	0.0076	0.0066	0.0067	0.0108	0.0103	0.0122
D183	0.0372	0.0373	0.0358	0.0354	0.0393	0.0339	0.0334	0.0354	0.0349	0.0344	0.034	0.0326	0.0358	0.0373	0.0335	0.0368

	A49	A50	A51	A52	A53	A54	A55	A56	A57	A58	A59	A60	A61	A62	A63	A64
A49	0															
A50	0.0029	0														
A51	0.0074	0.0075	0													
A52	0.0085	0.0065	0.0009	0												
A53	0.0102	0.0103	0.0024	0.0034	0											
A54	0.009	0.0091	0.0014	0.0024	0.0038	0										
A55	0.0058	0.007	0.0033	0.0024	0.0058	0.0048	0									
A56	0.0074	0.0108	0.0048	0.0058	0.0024	0.0063	0.0033	0								
A57	0.008	0.005	0.0024	0.0014	0.0049	0.0038	0.0019	0.0054	0							
A58	0.008	0.006	0.0014	0.0005	0.0039	0.0029	0.0019	0.0054	0.001	0						
A59	0.0083	0.0063	0.0047	0.0038	0.0073	0.0062	0.0062	0.0098	0.0053	0.0043	0					
A60	0.0099	0.01	0.0062	0.0073	0.0038	0.0077	0.0098	0.0062	0.0089	0.0078	0.0032	0.0037	0			
A61	0.0088	0.0089	0.0052	0.0062	0.0077	0.0037	0.0087	0.0103	0.0077	0.0067	0.0067	0.0032	0.0046	0.0036	0	
A62	0.0047	0.0078	0.0062	0.0072	0.0088	0.0098	0.0087	0.0047	0.0062	0.0057	0.0057	0.0023	0.0056	0.0046	0.0009	0
A63	0.0057	0.0068	0.0072	0.0062	0.0098	0.0087	0.0037	0.0072	0.0057	0.0057	0.0014	0.0047	0.0037	0.0027	0.0018	0
A64	0.0078	0.0048	0.0062	0.0053	0.0089	0.0077	0.0057	0.0093	0.0038	0.0048	0.0047	0.0014	0.0051	0.0042	0.0051	0.0033
A65	0.0094	0.0085	0.0078	0.0089	0.0053	0.0093	0.0093	0.0058	0.0073	0.0084	0.0047	0.0014	0.0028	0.0018	0.0018	0.0027
A66	0.0068	0.0069	0.0043	0.0053	0.0068	0.0057	0.0067	0.0083	0.0058	0.0048	0.0005	0.0037	0.0027	0.0027	0.0018	0.0009
A67	0.0078	0.0058	0.0053	0.0043	0.0078	0.0067	0.0057	0.0093	0.0038	0.0028	0.0042	0.0057	0.0046	0.0056	0.0066	0.0057
A68	0.0078	0.0079	0.0014	0.0023	0.0038	0.0028	0.0038	0.0073	0.0028	0.0019	0.0032	0.0066	0.0056	0.0066	0.0056	0.0047
A69	0.0089	0.0069	0.0023	0.0014	0.0048	0.0038	0.0038	0.0073	0.0028	0.0019	0.0066	0.0033	0.0071	0.0081	0.0091	0.0082
A70	0.0105	0.0106	0.0038	0.0048	0.0014	0.0053	0.0073	0.0038	0.0063	0.0053	0.0047	0.0082	0.0071	0.0061	0.0051	0.0033
A71	0.0084	0.0054	0.0038	0.0028	0.0063	0.0053	0.0033	0.0068	0.0014	0.0024	0.0019	0.0053	0.0042	0.0052	0.0042	0.0033
A72	0.0064	0.0044	0.0029	0.0019	0.0054	0.0043	0.0043	0.0079	0.0034	0.0024	0.0019	0.0068	0.0057	0.0047	0.0038	0.0019
A73	0.0059	0.0029	0.0044	0.0034	0.007	0.0058	0.0038	0.0074	0.0019	0.0029	0.0033	0.0068	0.0057	0.0047	0.0038	0.0009
A74	0.0057	0.0068	0.0072	0.0062	0.0098	0.0087	0.0037	0.0072	0.0057	0.0057	0.0023	0.0093	0.011	0.0098	0.0108	0.0119
A75	0.007	0.007	0.0044	0.0054	0.007	0.0058	0.0079	0.0096	0.007	0.0059	0.0093	0.011	0.0098	0.0108	0.0119	0.0108
B76	0.008	0.006	0.0054	0.0044	0.008	0.0069	0.0069	0.0106	0.0059	0.0049	0.0083	0.0121	0.0108	0.0119	0.0108	0.0099
B77	0.0065	0.0066	0.0049	0.0059	0.0075	0.0064	0.0074	0.0091	0.0065	0.0054	0.0099	0.0116	0.0104	0.0104	0.0115	0.0105
B78	0.0078	0.0058	0.0093	0.0083	0.0121	0.0108	0.0108	0.0148	0.011	0.0138	0.0127	0.0076	0.0042	0.0081	0.0091	0.0101
B79	0.0094	0.0096	0.011	0.0121	0.0084	0.0125	0.0148	0.0135	0.0082	0.0119	0.0104	0.0104	0.0066	0.0101	0.009	0.0051
B80	0.0053	0.0063	0.0119	0.0108	0.0148	0.0135	0.0082	0.0119	0.0083	0.0143	0.0143	0.0101	0.0066	0.0106	0.0066	0.0076
B81	0.0068	0.01	0.0136	0.0148	0.011	0.0152	0.0119	0.0083	0.0143	0.0143	0.0087	0.0103	0.0091	0.0101	0.0112	0.0103
B82	0.0073	0.0074	0.0058	0.0068	0.0084	0.0073	0.0093	0.011	0.0084	0.0073	0.0087	0.0113	0.0101	0.0112	0.0101	0.0092
B83	0.0084	0.0064	0.0068	0.0058	0.0094	0.0083	0.0083	0.0121	0.0073	0.0119	0.0108	0.0099	0.0063	0.0104	0.0115	0.0125
B84	0.0075	0.0076	0.0091	0.0102	0.0065	0.0106	0.0128	0.0091	0.0119	0.0102	0.0091	0.0093	0.011	0.0067	0.0098	0.0108
B85	0.0059	0.006	0.0085	0.0096	0.0112	0.0069	0.0111	0.0128	0.0102	0.0091	0.0093	0.0082	0.0119	0.0107	0.0066	0.0057
B86	0.0079	0.0091	0.0053	0.0043	0.0079	0.0068	0.0019	0.0053	0.0038	0.0038	0.0082	0.0119	0.0083	0.0124	0.0082	0.0092
B87	0.00099	0.013	0.0069	0.0079	0.0044	0.0084	0.0053	0.0019	0.0074	0.0074	0.0119	0.0083	0.0027	0.0042	0.0032	0.0041
B88	0.0093	0.0094	0.0057	0.0067	0.0083	0.0072	0.0092	0.0108	0.0083	0.0073	0.0027	0.0042	0.0018	0.0056	0.0066	0.0076
B89	0.0121	0.0122	0.0083	0.0093	0.0058	0.0098	0.0119	0.0083	0.011	0.0099	0.0051	0.0042	0.008	0.0041	0.0051	0.0071
B90	0.0093	0.0127	0.0108	0.0119	0.0083	0.0124	0.0092	0.0077	0.0115	0.0068	0.0058	0.0023	0.0057	0.0046	0.0046	0.0037
B91	0.0099	0.0079	0.0073	0.0062	0.0099	0.0088	0.0077	0.0115	0.0068	0.0048	0.0061	0.0076	0.0066	0.0076	0.0086	0.0076
B92	0.0099	0.01	0.0033	0.0043	0.0058	0.0047	0.0067	0.0083	0.0058	0.0038	0.0051	0.0067	0.0076	0.0086	0.0076	0.0066
B93	0.011	0.009	0.0043	0.0033	0.0068	0.0057	0.0057	0.0093	0.0048	0.0038	0.0053	0.0076	0.0092	0.0081	0.0071	0.0081
B94	0.0094	0.0085	0.0048	0.0058	0.0073	0.0062	0.0062	0.0078	0.0043	0.0053	0.0078	0.0094	0.0083	0.0093	0.0104	0.0094
B95	0.0044	0.0045	0.0029	0.0039	0.0054	0.0044	0.0064	0.008	0.0054	0.0044	0.0078	0.0105	0.0093	0.0104	0.0093	0.0084
B96	0.0054	0.0035	0.0039	0.0029	0.0065	0.0054	0.0054	0.0091	0.0044	0.0034	0.0068	0.0132	0.0119	0.0077	0.0067	0.0089
B97	0.0029	0.0039	0.0064	0.0054	0.0091	0.0079	0.0029	0.0064	0.0049	0.0049	0.0093	0.0122	0.011	0.0099	0.0089	0.0069
B98	0.005	0.002	0.0054	0.0044	0.0081	0.007	0.0049	0.0086	0.0029	0.0039	0.0037	0.0052	0.0042	0.0051	0.0061	0.0052
B99	0.0043	0.0044	0.0068	0.0078	0.0094	0.0083	0.0104	0.0121	0.0094	0.0084	0.0037	0.0061	0.0076	0.0066	0.0027	0.0037
B100	0.0019	0.0048	0.0093	0.0104	0.0121	0.0108	0.0077	0.0093	0.0099	0.0099	0.0061	0.0087	0.0076	0.0037	0.0027	0.0047
B101	0.0028	0.0038	0.0104	0.0093	0.0132	0.0119	0.0067	0.0104	0.0068	0.0127	0.0127	0.0087	0.0052	0.0091	0.0051	0.0061
B102	0.0043	0.0074	0.0121	0.0132	0.0094	0.0136	0.0104	0.0089	0.0127	0.0069	0.0079	0.0042	0.0077	0.0066	0.0057	0.0047
B103	0.0048	0.0019	0.0094	0.0084	0.0122	0.011	0.0089	0.0127	0.0069	0.0106	0.0117	0.0077	0.0043	0.0082	0.0072	0.0082
B104	0.0064	0.0054	0.0111	0.0122	0.0085	0.0127	0.0127	0.009	0.0106	0.0117	0.0077	0.0033	0.0067	0.0057	0.0057	0.0047
B105	0.0048	0.0029	0.0084	0.0073	0.0111	0.0099	0.0089	0.0127	0.0079	0.0069	0.0033	0.0067	0.0033	0.0072	0.0072	0.0082
B106	0.0064	0.0065	0.01	0.0111	0.0074	0.0116	0.0127	0.009	0.0117	0.0106	0.0067	0.0033	0.0072	0.0072	0.0082	0.0073
B107	0.0058	0.0039	0.0053	0.0043	0.0079	0.0068	0.0068	0.0105	0.0058	0.0048	0.0062	0.0098	0.0087	0.0097	0.0087	0.0077
B108	0.0024	0.0054	0.0068	0.0078	0.0094	0.0083	0.0053	0.0068	0.0073	0.0073	0.0097	0.0113	0.0101	0.0061	0.0071	0.0092
B109	0.005	0.005	0.0075	0.0086	0.005	0.0091	0.0112	0.0075	0.0103	0.0092	0.0084	0.0048	0.0089	0.0099	0.011	0.01

	A49	A50	A51	A52	A53	A54	A55	A56	A57	A58	A59	A60	A61	A62	A63	A64
B110	0.001	0.0019	0.0085	0.0074	0.0112	0.01	0.0048	0.0085	0.007	0.007	0.0073	0.011	0.0098	0.0057	0.0047	0.0068
B111	0.0074	0.0075	0	0.0009	0.0024	0.0014	0.0033	0.0048	0.0024	0.0014	0.0047	0.0062	0.0052	0.0062	0.0072	0.0062
B112	0.0085	0.0065	0.0009	0	0.0034	0.0024	0.0024	0.0058	0.0014	0.0005	0.0038	0.0073	0.0062	0.0072	0.0062	0.0053
B113	0.0102	0.0103	0.0024	0.0034	0	0.0038	0.0058	0.0024	0.0049	0.0039	0.0073	0.0038	0.0077	0.0088	0.0098	0.0089
B114	0.0058	0.007	0.0033	0.0024	0.0058	0.0048	0	0.0033	0.0019	0.0019	0.0062	0.0098	0.0087	0.0047	0.0037	0.0057
B115	0.0074	0.0108	0.0048	0.0058	0.0024	0.0063	0.0033	0	0.0054	0.0054	0.0098	0.0062	0.0103	0.0062	0.0072	0.0093
B116	0.008	0.005	0.0024	0.0014	0.0049	0.0038	0.0019	0.0054	0	0.001	0.0053	0.0089	0.0077	0.0067	0.0057	0.0038
B117	0.008	0.006	0.0014	0.0005	0.0039	0.0029	0.0019	0.0054	0.001	0	0.0043	0.0078	0.0067	0.0067	0.0057	0.0048
B118	0.0097	0.0098	0.0029	0.0039	0.0005	0.0044	0.0054	0.0019	0.0044	0.0034	0.0078	0.0043	0.0083	0.0083	0.0093	0.0084
B119	0.0083	0.0063	0.0047	0.0038	0.0073	0.0062	0.0062	0.0098	0.0053	0.0043	0	0.0032	0.0023	0.0032	0.0023	0.0014
B120	0.0047	0.0078	0.0062	0.0072	0.0088	0.0076	0.0047	0.0062	0.0067	0.0067	0.0032	0.0046	0.0036	0	0.0009	0.0027
B121	0.0057	0.0068	0.0072	0.0062	0.0098	0.0087	0.0037	0.0072	0.0057	0.0057	0.0023	0.0056	0.0046	0.0009	0	0.0018
B122	0.0068	0.0058	0.0053	0.0062	0.0078	0.0067	0.0067	0.0083	0.0048	0.0058	0.0023	0.0037	0.0027	0.0018	0.0027	0.0009
B123	0.0089	0.0069	0.0023	0.0014	0.0048	0.0038	0.0038	0.0073	0.0028	0.0019	0.0032	0.0066	0.0056	0.0066	0.0056	0.0047
B124	0.0084	0.0054	0.0038	0.0028	0.0063	0.0053	0.0033	0.0068	0.0014	0.0024	0.0047	0.0082	0.0071	0.0061	0.0051	0.0033
D125	0.01	0.0091	0.0053	0.0063	0.0029	0.0068	0.0068	0.0033	0.0048	0.0058	0.0082	0.0047	0.0087	0.0076	0.0087	0.0067
D126	0.0075	0.0045	0.007	0.0059	0.0097	0.0085	0.0064	0.0102	0.0044	0.0054	0.0099	0.0138	0.0125	0.0115	0.0104	0.0084
D127	0.0075	0.0055	0.0059	0.0049	0.0086	0.0074	0.0064	0.0102	0.0054	0.0044	0.0089	0.0127	0.0115	0.0115	0.0104	0.0094
D128	0.0053	0.0063	0.0119	0.0108	0.0148	0.0135	0.0082	0.0119	0.0104	0.0104	0.0066	0.0101	0.009	0.0051	0.0041	0.0061
D129	0.0073	0.0044	0.011	0.0099	0.0138	0.0125	0.0104	0.0143	0.0064	0.0094	0.0057	0.0092	0.0081	0.0071	0.0061	0.0042
D139	0.0084	0.0064	0.0068	0.0058	0.0094	0.0083	0.0083	0.0121	0.0073	0.0063	0.0076	0.0113	0.0092	0.0112	0.0101	0.0092
D131	0.0054	0.0024	0.0091	0.008	0.0119	0.0106	0.0085	0.0124	0.0065	0.0075	0.0078	0.0116	0.0104	0.0093	0.0083	0.0063
D132	0.0124	0.0125	0.0044	0.0054	0.0019	0.0058	0.0079	0.0044	0.007	0.0059	0.0093	0.0058	0.0098	0.0108	0.0119	0.011
D133	0.0079	0.0091	0.0053	0.0043	0.0079	0.0068	0.0019	0.0053	0.0038	0.0038	0.0082	0.0119	0.0107	0.0066	0.0057	0.0077
D134	0.0102	0.007	0.0044	0.0034	0.007	0.0058	0.0038	0.0074	0.0019	0.0029	0.0073	0.011	0.0098	0.0088	0.0077	0.0058
D135	0.0093	0.0094	0.0057	0.0067	0.0083	0.0072	0.0092	0.0108	0.0083	0.0073	0.0027	0.0042	0.0032	0.0041	0.0051	0.0042
D136	0.0077	0.0089	0.0092	0.0082	0.0119	0.0107	0.0057	0.0092	0.0077	0.0077	0.0041	0.0076	0.0065	0.0027	0.0018	0.0037
D137	0.0099	0.0069	0.0083	0.0073	0.011	0.0098	0.0077	0.0115	0.0058	0.0068	0.0032	0.0066	0.0056	0.0046	0.0037	0.0018
D138	0.0116	0.0106	0.0099	0.011	0.0073	0.0115	0.0115	0.0078	0.0094	0.0105	0.0066	0.0033	0.0071	0.0061	0.0071	0.0052
D139	0.0104	0.0094	0.0088	0.0098	0.0115	0.0072	0.0103	0.0119	0.0083	0.0093	0.0056	0.0071	0.0032	0.0051	0.006	0.0042
D140	0.011	0.009	0.0043	0.0033	0.0068	0.0057	0.0057	0.0093	0.0048	0.0038	0.0051	0.0087	0.0076	0.0086	0.0076	0.0066
D141	0.0122	0.0112	0.0073	0.0084	0.0048	0.0089	0.0089	0.0053	0.0069	0.0079	0.0103	0.0067	0.0107	0.0097	0.0107	0.0088
D142	0.0085	0.0065	0.0048	0.0038	0.0074	0.0063	0.0063	0.01	0.0054	0.0044	0.0038	0.0073	0.0062	0.0072	0.0062	0.0053
D143	0.0048	0.008	0.0063	0.0073	0.009	0.0078	0.0048	0.0063	0.0069	0.0069	0.0072	0.0088	0.0076	0.0037	0.0047	0.0067
D144	0.007	0.0071	0.0054	0.0065	0.0029	0.007	0.0091	0.0054	0.0081	0.007	0.0105	0.0069	0.011	0.0121	0.0132	0.0122
D145	0.0029	0.0039	0.0064	0.0054	0.0091	0.0079	0.0029	0.0064	0.0049	0.0049	0.0093	0.0132	0.0119	0.0077	0.0067	0.0089
D146	0.0066	0.0056	0.007	0.0081	0.0045	0.0086	0.0086	0.005	0.0066	0.0076	0.0122	0.0085	0.0127	0.0116	0.0127	0.0106
D147	0.0054	0.0045	0.0059	0.007	0.0086	0.0044	0.0074	0.0091	0.0054	0.0065	0.011	0.0127	0.0083	0.0104	0.0115	0.0094
D148	0.0066	0.0066	0.006	0.007	0.0035	0.0075	0.0086	0.005	0.0076	0.0066	0.0111	0.0074	0.0116	0.0116	0.0127	0.0117
D149	0.0028	0.0038	0.0104	0.0093	0.0132	0.0119	0.0067	0.0104	0.0089	0.0089	0.0051	0.0087	0.0076	0.0037	0.0027	0.0047
D150	0.0043	0.0074	0.0121	0.0132	0.0094	0.0136	0.0104	0.0068	0.0127	0.0127	0.0087	0.0052	0.0091	0.0054	0.0061	0.0082
D151	0.0048	0.0049	0.0043	0.0053	0.0069	0.0058	0.0078	0.0094	0.0069	0.0058	0.0072	0.0088	0.0076	0.0087	0.0097	0.0088
D152	0.0058	0.0039	0.0053	0.0043	0.0079	0.0068	0.0068	0.0105	0.0058	0.0048	0.0062	0.0098	0.0087	0.0097	0.0087	0.0077
D153	0.0033	0.0044	0.0078	0.0068	0.0105	0.0093	0.0043	0.0078	0.0063	0.0063	0.0087	0.0124	0.0112	0.0071	0.0061	0.0082
D154	0.007	0.006	0.0085	0.0096	0.0059	0.01	0.01	0.0064	0.008	0.0091	0.0115	0.0078	0.0119	0.0108	0.0119	0.0099
D155	0.0045	0.0035	0.0092	0.0103	0.0066	0.0108	0.0108	0.007	0.0087	0.0098	0.01	0.0064	0.0105	0.0094	0.0105	0.0085
D156	0.0074	0.0075	0	0.0009	0.0024	0.0014	0.0033	0.0048	0.0024	0.0014	0.0047	0.0062	0.0052	0.0062	0.0072	0.0062
D157	0.0085	0.0065	0.0009	0	0.0034	0.0024	0.0024	0.0058	0.0014	0.0005	0.0038	0.0073	0.0062	0.0072	0.0062	0.0053
D158	0.0102	0.0103	0.0024	0.0034	0	0.0038	0.0058	0.0024	0.0049	0.0039	0.0073	0.0038	0.0077	0.0088	0.0098	0.0089
D159	0.0048	0.008	0.0024	0.0033	0.0048	0.0038	0.0009	0.0024	0.0029	0.0029	0.0072	0.0088	0.0076	0.0037	0.0047	0.0067
D160	0.0058	0.007	0.0033	0.0024	0.0058	0.0048	0	0.0033	0.0019	0.0019	0.0062	0.0098	0.0087	0.0047	0.0037	0.0057
D161	0.007	0.006	0.0014	0.0024	0.0039	0.0029	0.0029	0.0044	0.001	0.0019	0.0062	0.0078	0.0067	0.0057	0.0067	0.0048
D162	0.008	0.005	0.0024	0.0014	0.0049	0.0038	0.0019	0.0054	0	0.001	0.0053	0.0089	0.0077	0.0067	0.0057	0.0038
D163	0.0097	0.0087	0.0039	0.0049	0.0014	0.0054	0.0054	0.0019	0.0034	0.0044	0.0089	0.0053	0.0093	0.0083	0.0093	0.0073
D164	0.0083	0.0063	0.0047	0.0038	0.0073	0.0062	0.0062	0.0098	0.0053	0.0043	0	0.0032	0.0023	0.0032	0.0023	0.0014
D165	0.0099	0.01	0.0062	0.0073	0.0038	0.0077	0.0098	0.0062	0.0089	0.0078	0.0032	0	0.0037	0.0046	0.0056	0.0047
D166	0.0047	0.0078	0.0062	0.0072	0.0088	0.0076	0.0047	0.0062	0.0067	0.0067	0.0032	0.0046	0.0036	0	0.0009	0.0027
D167	0.0057	0.0068	0.0072	0.0062	0.0098	0.0087	0.0037	0.0072	0.0057	0.0057	0.0023	0.0056	0.0046	0.0009	0	0.0018
D168	0.0073	0.0105	0.0088	0.0098	0.0062	0.0103	0.0072	0.0038	0.0093	0.0093	0.0056	0.0023	0.006	0.0023	0.0032	0.0051
D169	0.0068	0.0058	0.0053	0.0062	0.0078	0.0067	0.0067	0.0083	0.0048	0.0058	0.0023	0.0037	0.0027	0.0018	0.0027	0.0009
D170	0.0078	0.0048	0.0062	0.0053	0.0089	0.0077	0.0057	0.0093	0.0038	0.0048	0.0014	0.0047	0.0037	0.0027	0.0018	0
D171	0.0078	0.0048	0.0062	0.0053	0.0089	0.0077	0.0057	0.0093	0.0038	0.0048	0.0014	0.0047	0.0037	0.0027	0.0018	0

	A49	A50	A51	A52	A53	A54	A55	A56	A57	A58	A59	A60	A61	A62	A63	A64
D172	0.0094	0.0085	0.0078	0.0089	0.0053	0.0093	0.0093	0.0058	0.0073	0.0084	0.0047	0.0014	0.0051	0.0042	0.0051	0.0033
D173	0.0083	0.0073	0.0067	0.0077	0.0093	0.0052	0.0082	0.0098	0.0062	0.0073	0.0037	0.0051	0.0013	0.0032	0.0041	0.0023
D174	0.0094	0.0096	0.0068	0.0078	0.0043	0.0083	0.0093	0.0058	0.0084	0.0073	0.0037	0.0005	0.0042	0.0042	0.0051	0.0042
D175	0.0073	0.0064	0.0028	0.0038	0.0053	0.0043	0.0043	0.0058	0.0024	0.0033	0.0057	0.0072	0.0061	0.0051	0.0061	0.0042
D176	0.01	0.0091	0.0053	0.0063	0.0029	0.0068	0.0068	0.0033	0.0048	0.0058	0.0082	0.0047	0.0087	0.0076	0.0087	0.0067
D177	0.0084	0.0064	0.0028	0.0019	0.0053	0.0043	0.0033	0.0068	0.0024	0.0014	0.0037	0.0072	0.0061	0.0061	0.0051	0.0042
D178	0.0049	0.0039	0.0034	0.0044	0.0059	0.0048	0.0048	0.0064	0.0029	0.0039	0.0043	0.0058	0.0047	0.0038	0.0047	0.0028
D179	0.0075	0.0066	0.0059	0.007	0.0034	0.0074	0.0074	0.0039	0.0054	0.0065	0.0068	0.0033	0.0073	0.0062	0.0073	0.0053
D180	0.0099	0.0069	0.0083	0.0073	0.011	0.0098	0.0077	0.0115	0.0058	0.0068	0.0032	0.0066	0.0056	0.0046	0.0037	0.0018
D181	0.0099	0.0069	0.0083	0.0073	0.011	0.0098	0.0077	0.0115	0.0058	0.0068	0.0032	0.0066	0.0056	0.0046	0.0037	0.0018
D182	0.0099	0.0069	0.0083	0.0073	0.011	0.0098	0.0077	0.0115	0.0058	0.0068	0.0032	0.0066	0.0056	0.0046	0.0037	0.0018
D183	0.0363	0.0354	0.0413	0.0398	0.0393	0.0433	0.0373	0.0368	0.0378	0.0393	0.0387	0.0382	0.0421	0.0377	0.0363	0.0368

	A65	A66	A67	A68	A69	A70	A71	A72	A73	A74	A75	B76	B77	B78	B79	B80
A65	0															
A66	0.0033	0														
A67	0.0042	0.0009	0													
A68	0.0072	0.0037	0.0047	0												
A69	0.0082	0.0047	0.0037	0.0009	0											
A70	0.0047	0.0062	0.0072	0.0023	0.0033	0										
A71	0.0067	0.0052	0.0042	0.0023	0.0014	0.0047	0									
A72	0.0068	0.0033	0.0023	0.0033	0.0023	0.0058	0.0038	0								
A73	0.0053	0.0038	0.0028	0.0048	0.0038	0.0073	0.0024	0.0014	0							
A74	0.0051	0.0027	0.0018	0.0066	0.0056	0.0091	0.0051	0.0042	0.0038	0						
A75	0.0127	0.0089	0.0099	0.0058	0.0068	0.0084	0.0084	0.0074	0.0091	0.0119	0					
B76	0.0138	0.0099	0.089	0.0068	0.0058	0.0094	0.0073	0.0064	0.008	0.0108	0.001	0				
B77	0.0122	0.0084	0.0094	0.0063	0.0073	0.009	0.0079	0.008	0.0086	0.0115	0.0005	0.0014	0			
B78	0.0092	0.0057	0.0047	0.0087	0.0076	0.0113	0.0092	0.0062	0.0078	0.0066	0.0048	0.0038	0.0053	0		
B79	0.0057	0.0072	0.0082	0.0103	0.0113	0.0077	0.013	0.0099	0.0116	0.0101	0.0063	0.0073	0.0069	0.0033	0	
B80	0.0097	0.0071	0.0061	0.0112	0.0101	0.0139	0.0097	0.0088	0.0083	0.0041	0.0073	0.0062	0.0068	0.0023	0.0057	0
B81	0.0062	0.0087	0.0097	0.0129	0.0139	0.0103	0.0135	0.0125	0.0121	0.0076	0.0089	0.0099	0.0084	0.0057	0.0023	0.0032
B82	0.0119	0.0082	0.0092	0.0042	0.0052	0.0067	0.0067	0.0078	0.0094	0.0112	0.0014	0.0024	0.0019	0.0042	0.0057	0.0066
B83	0.013	0.0092	0.0082	0.0052	0.0042	0.0077	0.0057	0.0068	0.0084	0.0101	0.0024	0.0014	0.0029	0.0033	0.0067	0.0057
B84	0.0079	0.0094	0.0105	0.0094	0.0105	0.0069	0.0122	0.008	0.0097	0.0125	0.0044	0.0054	0.005	0.0053	0.0019	0.0078
B85	0.0116	0.0078	0.0089	0.0089	0.0099	0.0116	0.0105	0.0074	0.008	0.0108	0.0039	0.0049	0.0034	0.0048	0.0063	0.0062
B86	0.0115	0.0088	0.0077	0.0067	0.0057	0.0093	0.0053	0.0063	0.0058	0.0057	0.0058	0.0048	0.0054	0.0088	0.0125	0.0062
B87	0.0078	0.0104	0.0115	0.0083	0.0093	0.0058	0.0089	0.01	0.0096	0.0092	0.0074	0.0085	0.007	0.0125	0.0089	0.0098
B88	0.0057	0.0023	0.0032	0.0051	0.0061	0.0076	0.0076	0.0047	0.0062	0.0051	0.0062	0.0073	0.0068	0.0032	0.0047	0.0056
B89	0.0033	0.0047	0.0057	0.0076	0.0087	0.0052	0.0103	0.0073	0.0089	0.0076	0.0089	0.0099	0.0094	0.0057	0.0023	0.0081
B90	0.0037	0.0061	0.0071	0.0101	0.0112	0.0076	0.0107	0.0098	0.0093	0.0051	0.0115	0.0125	0.011	0.0081	0.0047	0.0056
B91	0.0062	0.0028	0.0018	0.0066	0.0057	0.0092	0.0062	0.0043	0.0048	0.0037	0.0078	0.0068	0.0073	0.0028	0.0062	0.0042
B92	0.0092	0.0057	0.0066	0.0018	0.0028	0.0042	0.0042	0.0053	0.0068	0.0086	0.0038	0.0048	0.0043	0.0066	0.0082	0.0091
B93	0.0103	0.0066	0.0057	0.0028	0.0018	0.0052	0.0033	0.0043	0.0058	0.0076	0.0048	0.0038	0.0053	0.0057	0.0092	0.0081
B94	0.0077	0.0062	0.0072	0.0033	0.0042	0.0057	0.0028	0.0068	0.0053	0.0081	0.0053	0.0063	0.0048	0.0082	0.0098	0.0087
B95	0.0111	0.0073	0.0084	0.0043	0.0053	0.0069	0.0069	0.0059	0.0075	0.0104	0.0024	0.0034	0.0029	0.0073	0.009	0.0099
B96	0.0122	0.0084	0.0073	0.0053	0.0043	0.0079	0.0058	0.0049	0.0065	0.0093	0.0034	0.0024	0.0039	0.0063	0.01	0.099
B97	0.0127	0.0099	0.0089	0.0078	0.0068	0.0105	0.0063	0.0074	0.007	0.0067	0.0059	0.0049	0.0054	0.0089	0.0127	0.0062
B98	0.0106	0.009	0.0079	0.0069	0.0058	0.0096	0.0044	0.0065	0.005	0.0089	0.005	0.0039	0.0045	0.0079	0.0117	0.0084
B99	0.0067	0.0033	0.0042	0.0062	0.0072	0.0088	0.0088	0.0058	0.0073	0.0061	0.0063	0.0073	0.0069	0.0033	0.0047	0.0057
B100	0.0072	0.0047	0.0057	0.0087	0.0097	0.0113	0.0092	0.0083	0.0078	0.0037	0.0089	0.0099	0.0084	0.0057	0.0072	0.0032
B101	0.0082	0.0057	0.0047	0.0097	0.0087	0.0124	0.0082	0.0073	0.0068	0.0027	0.0099	0.0089	0.0094	0.0047	0.0082	0.0023
B102	0.0047	0.0072	0.0082	0.0113	0.0124	0.0088	0.0119	0.011	0.0105	0.0061	0.0116	0.0127	0.0111	0.0082	0.0047	0.0057
B103	0.0062	0.0047	0.0038	0.0088	0.0077	0.0115	0.0062	0.0063	0.0048	0.0047	0.009	0.0079	0.0085	0.0038	0.0073	0.0042
B104	0.0028	0.0062	0.0073	0.0104	0.0115	0.0078	0.0099	0.01	0.0085	0.0082	0.0106	0.0117	0.0102	0.0073	0.0038	0.0077
B105	0.0073	0.0038	0.0028	0.0077	0.0067	0.0104	0.0073	0.0053	0.0058	0.0047	0.0079	0.0069	0.0074	0.0028	0.0062	0.0042
B106	0.0038	0.0053	0.0062	0.0093	0.0104	0.0068	0.011	0.009	0.0096	0.0082	0.0096	0.0106	0.0091	0.0062	0.0028	0.0077
B107	0.0115	0.0077	0.0067	0.0038	0.0028	0.0062	0.0043	0.0053	0.0069	0.0087	0.0048	0.0038	0.0054	0.0057	0.0093	0.0082
B108	0.0108	0.0082	0.0092	0.0052	0.0062	0.0077	0.0057	0.0089	0.0084	0.0071	0.0063	0.0073	0.0058	0.0092	0.0108	0.0066
B109	0.0064	0.0079	0.009	0.0079	0.009	0.0054	0.0106	0.0065	0.0081	0.011	0.007	0.0081	0.0076	0.0079	0.0044	0.0105
B110	0.0105	0.0078	0.0068	0.0089	0.0078	0.0116	0.0073	0.0054	0.0049	0.0047	0.008	0.007	0.0075	0.0068	0.0105	0.0043
B111	0.0078	0.0043	0.0053	0.0014	0.0023	0.0038	0.0038	0.0029	0.0044	0.0072	0.0044	0.0054	0.0049	0.0093	0.011	0.0119
B112	0.0089	0.0053	0.0043	0.0023	0.0014	0.0048	0.0028	0.0019	0.0034	0.0062	0.0054	0.0044	0.0059	0.0083	0.0121	0.0108
B113	0.0053	0.0068	0.0078	0.0038	0.0048	0.0014	0.0063	0.0054	0.007	0.0098	0.007	0.008	0.0075	0.0121	0.0084	0.0148
B114	0.0093	0.0067	0.0057	0.0047	0.0038	0.0073	0.0033	0.0043	0.0038	0.0037	0.0079	0.0069	0.0074	0.0108	0.0148	0.0082
B115	0.0058	0.0083	0.0093	0.0062	0.0073	0.0038	0.0068	0.0079	0.0074	0.0072	0.0096	0.0106	0.0091	0.0148	0.011	0.0119
B116	0.0073	0.0058	0.0048	0.0038	0.0028	0.0063	0.0014	0.0034	0.0019	0.0057	0.007	0.0059	0.0065	0.0099	0.0138	0.0104
B117	0.0084	0.0048	0.0038	0.0028	0.0019	0.0053	0.0024	0.0024	0.0029	0.0057	0.0059	0.0049	0.0054	0.0089	0.0127	0.0104
B118	0.0048	0.0063	0.0073	0.0043	0.0053	0.0019	0.0058	0.0059	0.0065	0.0093	0.0075	0.0086	0.007	0.0127	0.009	0.0143
B119	0.0047	0.0014	0.0005	0.0042	0.0032	0.0066	0.0047	0.0019	0.0033	0.0023	0.0093	0.0083	0.0099	0.0042	0.0076	0.0066
B120	0.0042	0.0018	0.0027	0.0056	0.0066	0.0081	0.0061	0.0052	0.0047	0.0023	0.0093	0.0083	0.0099	0.0042	0.0091	0.0051
B121	0.0051	0.0027	0.0018	0.0066	0.0056	0.0091	0.0051	0.0042	0.0038	0	0.0119	0.0108	0.0115	0.0066	0.0101	0.0041
B122	0.0023	0.0009	0.0018	0.0047	0.0057	0.0072	0.0042	0.0043	0.0028	0.0027	0.0099	0.011	0.0094	0.0066	0.0082	0.0071
B123	0.0082	0.0047	0.0037	0.0009	0	0.0033	0.0014	0.0023	0.0038	0.0056	0.0068	0.0058	0.0073	0.0076	0.0113	0.0101
B124	0.0067	0.0052	0.0042	0.0023	0.0014	0.0047	0	0.0038	0.0024	0.0051	0.0084	0.0073	0.0079	0.0092	0.013	0.0097
D125	0.0033	0.0067	0.0077	0.0038	0.0047	0.0014	0.0033	0.0073	0.0058	0.0087	0.01	0.0111	0.0096	0.013	0.0093	0.0135

	A65	A66	A67	A68	A69	A70	A71	A72	A73	A74	A75	B76	B77	B78	B79	B80
D126	0.0122	0.0105	0.0094	0.0084	0.0073	0.0111	0.0058	0.008	0.0065	0.0104	0.0024	0.0014	0.0019	0.0053	0.009	0.0058
D127	0.0133	0.0094	0.0084	0.0073	0.0063	0.01	0.0069	0.007	0.0075	0.0104	0.0014	0.0005	0.001	0.0043	0.0079	0.0058
D128	0.0097	0.0071	0.0061	0.0112	0.0101	0.0139	0.0097	0.0088	0.0083	0.0041	0.0073	0.0062	0.0068	0.0023	0.0057	0
D129	0.0077	0.0062	0.0052	0.0103	0.0092	0.013	0.0077	0.0078	0.0063	0.0061	0.0063	0.0053	0.0058	0.0014	0.0047	0.0018
D130	0.013	0.0092	0.0082	0.0052	0.0042	0.0077	0.0057	0.0068	0.0084	0.0101	0.0024	0.0014	0.0029	0.0033	0.0067	0.0057
D131	0.01	0.0084	0.0073	0.0094	0.0084	0.0122	0.0069	0.0059	0.0044	0.0083	0.0044	0.0034	0.0039	0.0033	0.0069	0.0038
D132	0.0073	0.0089	0.0099	0.0058	0.0068	0.0033	0.0084	0.0074	0.0091	0.0119	0.0049	0.0059	0.0054	0.0099	0.0063	0.0125
D133	0.0115	0.0088	0.0077	0.0067	0.0057	0.0093	0.0053	0.0063	0.0058	0.0057	0.0058	0.0048	0.0054	0.0088	0.0125	0.0062
D134	0.0094	0.0078	0.0068	0.0058	0.0048	0.0084	0.0033	0.0054	0.0039	0.0077	0.0049	0.0039	0.0044	0.0078	0.0116	0.0083
D135	0.0057	0.0023	0.0032	0.0051	0.0061	0.0076	0.0076	0.0047	0.0062	0.0051	0.0062	0.0073	0.0068	0.0032	0.0047	0.0056
D136	0.0071	0.0046	0.0037	0.0086	0.0076	0.0112	0.0071	0.0062	0.0057	0.0018	0.0098	0.0088	0.0093	0.0046	0.0081	0.0023
D137	0.0052	0.0037	0.0028	0.0076	0.0066	0.0103	0.0052	0.0053	0.0038	0.0037	0.0089	0.0078	0.0084	0.0037	0.0072	0.0042
D138	0.0019	0.0052	0.0062	0.0092	0.0103	0.0067	0.0088	0.0089	0.0073	0.0071	0.0105	0.0116	0.01	0.0072	0.0038	0.0076
D139	0.0057	0.0042	0.0051	0.0081	0.0091	0.0107	0.0076	0.0077	0.0062	0.006	0.0093	0.0104	0.0089	0.0061	0.0076	0.0066
D140	0.0103	0.0066	0.0057	0.0028	0.0018	0.0052	0.0033	0.0043	0.0058	0.0076	0.0048	0.0038	0.0053	0.0057	0.0092	0.0081
D141	0.0053	0.0088	0.0098	0.0057	0.0067	0.0033	0.0053	0.0094	0.0079	0.0107	0.0079	0.009	0.0074	0.0108	0.0073	0.0113
D142	0.0089	0.0053	0.0043	0.0053	0.0043	0.0078	0.0058	0.0019	0.0034	0.0062	0.0054	0.0044	0.0059	0.0043	0.0078	0.0067
D143	0.0083	0.0057	0.0067	0.0067	0.0077	0.0093	0.0073	0.0053	0.0048	0.0047	0.0069	0.0079	0.0064	0.0077	0.0093	0.0052
D144	0.0085	0.01	0.0111	0.0069	0.0079	0.0044	0.0096	0.0086	0.0103	0.0132	0.005	0.006	0.0055	0.01	0.0064	0.0127
D145	0.0127	0.0099	0.0089	0.0078	0.0068	0.0105	0.0063	0.0074	0.007	0.0067	0.0059	0.0049	0.0054	0.0089	0.0127	0.0062
D146	0.007	0.0106	0.0117	0.0085	0.0096	0.0059	0.008	0.0103	0.0087	0.0127	0.0066	0.0076	0.0061	0.0117	0.008	0.0122
D147	0.0111	0.0094	0.0105	0.0073	0.0084	0.01	0.0069	0.0091	0.0075	0.0115	0.0054	0.0065	0.005	0.0105	0.0122	0.011
D148	0.008	0.0096	0.0106	0.0074	0.0085	0.0049	0.0091	0.0092	0.0098	0.0127	0.0055	0.0066	0.005	0.0106	0.007	0.0122
D149	0.0082	0.0057	0.0047	0.0097	0.0087	0.0124	0.0082	0.0073	0.0068	0.0027	0.0099	0.0089	0.0094	0.0047	0.0082	0.0023
D150	0.0047	0.0072	0.0082	0.0113	0.0124	0.0088	0.0119	0.011	0.0105	0.0061	0.0116	0.0127	0.0111	0.0082	0.0047	0.0057
D151	0.0104	0.0067	0.0077	0.0028	0.0038	0.0053	0.0053	0.0063	0.0079	0.0097	0.0038	0.0048	0.0044	0.0067	0.0083	0.0092
D152	0.0115	0.0077	0.0067	0.0038	0.0028	0.0062	0.0043	0.0053	0.0069	0.0087	0.0048	0.0038	0.0054	0.0057	0.0093	0.0082
D153	0.0119	0.0092	0.0082	0.0062	0.0052	0.0088	0.0047	0.0078	0.0073	0.0061	0.0073	0.0063	0.0069	0.0082	0.0119	0.0057
D154	0.0063	0.0099	0.011	0.0068	0.0078	0.0043	0.0063	0.0106	0.0091	0.0119	0.008	0.0091	0.0075	0.011	0.0073	0.0115
D155	0.0049	0.0085	0.0096	0.0096	0.0106	0.007	0.0091	0.0081	0.0066	0.0105	0.0087	0.0098	0.0082	0.0096	0.0059	0.01
D156	0.0078	0.0043	0.0053	0.0014	0.0023	0.0038	0.0038	0.0029	0.0044	0.0072	0.0044	0.0054	0.0049	0.0093	0.011	0.0119
D157	0.0089	0.0053	0.0043	0.0023	0.0014	0.0048	0.0028	0.0019	0.0034	0.0062	0.0054	0.0044	0.0059	0.0083	0.0121	0.0108
D158	0.0053	0.0068	0.0078	0.0038	0.0048	0.0014	0.0063	0.0054	0.007	0.0098	0.007	0.008	0.0075	0.0121	0.0084	0.0148
D159	0.0083	0.0057	0.0067	0.0038	0.0047	0.0062	0.0043	0.0053	0.0048	0.0047	0.0069	0.0079	0.0064	0.0119	0.0136	0.0092
D160	0.0093	0.0067	0.0057	0.0047	0.0038	0.0073	0.0033	0.0043	0.0038	0.0037	0.0079	0.0069	0.0074	0.0108	0.0148	0.0082
D161	0.0063	0.0048	0.0058	0.0028	0.0038	0.0053	0.0024	0.0044	0.0029	0.0067	0.0059	0.007	0.0054	0.011	0.0127	0.0115
D162	0.0073	0.0058	0.0048	0.0038	0.0028	0.0063	0.0014	0.0034	0.0019	0.0057	0.007	0.0059	0.0065	0.0099	0.0138	0.0104
D163	0.0038	0.0073	0.0084	0.0053	0.0063	0.0029	0.0048	0.007	0.0054	0.0093	0.0086	0.0097	0.0081	0.0138	0.01	0.0143
D164	0.0047	0.0014	0.0005	0.0042	0.0032	0.0066	0.0047	0.0019	0.0033	0.0023	0.0093	0.0083	0.0099	0.0042	0.0076	0.0066
D165	0.0014	0.0028	0.0037	0.0057	0.0066	0.0033	0.0082	0.0053	0.0068	0.0056	0.011	0.0121	0.0116	0.0076	0.0042	0.0101
D166	0.0042	0.0018	0.0027	0.0056	0.0066	0.0081	0.0061	0.0052	0.0047	0.0009	0.0108	0.0119	0.0104	0.0076	0.0091	0.0051
D167	0.0018	0.0042	0.0051	0.0081	0.0091	0.0057	0.0087	0.0077	0.0073	0.0032	0.0136	0.0148	0.0132	0.0101	0.0066	0.0076
D168	0.0023	0.0009	0.0018	0.0047	0.0057	0.0072	0.0042	0.0043	0.0028	0.0027	0.0099	0.011	0.0094	0.0066	0.0082	0.0071
D169	0.0061	0.0101	0.0083	0.0119	0.0057	0.0077	0.0051	0.0018	0.0037	0.0071	0.006	0.0076	0.0107	0.0062	0.0047	0.0132
D170	0.0033	0.0018	0.0009	0.0057	0.0047	0.0082	0.0033	0.0033	0.0019	0.0018	0.011	0.0099	0.0105	0.0057	0.0092	0.0061
D171	0.0033	0.0018	0.0009	0.0057	0.0047	0.0082	0.0033	0.0033	0.0019	0.0018	0.011	0.0099	0.0105	0.0057	0.0092	0.0061
D172	0	0.0033	0.0042	0.0072	0.0082	0.0047	0.0067	0.0068	0.0053	0.0051	0.0127	0.0138	0.0122	0.0092	0.0057	0.0097
D173	0.0037	0.0023	0.0032	0.0061	0.0071	0.0087	0.0057	0.0057	0.0043	0.0041	0.0115	0.0125	0.011	0.0081	0.0097	0.0086
D174	0.0009	0.0023	0.0033	0.0062	0.0072	0.0038	0.0077	0.0058	0.0063	0.0051	0.0116	0.0127	0.0111	0.0082	0.0047	0.0097
D175	0.0057	0.0042	0.0052	0.0014	0.0023	0.0038	0.0009	0.0048	0.0033	0.0061	0.0073	0.0084	0.0069	0.0103	0.0119	0.0107
D176	0.0033	0.0067	0.0077	0.0038	0.0047	0.0014	0.0033	0.0073	0.0058	0.0087	0.01	0.0111	0.0096	0.013	0.0093	0.0135
D177	0.0077	0.0042	0.0033	0.0014	0.0005	0.0038	0.0009	0.0028	0.0033	0.0051	0.0073	0.0063	0.0069	0.0082	0.0119	0.0097
D178	0.0043	0.0028	0.0038	0.0038	0.0048	0.0063	0.0033	0.0024	0.001	0.0047	0.008	0.0091	0.0075	0.0089	0.0105	0.0093
D179	0.0019	0.0053	0.0063	0.0063	0.0073	0.0038	0.0058	0.0049	0.0034	0.0073	0.0108	0.0119	0.0103	0.0116	0.0079	0.0121
D180	0.0052	0.0037	0.0028	0.0076	0.0066	0.0103	0.0052	0.0053	0.0038	0.0037	0.0089	0.0078	0.0084	0.0037	0.0072	0.0042
D181	0.0052	0.0037	0.0028	0.0076	0.0066	0.0103	0.0052	0.0053	0.0038	0.0037	0.0089	0.0078	0.0084	0.0037	0.0072	0.0042
D182	0.0052	0.0037	0.0028	0.0076	0.0066	0.0103	0.0052	0.0053	0.0038	0.0037	0.0089	0.0078	0.0084	0.0037	0.0072	0.0042
D183	0.0363	0.0397	0.0382	0.0412	0.0397	0.0392	0.0377	0.0413	0.0393	0.0363	0.0378	0.0363	0.0373	0.0353	0.0349	0.033

	B81	B82	B83	B84	B85	B86	B87	B88	B89	B90	B91	B92	B93	B94	B95	B96
B81	0															
B82	0.0082	0														
B83	0.0092	0.0009	0													
B84	0.0043	0.0048	0.0058	0												
B85	0.0078	0.0043	0.0053	0.0044	0											
B86	0.0098	0.0073	0.0062	0.0106	0.009	0										
B87	0.0062	0.0089	0.0099	0.0C7	0.0106	0.0033	0									
B88	0.0071	0.0057	0.0066	0.0068	0.0062	0.0072	0.0088	0								
B89	0.0047	0.0082	0.0092	0.0043	0.0089	0.0098	0.0062	0.0023	0							
B90	0.0023	0.0107	0.0118	0.0068	0.0104	0.0072	0.0038	0.0046	0.0023	0						
B91	0.0076	0.0072	0.0062	0.0084	0.0068	0.0057	0.0093	0.0014	0.0037	0.0051	0					
B92	0.0107	0.0023	0.0033	0.0073	0.0068	0.0047	0.0062	0.0032	0.0057	0.0081	0.0047	0				
B93	0.0118	0.0033	0.0023	0.0084	0.0078	0.0038	0.0073	0.0042	0.0066	0.0091	0.0037	0.0009	0			
B94	0.0103	0.0038	0.0047	0.009	0.0073	0.0043	0.0058	0.0047	0.0072	0.0076	0.0052	0.0014	0.0023	0		
B95	0.0116	0.0038	0.0048	0.007	0.0065	0.0085	0.0102	0.0089	0.0116	0.0143	0.0105	0.0063	0.0073	0.0079	0	
B96	0.0127	0.0048	0.0038	0.0081	0.0075	0.0074	0.0112	0.0099	0.0127	0.0154	0.0094	0.0073	0.0063	0.009	0.001	0
B97	0.0099	0.0073	0.0063	0.0108	0.0091	0.0048	0.0085	0.0125	0.0154	0.0125	0.011	0.0099	0.0089	0.0094	0.0034	0.0024
B98	0.0122	0.0064	0.0054	0.0098	0.0081	0.007	0.0108	0.0116	0.0145	0.0149	0.01	0.009	0.0079	0.0074	0.0024	0.0015
B99	0.0072	0.0057	0.0067	0.0069	0.0063	0.0125	0.0143	0.0047	0.0072	0.0097	0.0062	0.0082	0.0092	0.0098	0.0038	0.0048
B100	0.0047	0.0082	0.0092	0.0094	0.0078	0.0098	0.0115	0.0071	0.0097	0.0071	0.0076	0.0107	0.0118	0.0103	0.0063	0.0073
B101	0.0057	0.0092	0.0082	0.0105	0.0089	0.0088	0.0125	0.0081	0.0107	0.0081	0.0066	0.0118	0.0107	0.0113	0.0073	0.0063
B102	0.0023	0.0108	0.0119	0.0069	0.0105	0.0125	0.0089	0.0097	0.0072	0.0047	0.0103	0.0135	0.0146	0.013	0.009	0.01
B103	0.0077	0.0083	0.0073	0.0096	0.0079	0.011	0.0149	0.0072	0.0098	0.0103	0.0057	0.0108	0.0098	0.0093	0.0064	0.0054
B104	0.0043	0.0099	0.011	0.0059	0.0096	0.0149	0.0111	0.0088	0.0062	0.0067	0.0093	0.0125	0.0136	0.011	0.008	0.0091
B105	0.0077	0.0073	0.0062	0.0085	0.0069	0.011	0.0149	0.0062	0.0088	0.0103	0.0047	0.0098	0.0088	0.0104	0.0054	0.0044
B106	0.0043	0.0089	0.0099	0.0049	0.0085	0.0149	0.0111	0.0077	0.0053	0.0067	0.0083	0.0115	0.0125	0.0121	0.007	0.008
B107	0.0119	0.0033	0.0023	0.0085	0.0079	0.0089	0.0127	0.0092	0.0119	0.0146	0.0088	0.0057	0.0047	0.0073	0.0024	0.0014
B108	0.0082	0.0047	0.0057	0.01	0.0084	0.0076	0.0089	0.0107	0.0135	0.0107	0.0113	0.0072	0.0082	0.0067	0.0038	0.0048
B109	0.0069	0.0074	0.0085	0.0024	0.007	0.0135	0.0097	0.0094	0.0069	0.0094	0.0111	0.01	0.0111	0.0117	0.0045	0.0055
B110	0.0078	0.0084	0.0073	0.0086	0.007	0.0069	0.0106	0.0104	0.0132	0.0104	0.0089	0.011	0.0099	0.0105	0.0054	0.0044
B111	0.0136	0.0058	0.0068	0.0091	0.0085	0.0053	0.0069	0.0057	0.0083	0.0108	0.0073	0.0033	0.0043	0.0048	0.0029	0.0039
B112	0.0148	0.0068	0.0058	0.0102	0.0096	0.0043	0.0079	0.0067	0.0093	0.0119	0.0062	0.0043	0.0033	0.0058	0.0039	0.0029
B113	0.011	0.0084	0.0094	0.0065	0.0112	0.0079	0.0044	0.0083	0.0058	0.0083	0.0099	0.0058	0.0068	0.0073	0.0054	0.0065
B114	0.0119	0.0093	0.0083	0.0128	0.0111	0.0019	0.0053	0.0092	0.0119	0.0092	0.0077	0.0067	0.0057	0.0062	0.0064	0.0054
B115	0.0083	0.011	0.0121	0.0091	0.0128	0.0053	0.0019	0.0108	0.0083	0.0057	0.0115	0.0083	0.0093	0.0078	0.008	0.0091
B116	0.0143	0.0084	0.0073	0.0119	0.0102	0.0038	0.0074	0.0083	0.011	0.0115	0.0068	0.0058	0.0048	0.0043	0.0054	0.0044
B117	0.0143	0.0073	0.0063	0.0108	0.0091	0.0038	0.0074	0.0073	0.0099	0.0115	0.0058	0.0048	0.0038	0.0053	0.0044	0.0034
B118	0.0105	0.009	0.01	0.007	0.0108	0.0074	0.0039	0.0089	0.0063	0.0078	0.004	0.0063	0.0073	0.0069	0.006	0.007
B119	0.0101	0.0087	0.0076	0.0099	0.0093	0.0082	0.0119	0.0027	0.0051	0.0076	0.0023	0.0061	0.0051	0.0076	0.0078	0.0068
B120	0.0066	0.0101	0.0112	0.0115	0.0098	0.0066	0.0082	0.0041	0.0066	0.0041	0.0046	0.0076	0.0086	0.0071	0.0093	0.0104
B121	0.0076	0.0112	0.0101	0.0125	0.0108	0.0057	0.0092	0.0051	0.0076	0.0051	0.0037	0.0086	0.0076	0.0081	0.0104	0.0093
B122	0.0087	0.0092	0.0103	0.0105	0.0089	0.0088	0.0104	0.0032	0.0057	0.0061	0.0037	0.0066	0.0076	0.0052	0.0084	0.0094
B123	0.0139	0.0052	0.0042	0.0105	0.0099	0.0057	0.0093	0.0061	0.0087	0.0112	0.0057	0.0028	0.0018	0.0042	0.0053	0.0043
B124	0.0135	0.0067	0.0057	0.0122	0.0105	0.0053	0.0089	0.0076	0.0103	0.0107	0.0062	0.0042	0.0033	0.0028	0.0069	0.0058
D125	0.0098	0.0083	0.0093	0.0085	0.0122	0.0089	0.0053	0.0092	0.0067	0.0072	0.0098	0.0057	0.0067	0.0043	0.0085	0.0096
D126	0.0094	0.0038	0.0029	0.007	0.0054	0.0044	0.008	0.0089	0.0116	0.0121	0.0073	0.0063	0.0053	0.0048	0.005	0.0039
D127	0.0094	0.0029	0.0019	0.006	0.0044	0.0044	0.008	0.0078	0.0105	0.0121	0.0063	0.0053	0.0043	0.0058	0.0039	0.0029
D128	0.0032	0.0066	0.0057	0.0078	0.0062	0.0062	0.0098	0.0056	0.0081	0.0056	0.0042	0.0091	0.0081	0.0087	0.0099	0.0089
D129	0.0052	0.0057	0.0047	0.0069	0.0053	0.0083	0.0121	0.0047	0.0072	0.0076	0.0033	0.0082	0.0072	0.0067	0.009	0.0079
D130	0.0092	0.0009	0	0.0058	0.0053	0.0062	0.0099	0.0066	0.0092	0.0118	0.0062	0.0033	0.0023	0.0047	0.0048	0.0038
D131	0.0073	0.0048	0.0038	0.005	0.0034	0.0064	0.0102	0.0068	0.0094	0.0099	0.0053	0.0073	0.0063	0.0058	0.007	0.006
D132	0.0089	0.0063	0.0073	0.0044	0.0091	0.0058	0.0024	0.0062	0.0038	0.0062	0.0078	0.0038	0.0048	0.0053	0.0075	0.0086
D133	0.0098	0.0073	0.0062	0.0106	0.009	0	0.0033	0.0072	0.008	0.0072	0.0057	0.0047	0.0038	0.0043	0.0085	0.0074
D134	0.0121	0.0063	0.0053	0.0097	0.008	0.0019	0.0054	0.0062	0.0089	0.0093	0.0048	0.0038	0.0028	0.0024	0.0075	0.0065
D135	0.0071	0.0057	0.0066	0.00678	0.0062	0.0072	0.0088	0	0.0023	0.0046	0.0014	0.0032	0.0042	0.0047	0.0089	0.0099
D136	0.0056	0.0091	0.0081	0.0104	0.0088	0.0037	0.0072	0.0032	0.0056	0.0032	0.0018	0.0066	0.0056	0.0061	0.0125	0.0115
D137	0.0076	0.0082	0.0072	0.0094	0.0078	0.0057	0.0093	0.0023	0.0047	0.0051	0.0009	0.0057	0.0047	0.0042	0.0116	0.0105
D138	0.0042	0.0098	0.0108	0.0058	0.0094	0.0093	0.0058	0.0037	0.0014	0.0018	0.0042	0.00721	0.0082	0.0057	0.0133	0.0145
D139	0.0081	0.0087	0.0097	0.0099	0.0053	0.0082	0.0098	0.0027	0.0051	0.0056	0.0032	0.0061	0.0071	0.0047	0.0121	0.0132
D140	0.0118	0.0033	0.0023	0.0084	0.0078	0.0038	0.0073	0.0042	0.0066	0.0091	0.0037	0.0009	0	0.0023	0.0073	0.0063
D141	0.0077	0.0062	0.0073	0.0064	0.01	0.0068	0.0033	0.0072	0.0047	0.00521	0.0077	0.0038	0.0047	0.0023	0.0106	0.0117

	B81	B82	B83	B84	B85	B86	B87	B88	B89	B90	B91	B92	B93	B94	B95	B96
D142	0.0104	0.0058	0.0048	0.0059	0.0054	0.0043	0.0079	0.0028	0.0053	0.0077	0.0023	0.0033	0.0023	0.0048	0.008	0.007
D143	0.0067	0.0073	0.0083	0.0074	0.0058	0.0028	0.0043	0.0042	0.0067	0.0042	0.0047	0.0047	0.0057	0.0043	0.0096	0.0106
D144	0.009	0.0064	0.0074	0.0045	0.0092	0.0112	0.0075	0.0116	0.009	0.0116	0.0133	0.009	0.01	0.0106	0.0024	0.0035
D145	0.0099	0.0073	0.0063	0.0108	0.0091	0.0048	0.0085	0.0125	0.0154	0.0125	0.011	0.0099	0.0089	0.0094	0.0034	0.0024
D146	0.0085	0.008	0.0091	0.0061	0.0098	0.0108	0.007	0.0133	0.0106	0.0111	0.014	0.0106	0.0117	0.0091	0.004	0.005
D147	0.0127	0.0069	0.0079	0.0103	0.0054	0.0096	0.0112	0.0121	0.0149	0.0154	0.0127	0.0094	0.0105	0.0079	0.0029	0.0039
D148	0.0085	0.007	0.008	0.005	0.0087	0.0108	0.007	0.0122	0.0096	0.0111	0.0128	0.0096	0.0106	0.0102	0.003	0.004
D149	0.0057	0.0092	0.0082	0.0105	0.0089	0.0088	0.0125	0.0081	0.0107	0.0081	0.0066	0.0118	0.0107	0.0113	0.0073	0.0063
D150	0.0023	0.0108	0.0119	0.0069	0.0105	0.0125	0.0089	0.0097	0.0072	0.0047	0.0103	0.0135	0.0146	0.013	0.009	0.01
D151	0.0108	0.0023	0.0033	0.0074	0.0069	0.0099	0.0116	0.0082	0.0108	0.0135	0.0098	0.0047	0.0057	0.0062	0.0014	0.0024
D152	0.0119	0.0033	0.0023	0.0085	0.0079	0.0089	0.0127	0.0092	0.0119	0.0146	0.0088	0.0057	0.0047	0.0073	0.0024	0.0014
D153	0.0092	0.0057	0.0047	0.0111	0.0094	0.0062	0.0099	0.0118	0.0146	0.0118	0.0103	0.0082	0.0072	0.0077	0.0048	0.0038
D154	0.0078	0.0063	0.0073	0.0065	0.0102	0.0122	0.0085	0.0125	0.0099	0.0104	0.0132	0.0089	0.0099	0.0073	0.0054	0.0065
D155	0.0064	0.0091	0.0102	0.004	0.0076	0.013	0.0092	0.0111	0.0085	0.009	0.0117	0.0117	0.0128	0.0102	0.0061	0.0071
D156	0.0136	0.0058	0.0068	0.0091	0.0085	0.0053	0.0069	0.0057	0.0083	0.0108	0.0073	0.0033	0.0043	0.0048	0.0029	0.0039
D157	0.0148	0.0068	0.0058	0.0102	0.0096	0.0043	0.0079	0.0067	0.0093	0.0119	0.0062	0.0043	0.0033	0.0058	0.0039	0.0029
D158	0.011	0.0084	0.0094	0.0065	0.0112	0.0079	0.0044	0.0083	0.0058	0.0083	0.0099	0.0058	0.0068	0.0073	0.0054	0.0065
D159	0.0108	0.0083	0.0093	0.0117	0.01	0.0028	0.0043	0.0082	0.0108	0.0082	0.0088	0.0057	0.0067	0.0053	0.0054	0.0064
D160	0.0119	0.0093	0.0083	0.0128	0.0111	0.0019	0.0053	0.0092	0.0119	0.0092	0.0077	0.0067	0.0057	0.0062	0.0054	0.0054
D161	0.0132	0.0073	0.0084	0.0108	0.0091	0.0048	0.0064	0.0073	0.0099	0.0104	0.0078	0.0048	0.0058	0.0033	0.0044	0.0054
D162	0.0143	0.0084	0.0073	0.0119	0.0102	0.0038	0.0074	0.0083	0.011	0.0115	0.0068	0.0058	0.0048	0.0043	0.0054	0.0044
D163	0.0105	0.01	0.0111	0.0081	0.0119	0.0074	0.0039	0.0099	0.0073	0.0078	0.0105	0.0073	0.0084	0.0058	0.007	0.0081
D164	0.0101	0.0087	0.0076	0.0099	0.0093	0.0082	0.0119	0.0027	0.0051	0.0076	0.0023	0.0061	0.0051	0.0076	0.0078	0.0068
D165	0.0066	0.0103	0.0113	0.0063	0.011	0.0119	0.0083	0.0042	0.0018	0.0042	0.0057	0.0076	0.0087	0.0092	0.0094	0.0105
D166	0.0066	0.0101	0.0112	0.0115	0.0098	0.0066	0.0082	0.0041	0.0066	0.0041	0.0046	0.0076	0.0086	0.0071	0.0093	0.0104
D167	0.0051	0.0027	0.0018	0.0066	0.0056	0.0091	0.0051	0.0042	0.0038	0	0.0119	0.0108	0.0115	0.0066	0.0101	0.0041
D168	0.0042	0.0129	0.0139	0.0089	0.0125	0.0092	0.0057	0.0066	0.0042	0.0018	0.0071	0.0101	0.0112	0.0097	0.0121	0.0132
D169	0.0087	0.0092	0.0103	0.0105	0.0089	0.0088	0.0104	0.0032	0.0057	0.0061	0.0037	0.0066	0.0076	0.0052	0.0084	0.0094
D170	0.0097	0.0103	0.0092	0.0116	0.0099	0.0077	0.0115	0.0042	0.0066	0.0071	0.0028	0.0076	0.0066	0.0062	0.0094	0.0084
D171	0.0097	0.0103	0.0092	0.0116	0.0099	0.0077	0.0115	0.0042	0.0066	0.0071	0.0028	0.0076	0.0066	0.0062	0.0094	0.0084
D172	0.0062	0.0119	0.013	0.0079	0.0116	0.0115	0.0078	0.0057	0.0033	0.0037	0.0062	0.0092	0.0103	0.0077	0.0111	0.0122
D173	0.0101	0.0107	0.0118	0.0121	0.0073	0.0103	0.0119	0.0046	0.0071	0.0076	0.0051	0.0081	0.0091	0.0066	0.0099	0.011
D174	0.0062	0.0108	0.0119	0.0069	0.0105	0.0115	0.0078	0.0047	0.0023	0.0037	0.0052	0.0082	0.0092	0.0088	0.01	0.0111
D175	0.0124	0.0057	0.0067	0.0111	0.0094	0.0062	0.0078	0.0066	0.0092	0.0097	0.0072	0.0035	0.0042	0.0019	0.0058	0.0069
D176	0.0098	0.0083	0.0093	0.0085	0.0122	0.0089	0.0053	0.0092	0.0067	0.0072	0.0098	0.0057	0.0067	0.0043	0.0085	0.0096
D177	0.0135	0.0057	0.0047	0.0111	0.0094	0.0053	0.0089	0.0066	0.0092	0.0107	0.0052	0.0033	0.0023	0.0038	0.0058	0.0048
D178	0.011	0.0084	0.0094	0.0086	0.007	0.0069	0.0085	0.0053	0.0078	0.0083	0.0058	0.0058	0.0068	0.0043	0.0065	0.0075
D179	0.0084	0.0111	0.0122	0.006	0.0097	0.0096	0.0059	0.0078	0.0053	0.0058	0.0084	0.0084	0.0094	0.0069	0.0092	0.0103
D180	0.0076	0.0082	0.0072	0.0094	0.0078	0.0057	0.0093	0.0023	0.0047	0.0051	0.0009	0.0057	0.0047	0.0042	0.0116	0.0105
D181	0.0076	0.0082	0.0072	0.0094	0.0078	0.0057	0.0093	0.0023	0.0047	0.0051	0.0009	0.0057	0.0047	0.0042	0.0116	0.0105
D182	0.0076	0.0082	0.0072	0.0094	0.0078	0.0057	0.0093	0.0023	0.0047	0.0051	0.0009	0.0057	0.0047	0.0042	0.0116	0.0105
D183	0.0326	0.0377	0.0363	0.0373	0.0408	0.0373	0.0368	0.0402	0.0382	0.0358	0.0382	0.0412	0.0397	0.0392	0.0373	0.0358

	B97	B98	B99	B100	B101	B102	B013	B014	B105	B106	B107	B108	B109	B110	B111	B112
B97	0.0019	0														
B98	0.0073	0.0064	0													
B99	0.0048	0.0069	0.0023	0												
B100	0.0038	0.0058	0.0033	0.0009	0											
B101	0.0073	0.0096	0.0047	0.0023	0.0033	0										
B102	0.0058	0.0039	0.0023	0.0028	0.0019	0.0053	0									
B103	0.0096	0.0075	0.0038	0.0043	0.0053	0.0019	0.0033	0								
B104	0.0058	0.0049	0.0014	0.0028	0.0019	0.0053	0.0009	0.0043	0							
B105	0.0096	0.0086	0.0028	0.0043	0.0053	0.0019	0.0043	0.0009	0.0033	0						
B106	0.0038	0.0029	0.0043	0.0067	0.0057	0.0093	0.0048	0.0084	0.0038	0.0073	0					
B107	0.0024	0.0044	0.0057	0.0033	0.0042	0.0057	0.0062	0.0078	0.0062	0.0078	0.0033	0				
B108	0.0081	0.0071	0.0044	0.0069	0.0079	0.0044	0.007	0.0034	0.0059	0.0024	0.0059	0.0074	0			
B109	0.0019	0.0039	0.0053	0.0028	0.0019	0.0053	0.0038	0.0074	0.0038	0.0074	0.0048	0.0033	0.006	0		
B110	0.0064	0.0054	0.0068	0.0093	0.0104	0.0121	0.0094	0.0111	0.0084	0.01	0.0053	0.0068	0.0075	0.0085	0	
B111	0.0054	0.0044	0.0078	0.0104	0.0093	0.0132	0.0084	0.0122	0.0073	0.0111	0.0043	0.0078	0.0086	0.0074	0.0009	0
B112	0.0091	0.0081	0.0094	0.0121	0.0132	0.0094	0.0122	0.0085	0.0111	0.0074	0.0079	0.0094	0.005	0.0112	0.0024	0.0034
B113	0.0029	0.0049	0.0104	0.0077	0.0067	0.0104	0.0089	0.0127	0.0089	0.0127	0.0068	0.0053	0.0112	0.0048	0.0033	0.0024
B114	0.0064	0.0086	0.0121	0.0093	0.0104	0.0068	0.0127	0.009	0.0127	0.009	0.0105	0.0068	0.0075	0.0085	0.0048	0.0058
B115	0.0049	0.0029	0.0094	0.0099	0.0089	0.0127	0.0069	0.0106	0.0079	0.0117	0.0058	0.0073	0.0103	0.007	0.0024	0.0014
B116	0.0049	0.0039	0.0084	0.0099	0.0089	0.0127	0.0079	0.0117	0.0069	0.0106	0.0048	0.0073	0.0092	0.007	0.0014	0.0005
B117	0.0086	0.0076	0.01	0.0116	0.0127	0.009	0.0117	0.008	0.0106	0.007	0.0085	0.009	0.0055	0.0108	0.0029	0.0039
B118	0.0093	0.0084	0.0037	0.0061	0.0051	0.0087	0.0042	0.0077	0.0033	0.0067	0.0062	0.0097	0.0084	0.0073	0.0047	0.0038
B119	0.0077	0.0099	0.0051	0.0027	0.0037	0.0051	0.0057	0.0072	0.0057	0.0072	0.0097	0.0061	0.0099	0.0057	0.0062	0.0072
B120	0.0067	0.0089	0.0061	0.0037	0.0027	0.0061	0.0047	0.0082	0.0047	0.0082	0.0087	0.0071	0.011	0.0047	0.0072	0.0062
B121	0.0099	0.0079	0.0042	0.0047	0.0057	0.0072	0.0038	0.0053	0.0047	0.0062	0.0088	0.0082	0.009	0.0078	0.0053	0.0062
B122	0.0068	0.0058	0.0072	0.0097	0.0087	0.0124	0.0077	0.0115	0.0067	0.0104	0.0028	0.0062	0.009	0.0078	0.0023	0.0014
B123	0.0063	0.0044	0.0088	0.0092	0.0082	0.0119	0.0062	0.0099	0.0073	0.011	0.0043	0.0057	0.0106	0.0073	0.0038	0.0028
B124	0.01	0.008	0.0104	0.0108	0.0119	0.0083	0.0099	0.0063	0.011	0.0073	0.0078	0.0073	0.007	0.0111	0.0053	0.0063
D125	0.0044	0.0024	0.009	0.0094	0.0084	0.0122	0.0064	0.0102	0.0074	0.0112	0.0054	0.0069	0.0098	0.0065	0.007	0.0059
D126	0.0044	0.0035	0.0079	0.0094	0.0084	0.0122	0.0064	0.0102	0.0044	0.0069	0.0087	0.0065	0.0059	0.0049		
D127	0.0062	0.0084	0.0057	0.0032	0.0023	0.0057	0.0042	0.0077	0.0042	0.0077	0.0082	0.0066	0.0105	0.0043	0.0119	0.0108
D128	0.0084	0.0064	0.0047	0.0052	0.0042	0.0077	0.0023	0.0058	0.0033	0.0068	0.0073	0.0088	0.0096	0.0063	0.011	0.0099
D129	0.0063	0.0054	0.0067	0.0092	0.0082	0.0119	0.0073	0.011	0.0062	0.0099	0.0023	0.0057	0.0085	0.0073	0.0068	0.0058
D130	0.0065	0.0045	0.0069	0.0073	0.0063	0.01	0.0044	0.008	0.0054	0.0091	0.0064	0.0079	0.0076	0.0044	0.0091	0.008
D131	0.0112	0.0103	0.0116	0.0143	0.0154	0.0116	0.0145	0.0106	0.0133	0.0096	0.01	0.0116	0.007	0.0135	0.0044	0.0054
D132	0.0048	0.007	0.0125	0.0098	0.0088	0.0125	0.011	0.0149	0.011	0.0149	0.0089	0.0073	0.0135	0.0069	0.0053	0.0043
D133	0.007	0.005	0.0116	0.0121	0.011	0.0149	0.009	0.0128	0.01	0.014	0.0079	0.0094	0.0125	0.0091	0.0044	0.0034
D134	0.0125	0.0116	0.0047	0.0071	0.0081	0.0097	0.0072	0.0088	0.0062	0.0077	0.0092	0.0107	0.0094	0.0104	0.0057	0.0067
D135	0.0088	0.011	0.0081	0.0056	0.0046	0.0081	0.0066	0.0103	0.0066	0.0103	0.0107	0.0091	0.0132	0.0067	0.0092	0.0082
D136	0.011	0.009	0.0072	0.0076	0.0066	0.0103	0.0047	0.0083	0.0057	0.0093	0.0098	0.0113	0.0122	0.0089	0.0083	0.0073
D137	0.0149	0.0128	0.0088	0.0092	0.0103	0.0067	0.0083	0.0048	0.0093	0.0058	0.0136	0.013	0.0085	0.0127	0.0099	0.011
D138	0.0136	0.0116	0.0076	0.0081	0.0091	0.0107	0.0072	0.0088	0.0082	0.0098	0.0124	0.0118	0.0127	0.0115	0.0088	0.0098
D139	0.0089	0.0079	0.0092	0.0118	0.0107	0.0146	0.0098	0.0136	0.0088	0.0125	0.0047	0.0082	0.0111	0.0099	0.0043	0.0033
D140	0.0122	0.0102	0.0125	0.013	0.0141	0.0104	0.0121	0.0084	0.0132	0.0094	0.0099	0.0093	0.0091	0.0133	0.0073	0.0084
D141	0.0096	0.0086	0.0078	0.0104	0.0093	0.0132	0.0084	0.0122	0.0073	0.0111	0.0073	0.011	0.0086	0.0074	0.0048	0.0038
D142	0.0079	0.0102	0.0093	0.0067	0.0077	0.0093	0.0099	0.0116	0.0099	0.0116	0.011	0.0073	0.0102	0.0058	0.0063	0.0073
D143	0.006	0.005	0.0064	0.009	0.01	0.0064	0.0091	0.0054	0.008	0.0044	0.0049	0.0064	0.002	0.0081	0.0054	0.0065
D144	0	0.0019	0.0073	0.0048	0.0038	0.0073	0.0058	0.0096	0.0058	0.0096	0.0038	0.0024	0.0081	0.0019	0.0064	0.0054
D145	0.0055	0.0035	0.008	0.0085	0.0096	0.0059	0.0075	0.0039	0.0086	0.005	0.0065	0.0059	0.0035	0.0076	0.007	0.0081
D146	0.0044	0.0024	0.0069	0.0073	0.0084	0.01	0.0064	0.008	0.0074	0.0091	0.0054	0.0048	0.0076	0.0065	0.0059	0.007
D147	0.0055	0.0045	0.007	0.0085	0.0096	0.0059	0.0086	0.005	0.0075	0.0039	0.0054	0.0059	0.0025	0.0076	0.006	0.007
D148	0.0038	0.0058	0.0033	0.0009	0	0.0033	0.0019	0.0053	0.0019	0.0053	0.0057	0.0042	0.0079	0.0019	0.0104	0.0093
D149	0.0073	0.0096	0.0047	0.0023	0.0033	0	0.0053	0.0019	0.0053	0.0019	0.0093	0.0057	0.0044	0.0053	0.0121	0.0132
D150	0.0048	0.0039	0.0033	0.0057	0.0067	0.0083	0.0058	0.0073	0.0048	0.0063	0.0009	0.0023	0.0049	0.0058	0.0043	0.0053
D151	0.0038	0.0029	0.0043	0.0067	0.0057	0.0093	0.0048	0.0084	0.0038	0.0073	0	0.0033	0.0059	0.0048	0.0053	0.0043
D152	0.0014	0.0034	0.0067	0.0042	0.0033	0.0067	0.0053	0.0089	0.0053	0.0089	0.0023	0.0009	0.0085	0.0024	0.0078	0.0068
D153	0.007	0.005	0.0073	0.0078	0.0089	0.0053	0.0069	0.0034	0.0079	0.0044	0.0048	0.0043	0.0039	0.008	0.0085	0.0096
D154	0.0076	0.0056	0.0059	0.0064	0.0074	0.0039	0.0054	0.0019	0.0065	0.0029	0.0075	0.007	0.0015	0.0055	0.0092	0.0103
D155	0.0064	0.0054	0.0068	0.0093	0.0104	0.0121	0.0094	0.0111	0.0084	0.01	0.0053	0.0068	0.0075	0.0085	0	0.0009
D156	0.0054	0.0044	0.0078	0.0104	0.0093	0.0132	0.0084	0.0122	0.0073	0.0111	0.0043	0.0078	0.0086	0.0074	0.0009	0

	B97	B98	B99	B100	B101	B102	B013	B014	B105	B106	B107	B108	B109	B110	B111	B112
D157	0.0091	0.0081	0.0094	0.0121	0.0132	0.0094	0.0122	0.0085	0.0111	0.0074	0.0079	0.0094	0.005	0.0112	0.0024	0.0034
D158	0.0038	0.0059	0.0093	0.0067	0.0077	0.0093	0.0099	0.0116	0.0099	0.0116	0.0078	0.0043	0.0102	0.0058	0.0024	0.0033
D159	0.0029	0.0049	0.0104	0.0077	0.0067	0.0104	0.0089	0.0127	0.0089	0.0127	0.0068	0.0053	0.0112	0.0048	0.0033	0.0024
D160	0.0059	0.0039	0.0084	0.0089	0.0099	0.0116	0.0079	0.0096	0.009	0.0106	0.0069	0.0063	0.0092	0.008	0.0014	0.0024
D161	0.0049	0.0029	0.0094	0.0099	0.0089	0.0127	0.0069	0.0106	0.0079	0.0117	0.0058	0.0073	0.0103	0.007	0.0024	0.0014
D162	0.0086	0.0066	0.0111	0.0116	0.0127	0.009	0.0106	0.007	0.0117	0.008	0.0096	0.009	0.0066	0.0108	0.0039	0.0049
D163	0.0093	0.0084	0.0037	0.0061	0.0051	0.0087	0.0042	0.0077	0.0033	0.0067	0.0062	0.0097	0.0084	0.0073	0.0047	0.0038
D164	0.0132	0.0122	0.0052	0.0076	0.0087	0.0052	0.0077	0.0043	0.0067	0.0033	0.0098	0.0113	0.0048	0.011	0.0062	0.0073
D165	0.0077	0.0099	0.0051	0.0027	0.0037	0.0051	0.0057	0.0072	0.0057	0.0072	0.0097	0.0061	0.0099	0.0057	0.0062	0.0072
D166	0.0076	0.0112	0.0101	0.0125	0.0108	0.0057	0.0092	0.0051	0.0076	0.0051	0.0037	0.0086	0.0076	0.0081	0.0104	0.0093
D167	0.0104	0.0127	0.0076	0.0051	0.0061	0.0028	0.0082	0.0047	0.0082	0.0047	0.0124	0.0087	0.0073	0.0083	0.0088	0.0098
D168	0.0099	0.0079	0.0042	0.0047	0.0057	0.0072	0.0038	0.0053	0.0047	0.0062	0.0088	0.0082	0.009	0.0078	0.0053	0.0062
D169	0.0051	0.0084	0.0089	0.0099	0.0116	0.0079	0.0104	0.0089	0.0127	0.0089	0.0127	0.0068	0.0053	0.0117	0.0057	0.0072
D170	0.0089	0.0069	0.0052	0.0057	0.0047	0.0082	0.0028	0.0062	0.0038	0.0073	0.0077	0.0092	0.01	0.0068	0.0062	0.0053
D171	0.0089	0.0069	0.0052	0.0057	0.0047	0.0082	0.0028	0.0062	0.0038	0.0073	0.0077	0.0092	0.01	0.0068	0.0062	0.0053
D172	0.0127	0.0106	0.0067	0.0072	0.0082	0.0047	0.0062	0.0028	0.0073	0.0038	0.0115	0.0108	0.0064	0.0105	0.0078	0.0089
D173	0.0115	0.0094	0.0057	0.0061	0.0071	0.0087	0.0052	0.0067	0.0062	0.0077	0.0103	0.0097	0.0105	0.0093	0.0067	0.0077
D174	0.0127	0.0117	0.0057	0.0072	0.0082	0.0047	0.0073	0.0038	0.0062	0.0028	0.0104	0.0108	0.0054	0.0105	0.0068	0.0078
D175	0.0073	0.0054	0.0077	0.0082	0.0092	0.0108	0.0073	0.0089	0.0083	0.0099	0.0053	0.0047	0.0096	0.0084	0.0028	0.0038
D176	0.01	0.008	0.0104	0.0108	0.0119	0.0083	0.0099	0.0063	0.011	0.0073	0.0078	0.0073	0.007	0.0111	0.0053	0.0063
D177	0.0063	0.0054	0.0077	0.0092	0.0082	0.0119	0.0073	0.011	0.0062	0.0099	0.0033	0.0057	0.0096	0.0073	0.0028	0.0019
D178	0.008	0.006	0.0063	0.0068	0.0078	0.0094	0.0058	0.0074	0.0069	0.0085	0.0079	0.0073	0.007	0.0059	0.0034	0.0044
D179	0.0108	0.0087	0.009	0.0094	0.0105	0.0069	0.0085	0.0049	0.0096	0.0059	0.0106	0.01	0.0045	0.0086	0.0059	0.007
D180	0.011	0.009	0.0072	0.0076	0.0066	0.0103	0.0047	0.0083	0.0057	0.0093	0.0098	0.0113	0.0122	0.0089	0.0083	0.0073
D181	0.011	0.009	0.0072	0.0076	0.0066	0.0103	0.0047	0.0083	0.0057	0.0093	0.0098	0.0113	0.0122	0.0089	0.0083	0.0073
D182	0.011	0.009	0.0072	0.0076	0.0066	0.0103	0.0047	0.0083	0.0057	0.0093	0.0098	0.0113	0.0122	0.0089	0.0083	0.0073
D183	0.0335	0.0339	0.0363	0.034	0.0326	0.0321	0.033	0.0325	0.0344	0.0339	0.0358	0.0349	0.0368	0.0349	0.0413	0.0398

	B113	B114	B115	B116	B117	B118	B119	B120	B121	B122	B123	B124	D125	D126	D127	D128
B113	0															
B114	0.0058	0														
B115	0.0024	0.0033	0													
B116	0.0049	0.0019	0.0054	0												
B117	0.0039	0.0019	0.0054	0.001	0											
B118	0.0005	0.0054	0.0019	0.0044	0.0034	0										
B119	0.0073	0.0062	0.0098	0.0053	0.0043	0.0078	0									
B120	0.0088	0.0047	0.0062	0.0067	0.0067	0.0083	0.0032	0								
B121	0.0098	0.0037	0.0072	0.0057	0.0057	0.0093	0.0023	0.0009	0							
B122	0.0078	0.0067	0.0083	0.0048	0.0058	0.0073	0.0023	0.0018	0.0027	0						
B123	0.0048	0.0038	0.0073	0.0028	0.0019	0.0053	0.0032	0.0066	0.0056	0.0057	0					
B124	0.0063	0.0033	0.0068	0.0014	0.0024	0.0058	0.0047	0.0061	0.0051	0.0042	0.0014	0				
D125	0.0029	0.0068	0.0033	0.0048	0.0058	0.0024	0.0082	0.0076	0.0087	0.0057	0.0047	0.0033	0			
D126	0.0097	0.0064	0.0102	0.0044	0.0054	0.0092	0.0099	0.0115	0.0104	0.0094	0.0073	0.0058	0.0096	0		
D127	0.0086	0.0064	0.0102	0.0054	0.0044	0.0081	0.0089	0.0115	0.0104	0.0105	0.0063	0.0069	0.0106	0.001	0	
D128	0.0148	0.0082	0.0119	0.0104	0.0104	0.0143	0.0066	0.0051	0.0041	0.0071	0.0101	0.0097	0.0135	0.0058	0.0058	0
D129	0.0138	0.0104	0.0143	0.0084	0.0094	0.0133	0.0057	0.0071	0.0061	0.0052	0.0092	0.0077	0.0115	0.0038	0.0048	0.0018
D130	0.0094	0.0083	0.0121	0.0073	0.0063	0.01	0.0076	0.0112	0.0101	0.0103	0.0042	0.0057	0.0093	0.0029	0.0019	0.0057
D131	0.0119	0.0085	0.0124	0.0065	0.0075	0.0114	0.0078	0.0093	0.0083	0.0073	0.0084	0.0069	0.0106	0.0019	0.0029	0.0038
D132	0.0019	0.0079	0.0044	0.007	0.0059	0.0024	0.0093	0.0108	0.0119	0.0099	0.0068	0.0084	0.0048	0.0075	0.0065	0.0125
D133	0.0079	0.0019	0.0053	0.0038	0.0038	0.0074	0.0082	0.0066	0.0057	0.0088	0.0057	0.0053	0.0089	0.0044	0.0044	0.0062
D134	0.0047	0.0038	0.0074	0.0019	0.0029	0.0065	0.0073	0.0088	0.0077	0.0068	0.0048	0.0033	0.0069	0.0024	0.0034	0.0083
D135	0.0083	0.0092	0.0108	0.0083	0.0073	0.0089	0.0027	0.0041	0.0051	0.0032	0.0061	0.0076	0.0092	0.0089	0.0078	0.0056
D136	0.0119	0.0057	0.0092	0.0077	0.0077	0.0115	0.0041	0.0027	0.0018	0.0046	0.0076	0.0071	0.0107	0.0083	0.0083	0.0023
D137	0.011	0.0077	0.0115	0.0058	0.0068	0.0105	0.0032	0.0046	0.0037	0.0028	0.0066	0.0052	0.0088	0.0063	0.0073	0.0042
D138	0.0073	0.0115	0.0078	0.0094	0.0105	0.0069	0.0066	0.0061	0.0071	0.0042	0.0103	0.0088	0.0053	0.01	0.0111	0.0076
D139	0.0115	0.0103	0.0119	0.0083	0.0093	0.011	0.0056	0.0051	0.006	0.0032	0.0091	0.0076	0.0092	0.0089	0.0099	0.0066
D140	0.0068	0.0057	0.0093	0.0048	0.0038	0.0073	0.0051	0.0086	0.0076	0.0076	0.0018	0.0033	0.0067	0.0053	0.0043	0.0081
D141	0.0048	0.0089	0.0053	0.0069	0.0079	0.0044	0.0103	0.0097	0.0107	0.0077	0.0067	0.0053	0.0019	0.0074	0.0085	0.0113
D142	0.0074	0.0063	0.01	0.0054	0.0044	0.008	0.0038	0.0072	0.0062	0.0062	0.0043	0.0058	0.0058	0.0094	0.0059	0.0067
D143	0.009	0.0048	0.0063	0.0069	0.0069	0.0085	0.0072	0.0037	0.0047	0.0057	0.0077	0.0073	0.0089	0.0074	0.0074	0.0052
D144	0.0029	0.0091	0.0054	0.0081	0.007	0.0035	0.0105	0.0121	0.0132	0.0111	0.0079	0.0096	0.0059	0.0076	0.0066	0.0127
D145	0.0091	0.0029	0.0064	0.0049	0.0049	0.0086	0.0093	0.0077	0.0067	0.0099	0.0068	0.0063	0.01	0.0044	0.0044	0.0062
D146	0.0045	0.0086	0.005	0.0066	0.0076	0.004	0.0122	0.0116	0.0127	0.0096	0.0096	0.008	0.0044	0.0061	0.0071	0.0122
D147	0.0086	0.0074	0.0091	0.0054	0.0065	0.0081	0.011	0.0104	0.0115	0.0084	0.0084	0.0069	0.0085	0.005	0.006	0.011
D148	0.0035	0.0086	0.005	0.0076	0.0066	0.003	0.0111	0.0116	0.0127	0.0106	0.0085	0.0091	0.0054	0.0071	0.0061	0.0122
D149	0.0132	0.0067	0.0104	0.0089	0.0089	0.0127	0.0051	0.0037	0.0027	0.0057	0.0087	0.0082	0.0119	0.0084	0.0084	0.0023
D150	0.0094	0.0104	0.0068	0.0127	0.0127	0.009	0.0087	0.0051	0.0061	0.0072	0.0124	0.0119	0.0083	0.0122	0.0122	0.0057
D151	0.0069	0.0078	0.0094	0.0069	0.0058	0.0074	0.0072	0.0087	0.0097	0.0077	0.0038	0.0053	0.0068	0.0064	0.0054	0.0092
D152	0.0079	0.0068	0.0105	0.0058	0.0048	0.0085	0.0062	0.0097	0.0087	0.0088	0.0028	0.0043	0.0078	0.0054	0.0044	0.0082
D153	0.0105	0.0043	0.0078	0.0063	0.0063	0.01	0.0087	0.0071	0.0061	0.0092	0.0052	0.0047	0.0083	0.0058	0.0058	0.0057
D154	0.0059	0.01	0.0064	0.008	0.0091	0.0054	0.0115	0.0108	0.0119	0.0089	0.0078	0.0063	0.0029	0.0075	0.0086	0.0115
D155	0.0066	0.0108	0.007	0.0087	0.0098	0.0061	0.01	0.0094	0.0105	0.0074	0.0106	0.0091	0.0054	0.0082	0.0093	0.01
D156	0.0024	0.0033	0.0048	0.0024	0.0014	0.0029	0.0047	0.0062	0.0072	0.0053	0.0023	0.0038	0.0053	0.007	0.0059	0.0119
D157	0.0034	0.0024	0.0058	0.0014	0.0005	0.0039	0.0038	0.0072	0.0062	0.0062	0.0014	0.0028	0.0063	0.0059	0.0049	0.0108
D158	0	0.0058	0.0024	0.0049	0.0039	0.0005	0.0073	0.0088	0.0098	0.0078	0.0048	0.0063	0.0029	0.0097	0.0086	0.0148
D159	0.0048	0.0009	0.0024	0.0029	0.0029	0.0044	0.0072	0.0037	0.0047	0.0057	0.0047	0.0043	0.0058	0.0074	0.0074	0.0092
D160	0.0058	0	0.0033	0.0019	0.0019	0.0054	0.0062	0.0047	0.0037	0.0067	0.0038	0.0033	0.0068	0.0064	0.0064	0.0082
D161	0.0039	0.0029	0.0044	0.001		0.0019	0.0034	0.0062	0.0057	0.0067	0.0038	0.0038	0.0024	0.0038	0.0054	0.0065	0.0115
D162	0.0049	0.0019	0.0054	0	0.001	0.0044	0.0053	0.0067	0.0057	0.0048	0.0028	0.0014	0.0048	0.0044	0.0054	0.0104
D163	0.0014	0.0054	0.0019	0.0034	0.0044	0.001	0.0089	0.0083	0.0093	0.0063	0.0063	0.0048	0.0014	0.0081	0.0092	0.0143
D164	0.0073	0.0062	0.0098	0.0053	0.0043	0.0078	0	0.0032	0.0023	0.0023	0.0032	0.0047	0.0082	0.0099	0.0089	0.0066
D165	0.0038	0.0098	0.0062	0.0089	0.0078	0.0043	0.0032	0.0046	0.0056	0.0037	0.0066	0.0082	0.0047	0.0138	0.0127	0.0101
D166	0.0088	0.0047	0.0062	0.0067	0.0067	0.0083	0.0032	0	0.0009	0.0018	0.0066	0.0061	0.0076	0.0115	0.0115	0.0051
D167	0.0067	0.0089	0.0061	0.0037	0.0027	0.0061	0.0047	0.0082	0.0047	0.0082	0.0087	0.0071	0.011	0.0047	0.0072	0.0062
D168	0.0062	0.0072	0.0038	0.0093	0.0093	0.0058	0.0056	0.0023	0.0032	0.0042	0.0091	0.0087	0.0052	0.0143	0.0143	0.0076
D169	0.0078	0.0067	0.0083	0.0048	0.0058	0.0073	0.0023	0.0018	0.0027	0	0.0057	0.0042	0.0057	0.0094	0.0105	0.0071
D170	0.0089	0.0057	0.0093	0.0038	0.0048	0.0084	0.0014	0.0027	0.0018	0.0009	0.0047	0.0033	0.0067	0.0084	0.0094	0.0061
D171	0.0089	0.0057	0.0093	0.0038	0.0048	0.0084	0.0014	0.0027	0.0018	0.0009	0.0047	0.0033	0.0067	0.0084	0.0094	0.0061
D172	0.0053	0.0093	0.0058	0.0073	0.0084	0.0048	0.0047	0.0042	0.0051	0.0023	0.0082	0.0067	0.0033	0.0122	0.0133	0.0097
D173	0.0093	0.0082	0.0098	0.0062	0.0073	0.0089	0.0037	0.0032	0.0041	0.0014	0.0071	0.0057	0.0072	0.011	0.0121	0.0086

	B113	B114	B115	B116	B117	B118	B119	B120	B121	B122	B123	B124	D125	D126	D127	D128
D174	0.0043	0.0093	0.0058	0.0084	0.0073	0.0038	0.0037	0.0042	0.0051	0.0033	0.0072	0.0077	0.0043	0.0133	0.0122	0.0097
D175	0.0053	0.0043	0.0058	0.0024	0.0033	0.0048	0.0057	0.0051	0.0061	0.0033	0.0023	0.0009	0.0023	0.0069	0.0079	0.0107
D176	0.0029	0.0068	0.0033	0.0048	0.0058	0.0024	0.0082	0.0076	0.0087	0.0057	0.0047	0.0033	0	0.0096	0.0106	0.0135
D177	0.0053	0.0033	0.0068	0.0024	0.0014	0.0048	0.0037	0.0061	0.0051	0.0052	0.0005	0.0009	0.0043	0.0069	0.0058	0.0097
D178	0.0059	0.0048	0.0064	0.0029	0.0039	0.0054	0.0043	0.0038	0.0047	0.0019	0.0048	0.0033	0.0048	0.0075	0.0086	0.0093
D179	0.0034	0.0074	0.0039	0.0054	0.0065	0.0029	0.0068	0.0062	0.0073	0.0043	0.0073	0.0058	0.0024	0.0103	0.0114	0.0121
D180	0.011	0.0077	0.0115	0.0058	0.0068	0.0105	0.0032	0.0046	0.0037	0.0028	0.0066	0.0052	0.0088	0.0063	0.0073	0.0042
D181	0.011	0.0077	0.0115	0.0058	0.0068	0.0105	0.0032	0.0046	0.0037	0.0028	0.0066	0.0052	0.0088	0.0063	0.0073	0.0042
D182	0.011	0.0077	0.0115	0.0058	0.0068	0.0105	0.0032	0.0046	0.0037	0.0028	0.0066	0.0052	0.0088	0.0063	0.0073	0.0042
D183	0.0393	0.0373	0.0368	0.0378	0.0393	0.0388	0.0387	0.0377	0.0363	0.0382	0.0397	0.0377	0.0373	0.0344	0.0358	0.033

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	D129	D130	D131	D132	D133	D134	D135	D136	D137	D138	D139	D140	D141	D142	D143	D144
D129	0															
D130	0.0047	0														
D131	0.0019	0.0038	0													
D132	0.0116	0.0073	0.0097	0												
D133	0.0083	0.0062	0.0064	0.0058	0											
D134	0.0063	0.0053	0.0044	0.0049	0.0019	0										
D135	0.0047	0.0066	0.0068	0.0062	0.0072	0.0062	0									
D136	0.0042	0.0081	0.0062	0.0098	0.0037	0.0057	0.0032	0								
D137	0.0023	0.0072	0.0043	0.0089	0.0057	0.0038	0.0023	0.0018	0							
D138	0.0057	0.0108	0.0079	0.0053	0.0093	0.0073	0.0037	0.0051	0.0033	0						
D139	0.0047	0.0097	0.0068	0.0093	0.0082	0.0062	0.0027	0.0041	0.0023	0.0037	0					
D140	0.0072	0.0023	0.0063	0.0048	0.0038	0.0028	0.0042	0.0056	0.0047	0.0082	0.0071	0				
D141	0.0093	0.0073	0.0085	0.0029	0.0068	0.0048	0.0072	0.0087	0.0067	0.0033	0.0072	0.0047	0			
D142	0.0058	0.0048	0.0039	0.0054	0.0043	0.0034	0.0028	0.0042	0.0033	0.0068	0.0057	0.0023	0.0073	0		
D143	0.0073	0.0083	0.0054	0.0069	0.0028	0.0048	0.0042	0.0028	0.0047	0.00621	0.0052	0.0057	0.0068	0.0033	0	
D144	0.0117	0.0074	0.0098	0.005	0.0112	0.0103	0.0116	0.0154	0.0145	0.0106	0.0149	0.01	0.008	0.0108	0.0124	0
D145	0.0084	0.0063	0.0065	0.0112	0.0048	0.007	0.0125	0.0088	0.011	0.0149	0.0136	0.0089	0.0122	0.0096	0.0079	0.006
D146	0.0102	0.0091	0.0082	0.0066	0.0108	0.0087	0.0133	0.0149	0.0128	0.0091	0.0133	0.0117	0.0065	0.0125	0.0119	0.0015
D147	0.009	0.0079	0.007	0.0108	0.0096	0.0075	0.0121	0.0136	0.0116	0.0133	0.0089	0.0105	0.0106	0.0112	0.0106	0.0055
D148	0.0112	0.008	0.0093	0.0055	0.0108	0.0098	0.0122	0.0149	0.014	0.0102	0.0145	0.0106	0.0075	0.0114	0.0119	0.0005
D149	0.0042	0.0082	0.0063	0.0154	0.0088	0.011	0.0081	0.0046	0.0066	0.0103	0.0091	0.0107	0.0141	0.0093	0.0077	0.01
D150	0.0077	0.0119	0.01	0.0116	0.0125	0.0149	0.0097	0.0081	0.0103	0.0067	0.0107	0.0146	0.0104	0.0132	0.0093	0.0064
D151	0.0083	0.0033	0.0074	0.009	0.0099	0.009	0.0082	0.0118	0.0108	0.0125	0.0113	0.0057	0.0089	0.0084	0.0099	0.0039
D152	0.0073	0.0023	0.0064	0.01	0.0089	0.0079	0.0092	0.0107	0.0098	0.0136	0.0124	0.0047	0.0099	0.0073	0.011	0.0049
D153	0.0077	0.0047	0.0069	0.0127	0.0062	0.0084	0.0118	0.0081	0.0103	0.0141	0.0129	0.0072	0.0104	0.0099	0.0083	0.0074
D154	0.0094	0.0073	0.0086	0.008	0.0122	0.0102	0.0125	0.0141	0.0121	0.0084	0.0125	0.0099	0.0048	0.0128	0.0122	0.0029
D155	0.008	0.0102	0.0061	0.0087	0.013	0.0109	0.0111	0.0127	0.0106	0.007	0.0111	0.0128	0.0075	0.0103	0.0097	0.0035
D156	0.011	0.0068	0.0091	0.0044	0.0053	0.0044	0.0057	0.0092	0.0083	0.0099	0.0088	0.0043	0.0073	0.0048	0.0063	0.0054
D157	0.0099	0.0058	0.008	0.0054	0.0043	0.0034	0.0067	0.0082	0.0073	0.011	0.0098	0.0033	0.0084	0.0038	0.0073	0.0065
D158	0.0138	0.0094	0.0119	0.0019	0.0079	0.007	0.0083	0.0119	0.011	0.0073	0.0115	0.0068	0.0048	0.0074	0.009	0.0029
D159	0.0115	0.0093	0.0096	0.0069	0.0028	0.0048	0.0082	0.0066	0.0088	0.0104	0.0092	0.0067	0.0078	0.0073	0.0038	0.008
D160	0.0104	0.0083	0.0085	0.0079	0.0019	0.0038	0.0092	0.0057	0.0077	0.0115	0.0103	0.0057	0.0089	0.0063	0.0048	0.0091
D161	0.0094	0.0084	0.0075	0.0059	0.0048	0.0029	0.0073	0.0088	0.0068	0.0084	0.0073	0.0058	0.0058	0.0064	0.0058	0.007
D162	0.0084	0.0073	0.0065	0.007	0.0038	0.0019	0.0083	0.0077	0.0058	0.0094	0.0083	0.0048	0.0069	0.0054	0.0069	0.0081
D163	0.0122	0.0111	0.0103	0.0034	0.0074	0.0054	0.0099	0.0115	0.0094	0.0058	0.0099	0.0084	0.0034	0.0091	0.0085	0.0045
D164	0.0057	0.0076	0.0078	0.0093	0.0082	0.0073	0.0027	0.0041	0.0032	0.0066	0.0056	0.0051	0.0103	0.0038	0.0072	0.0105
D165	0.0092	0.0113	0.0116	0.0058	0.0119	0.011	0.0042	0.0076	0.0066	0.0033	0.0071	0.0087	0.0067	0.0073	0.0088	0.0069
D166	0.0071	0.0112	0.0093	0.0108	0.0066	0.0088	0.0041	0.0027	0.0046	0.0061	0.0051	0.0086	0.0097	0.0072	0.0037	0.0121
D167	0.0098	0.0037	0.0072	0.0057	0.0057	0.0093	0.0023	0.0009	0	0.0027	0.0056	0.0051	0.0087	0.0104	0.0104	0.0041
D168	0.0097	0.0139	0.0121	0.0083	0.0092	0.0115	0.0066	0.0051	0.0071	0.0037	0.0076	0.0112	0.0072	0.0098	0.0062	0.0094
D169	0.0052	0.0103	0.0073	0.0099	0.0088	0.0068	0.0032	0.0046	0.0028	0.0042	0.0032	0.0076	0.0077	0.0062	0.0057	0.0111
D170	0.0042	0.0092	0.0063	0.011	0.0077	0.0058	0.0042	0.0037	0.0018	0.0052	0.0042	0.0066	0.0088	0.0053	0.0067	0.0122
D171	0.0042	0.0092	0.0063	0.011	0.0077	0.0058	0.0042	0.0037	0.0018	0.0052	0.0042	0.0066	0.0088	0.0053	0.0067	0.0122
D172	0.0077	0.013	0.01	0.0073	0.0115	0.0094	0.0057	0.0071	0.0052	0.0019	0.0057	0.0103	0.0053	0.0089	0.0083	0.0085
D173	0.0066	0.0118	0.0089	0.0115	0.0103	0.0083	0.0046	0.006	0.0042	0.0057	0.0018	0.0091	0.0092	0.0077	0.0072	0.0127
D174	0.0088	0.0119	0.0111	0.0063	0.0115	0.0105	0.0047	0.0071	0.0062	0.0028	0.0066	0.0092	0.0062	0.0078	0.0083	0.0074
D175	0.0088	0.0067	0.0079	0.0073	0.0062	0.0043	0.0066	0.0081	0.0062	0.0077	0.0066	0.0042	0.0043	0.0068	0.0062	0.0085
D176	0.0115	0.0093	0.0106	0.0048	0.0089	0.0069	0.0092	0.0107	0.0088	0.0053	0.0092	0.0067	0.0019	0.0094	0.0089	0.0059
D177	0.0088	0.0047	0.0079	0.0073	0.0053	0.0043	0.0066	0.0071	0.0062	0.0098	0.0087	0.0023	0.0062	0.0048	0.0073	0.0085
D178	0.0073	0.0094	0.0054	0.008	0.0069	0.0049	0.0053	0.0067	0.0048	0.0063	0.0053	0.0068	0.0069	0.0044	0.0038	0.0092
D179	0.01	0.0122	0.0081	0.0054	0.0096	0.0075	0.0078	0.0093	0.0073	0.0038	0.0078	0.0094	0.0044	0.007	0.0064	0.0066
D180	0.0023	0.0072	0.0043	0.0089	0.0057	0.0038	0.0023	0.0018	0	0.0033	0.0023	0.0047	0.0067	0.0033	0.0047	0.0145
D181	0.0023	0.0072	0.0043	0.0089	0.0057	0.0038	0.0023	0.0018	0	0.0033	0.0023	0.0047	0.0067	0.0033	0.0047	0.0145
D182	0.0023	0.0072	0.0043	0.0089	0.0057	0.0038	0.0023	0.0018	0	0.0033	0.0023	0.0047	0.0067	0.0033	0.0047	0.0145
D183	0.0335	0.0363	0.0358	0.0393	0.0373	0.0378	0.0402	0.0363	0.0368	0.0363	0.0402	0.0397	0.0373	0.0413	0.0402	0.0354

	D145	D146	D147	D148	D149	D150	D151	D152	D153	D154	D155	D156	D157	D158	D159	D160
D145	0															
D146	0.0055	0														
D147	0.0044	0.004	0													
D148	0.0055	0.001	0.005	0												
D149	0.0038	0.0096	0.0084	0.0096	0											
D150	0.0073	0.0059	0.01	0.0059	0.0033	0										
D151	0.0048	0.0054	0.0044	0.0044	0.0067	0.0083	0									
D152	0.0038	0.0065	0.0054	0.0054	0.0057	0.0093	0.0009	0								
D153	0.0014	0.007	0.0058	0.007	0.0033	0.0067	0.0033	0.0023	0							
D154	0.007	0.0015	0.0054	0.0024	0.0089	0.0053	0.0038	0.0048	0.0053	0						
D155	0.0076	0.002	0.0061	0.003	0.0074	0.0039	0.0065	0.0075	0.008	0.0024	0					
D156	0.0064	0.007	0.0059	0.006	0.0104	0.0121	0.0043	0.0053	0.0078	0.0085	0.0092	0				
D157	0.0054	0.0081	0.007	0.007	0.0093	0.0132	0.0053	0.0043	0.0068	0.0096	0.0103	0.0009	0			
D158	0.0091	0.0045	0.0086	0.0035	0.0132	0.0094	0.0069	0.0079	0.0105	0.0059	0.0066	0.0024	0.0034	0		
D159	0.0038	0.0075	0.0064	0.0075	0.0077	0.0093	0.0068	0.0078	0.0053	0.009	0.0097	0.0024	0.0033	0.0048	0	
D160	0.0029	0.0086	0.0074	0.0086	0.0067	0.0104	0.0078	0.0068	0.0043	0.01	0.0108	0.0033	0.0024	0.0058	0.0009	0
D161	0.0059	0.0055	0.0044	0.0066	0.0099	0.0116	0.0058	0.0069	0.0073	0.007	0.0076	0.0014	0.0024	0.0039	0.0019	0.0029
D162	0.0049	0.0066	0.0054	0.0076	0.0089	0.0127	0.0069	0.0058	0.0063	0.008	0.0087	0.0024	0.0014	0.0049	0.0029	0.0019
D163	0.0086	0.003	0.007	0.004	0.0127	0.009	0.0085	0.0096	0.01	0.0044	0.005	0.0039	0.0049	0.0014	0.0044	0.0054
D164	0.0093	0.0122	0.011	0.0111	0.0051	0.0087	0.0072	0.0062	0.0087	0.0115	0.01	0.0047	0.0038	0.0073	0.0072	0.0062
D165	0.0132	0.0085	0.0127	0.0074	0.0087	0.0052	0.0088	0.0098	0.0124	0.0078	0.0064	0.0062	0.0073	0.0038	0.0088	0.0098
D166	0.0077	0.0116	0.0104	0.0116	0.0037	0.0051	0.0087	0.0097	0.0071	0.0108	0.0094	0.0062	0.0072	0.0088	0.0037	0.0047
D167	0.0067	0.0127	0.0115	0.0127	0.0027	0.0061	0.0097	0.0087	0.0061	0.0119	0.0105	0.0072	0.0062	0.0098	0.0047	0.0037
D168	0.0104	0.009	0.0132	0.009	0.0061	0.0028	0.0113	0.0124	0.0097	0.0083	0.0069	0.0088	0.0098	0.0062	0.0062	0.0072
D169	0.0099	0.0096	0.0084	0.0106	0.0057	0.0072	0.0077	0.0088	0.00921	0.0089	0.0074	0.0053	0.0062	0.0078	0.0057	0.0067
D170	0.0089	0.0106	0.0094	0.0117	0.0047	0.0082	0.0088	0.0077	0.0082	0.0099	0.0085	0.0062	0.0053	0.0089	0.0067	0.0057
D171	0.0089	0.0106	0.0094	0.0117	0.0047	0.0082	0.0088	0.0077	0.0082	0.0099	0.0085	0.0062	0.0053	0.0089	0.0067	0.0057
D172	0.0127	0.007	0.0111	0.008	0.0082	0.0047	0.0104	0.0115	0.0119	0.0063	0.0049	0.0078	0.0089	0.0053	0.0083	0.0093
D173	0.0115	0.0111	0.0068	0.0122	0.0071	0.0087	0.0092	0.0103	0.0107	0.0104	0.009	0.0067	0.0077	0.0093	0.0072	0.0082
D174	0.0127	0.008	0.0122	0.007	0.0082	0.0047	0.0093	0.0104	0.0119	0.0073	0.0059	0.0068	0.0078	0.0043	0.0083	0.0093
D175	0.0073	0.007	0.0058	0.008	0.0092	0.0108	0.0043	0.0053	0.0057	0.0053	0.008	0.0028	0.0038	0.0053	0.0033	0.0043
D176	0.01	0.0044	0.0085	0.0054	0.0119	0.0083	0.0068	0.0078	0.0083	0.0029	0.0054	0.0053	0.0063	0.0029	0.0058	0.0068
D177	0.0063	0.0091	0.0079	0.008	0.0082	0.0119	0.0043	0.0033	0.0047	0.0073	0.0102	0.0028	0.0019	0.0053	0.0043	0.0033
D178	0.008	0.0076	0.0065	0.0087	0.0078	0.0094	0.0069	0.0079	0.0084	0.008	0.0055	0.0034	0.0044	0.0059	0.0038	0.0048
D179	0.0108	0.005	0.0092	0.0061	0.0105	0.0069	0.0096	0.0106	0.0111	0.0054	0.003	0.0059	0.007	0.0034	0.0064	0.0074
D180	0.011	0.0128	0.0116	0.014	0.0066	0.0103	0.0108	0.0098	0.0103	0.0121	0.0106	0.0083	0.0073	0.011	0.0088	0.0077
D181	0.011	0.0128	0.0116	0.014	0.0066	0.0103	0.0108	0.0098	0.0103	0.0121	0.0106	0.0083	0.0073	0.011	0.0088	0.0077
D182	0.011	0.0128	0.0116	0.014	0.0066	0.0103	0.0108	0.0098	0.0103	0.0121	0.0106	0.0083	0.0073	0.011	0.0088	0.0077
D183	0.0335	0.0334	0.0373	0.0349	0.0326	0.0321	0.0373	0.0358	0.0335	0.0335	0.0349	0.0413	0.0398	0.0393	0.0387	0.0373

	D161	D162	D163	D164	D165	D166	D167	D168	D169	D170	D171	D172	D173	D174	D175	D176
D161	0															
D162	0.001	0														
D163	0.0024	0.0034	0													
D164	0.0062	0.0053	0.0089	0												
D165	0.0078	0.0089	0.0053	0.0032	0											
D166	0.0057	0.0067	0.0083	0.0032	0.0046	0										
D167	0.0067	0.0057	0.0093	0.0023	0.0056	0.0009	0									
D168	0.0083	0.0093	0.0058	0.0056	0.0023	0.0023	0.0032	0								
D169	0.0038	0.0048	0.0063	0.0023	0.0037	0.0018	0.0027	0.0042	0							
D170	0.0048	0.0038	0.0073	0.0014	0.0047	0.0027	0.0018	0.0051	0.0009	0						
D171	0.0048	0.0038	0.0073	0.0014	0.0047	0.0027	0.0018	0.0051	0.0009	0	0					
D172	0.0063	0.0073	0.0038	0.0047	0.0014	0.0042	0.0051	0.0018	0.0023	0.0033	0.0033	0				
D173	0.0053	0.0062	0.0078	0.0037	0.0051	0.0032	0.0041	0.0056	0.0014	0.0023	0.0023	0.0037	0			
D174	0.0073	0.0084	0.0048	0.0037	0.0005	0.0042	0.0051	0.0018	0.0033	0.0042	0.0042	0.0009	0.0047	0		
D175	0.0014	0.0024	0.0038	0.0057	0.0072	0.0051	0.0061	0.0076	0.0033	0.0042	0.0042	0.0057	0.0047	0.0067	0	
D176	0.0038	0.0048	0.0014	0.0082	0.0047	0.0076	0.0087	0.0052	0.0057	0.0067	0.0067	0.0033	0.0072	0.0043	0.0023	0
D177	0.0033	0.0024	0.0058	0.0037	0.0072	0.0061	0.0051	0.0087	0.0052	0.0042	0.0042	0.0077	0.0066	0.0067	0.0019	0.0043
D178	0.0019	0.0029	0.0044	0.0043	0.0058	0.0038	0.0047	0.0062	0.0019	0.0028	0.0028	0.0043	0.0033	0.0053	0.0024	0.0048
D179	0.0044	0.0054	0.0019	0.0068	0.0033	0.0062	0.0073	0.0038	0.0043	0.0053	0.0053	0.0019	0.0058	0.0029	0.0048	0.0024
D180	0.0068	0.0058	0.0094	0.0032	0.0066	0.0046	0.0037	0.0071	0.0028	0.0018	0.0018	0.0052	0.0042	0.0062	0.0062	0.0088
D181	0.0068	0.0058	0.0094	0.0032	0.0066	0.0046	0.0037	0.0071	0.0028	0.0018	0.0018	0.0052	0.0042	0.0062	0.0062	0.0088
D182	0.0068	0.0058	0.0094	0.0032	0.0066	0.0046	0.0037	0.0071	0.0028	0.0018	0.0018	0.0052	0.0042	0.0062	0.0062	0.0088
D183	0.0393	0.0378	0.0373	0.0387	0.0382	0.0377	0.0363	0.0358	0.0382	0.0368	0.0368	0.0363	0.0402	0.0377	0.0392	0.0373

	D177	D178	D179	D180	D181	D182	D183
D177	0						
D178	0.0043	0					
D179	0.0069	0.0024	0				
D180	0.0062	0.0048	0.0073	0			
D181	0.0062	0.0048	0.0073	0	0		
D182	0.0062	0.0048	0.0073	0	0	0	
D183	0.0392	0.0408	0.0388	0.0368	0.0368	0.0368	0

	A1	A2	A3	A4	A5	B1	B2	B3	C1	C2	C3	C4	C5	D1	D2	CB	GR	PR	SF
A1	0																		
A2	0.0033	0																	
A3	0.0033	0	0																
A4	0.0049	0.0049	0.0049	0															
A5	0.0017	0.0017	0.0017	0.0066	0														
B1	0.0049	0.0049	0.0049	0.0032	0.0066	0													
B2	0.0050	0.0050	0.0050	0.0065	0.0033	0.0032	0												
B3	0.0066	0.0066	0.0066	0.0048	0.0050	0.0048	0.0016	0											
C1	0.0050	0.0050	0.0050	0.0098	0.0033	0.0065	0.0033	0.0049	0										
C2	0.0065	0.0065	0.0065	0.0047	0.0082	0.0016	0.0048	0.0064	0.0048	0									
C3	0.0066	0.0066	0.0066	0.0081	0.0050	0.0048	0.0016	0.0032	0.0016	0.0032	0								
C4	0.0081	0.0081	0.0081	0.0031	0.0098	0.0031	0.0064	0.0047	0.0064	0.0015	0.0047	0							
C5	0.0082	0.0082	0.0082	0.0064	0.0066	0.0064	0.0032	0.0016	0.0032	0.0047	0.0016	0.0031	0						
D1	0.0081	0.0081	0.0081	0.0031	0.0098	0.0063	0.0097	0.0079	0.0097	0.0046	0.0079	0.0030	0.0063	0					
D2	0.0066	0.0066	0.0066	0.0114	0.0050	0.0114	0.0082	0.0098	0.0049	0.0097	0.0065	0.0112	0.0081	0.0079	0				
CB	0.0082	0.0082	0.0082	0.0064	0.0066	0.0064	0.0032	0.0016	0.0032	0.0047	0.0016	0.0031	0	0.0063	0.0081	0			
GR	0.0082	0.0082	0.0082	0.0064	0.0066	0.0064	0.0032	0.0016	0.0032	0.0047	0.0016	0.0031	0	0.0063	0.0081	0	0		
PR	0.0082	0.0082	0.0082	0.0064	0.0066	0.0064	0.0032	0.0016	0.0032	0.0047	0.0016	0.0031	0	0.0063	0.0081	0	0	0	
SF	0.0410	0.0410	0.0410	0.0378	0.0434	0.0378	0.0426	0.0401	0.0426	0.0394	0.0442	0.0371	0.0417	0.0409	0.0483	0.0417	0.0417	0.0417	0

APPENDIX C: PC 2 versus PC 3 component plots for Morphometric vs Meristic analyses
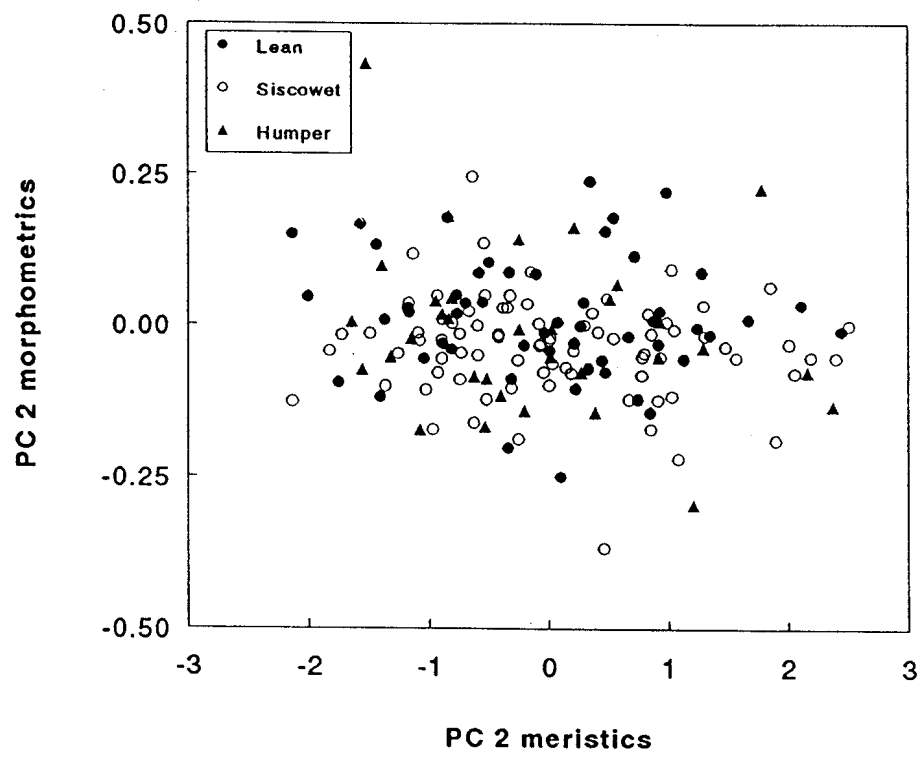
Figure C.1. PC 2 morphometrics vs PC 2 meristics for Lake Superior *S. namaycush*.
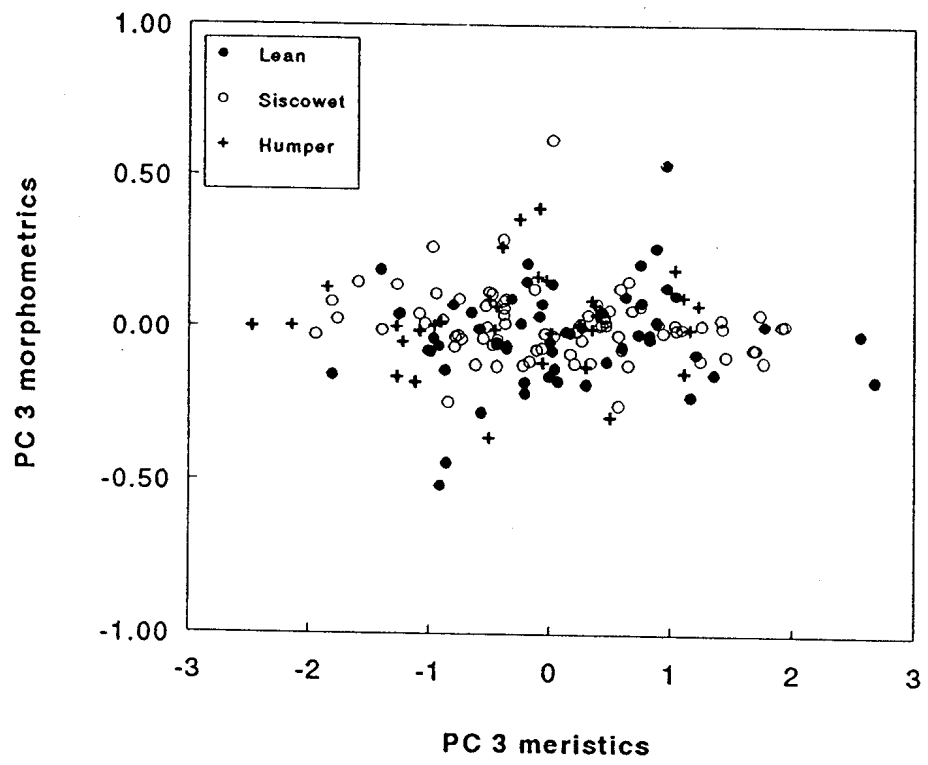
Figure C.2.   PC 3 morphometrics vs PC 3 meristics for Lake Superior *S. namaycush*.
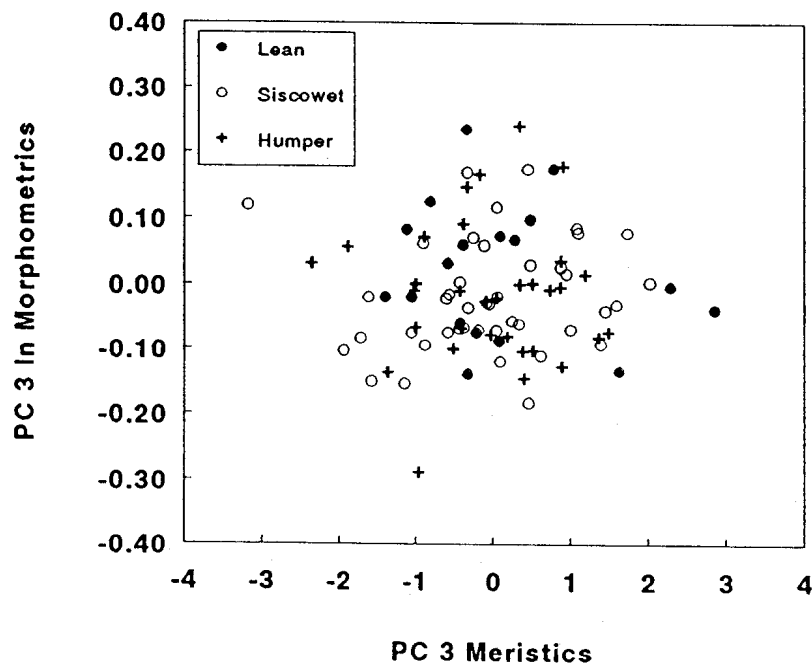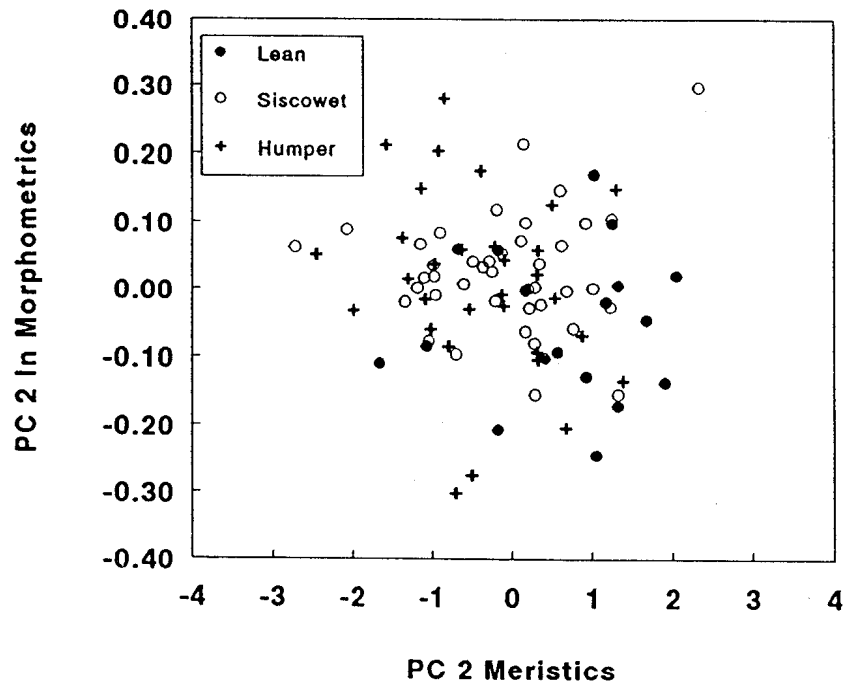
Figure C.3. PC 2 and PC 3 morphometrics vs meristics for Isle Royale populations.
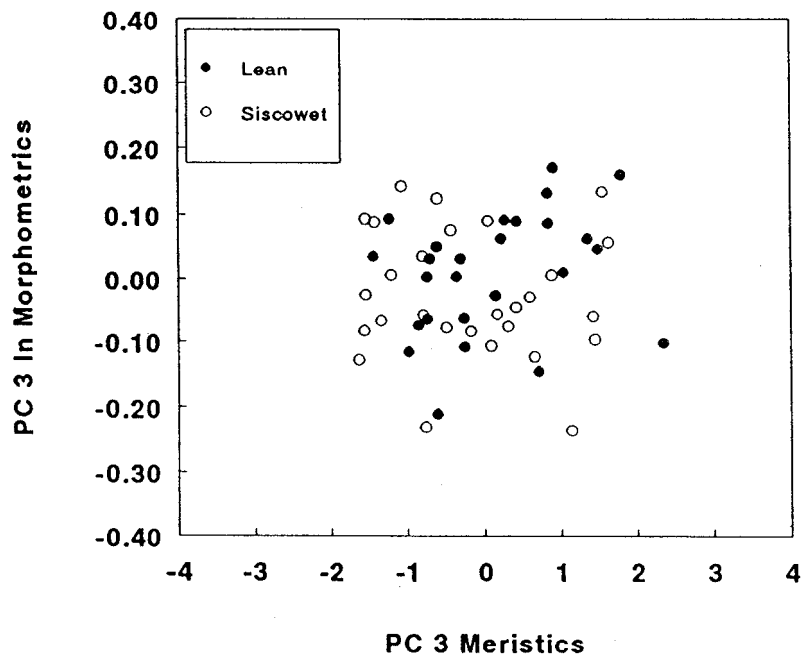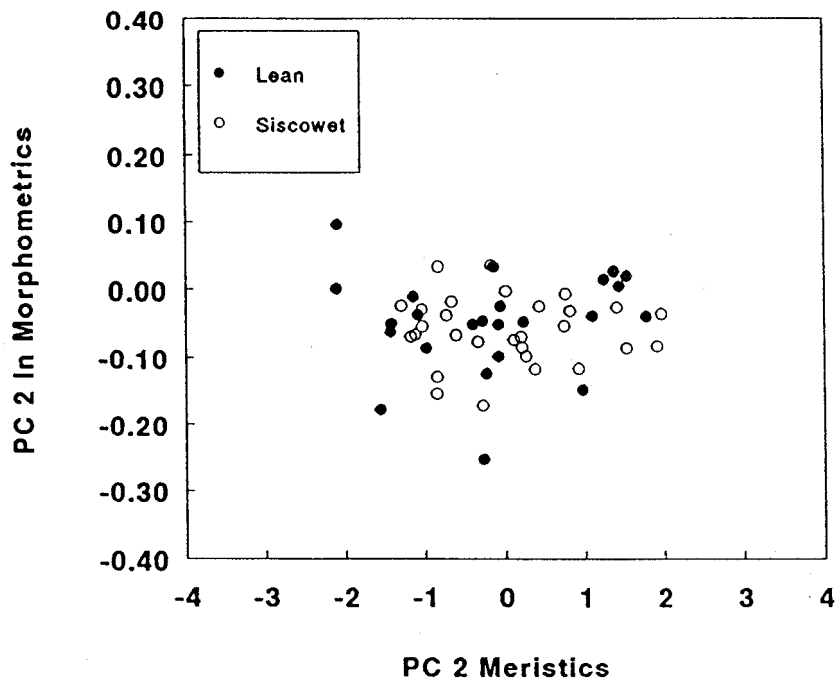
Figure C.4. PC 2 and PC 3 morphometrics vs meristics for Keweenaw Bay populations.
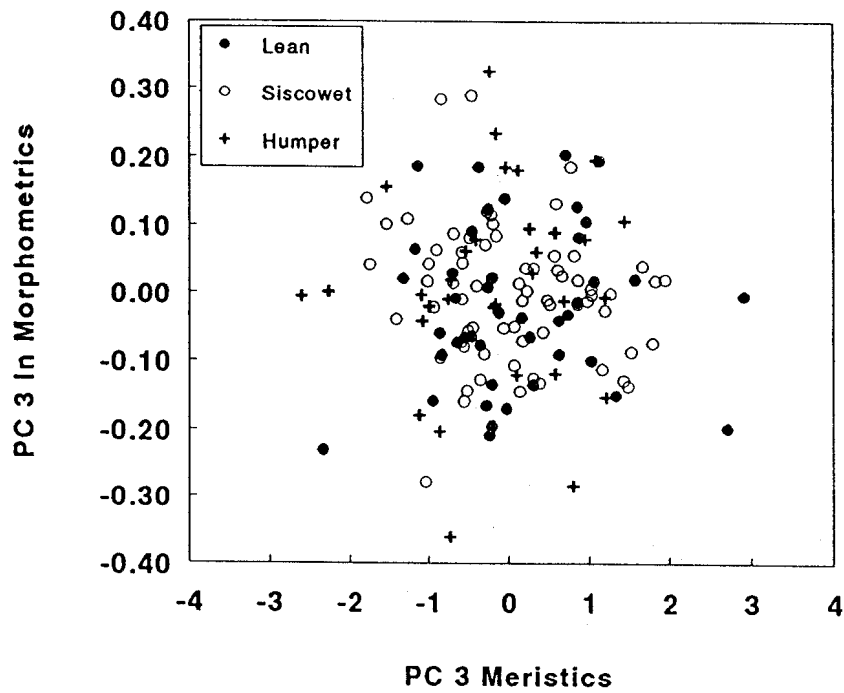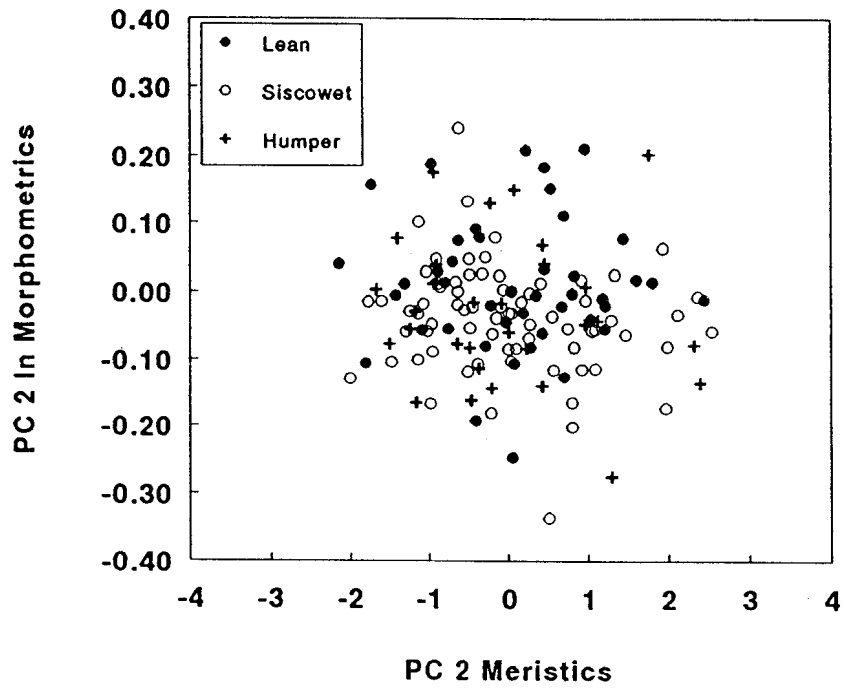
Figure C.5. PC 2 and PC 3 morphometrics vs meristics for north and south populations combined, identified by phenotype.
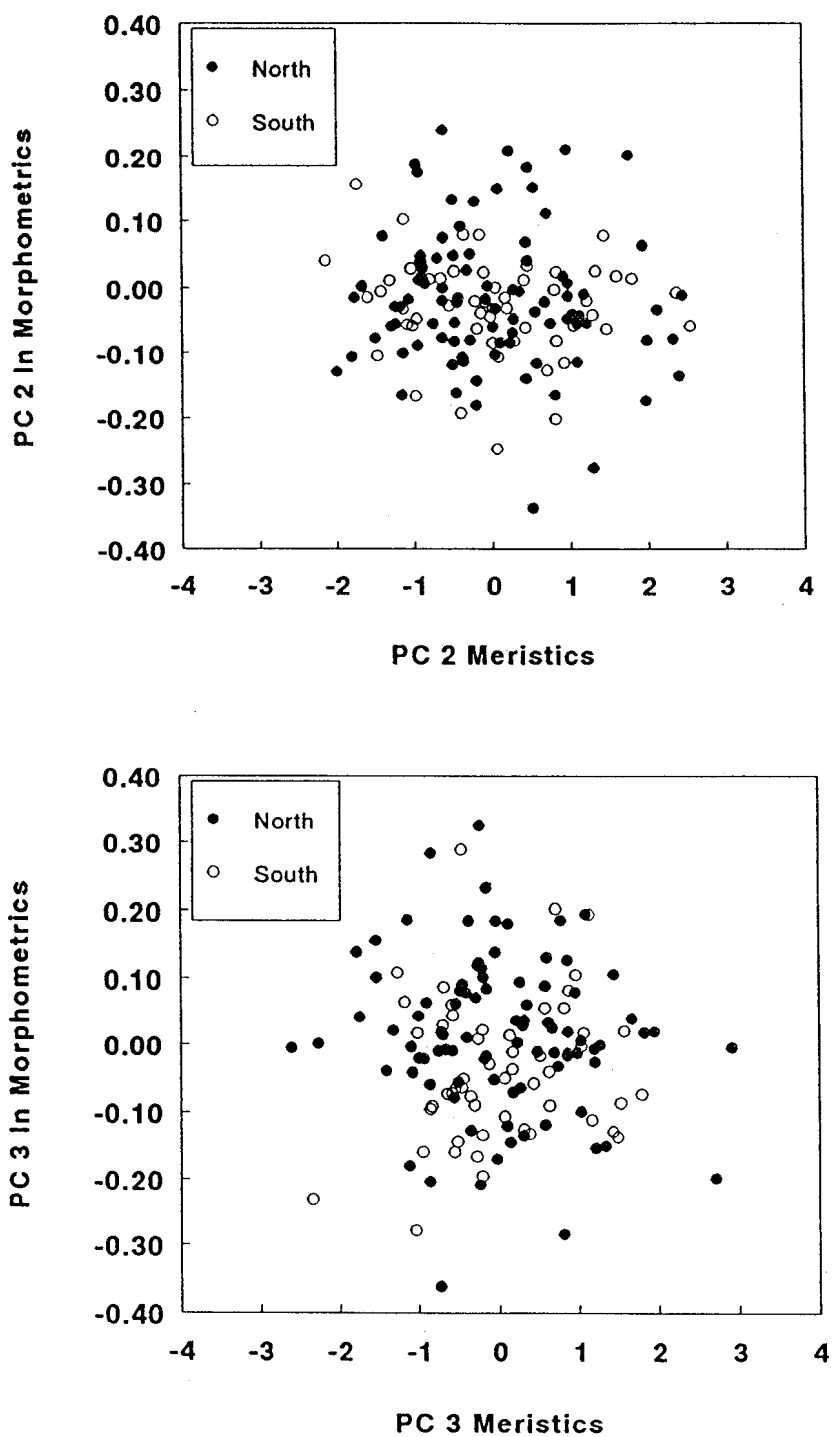
Figure C.6. PC 2 and PC 3 morphometrics vs meristics for north and south populations combined, identified by geographic locality.
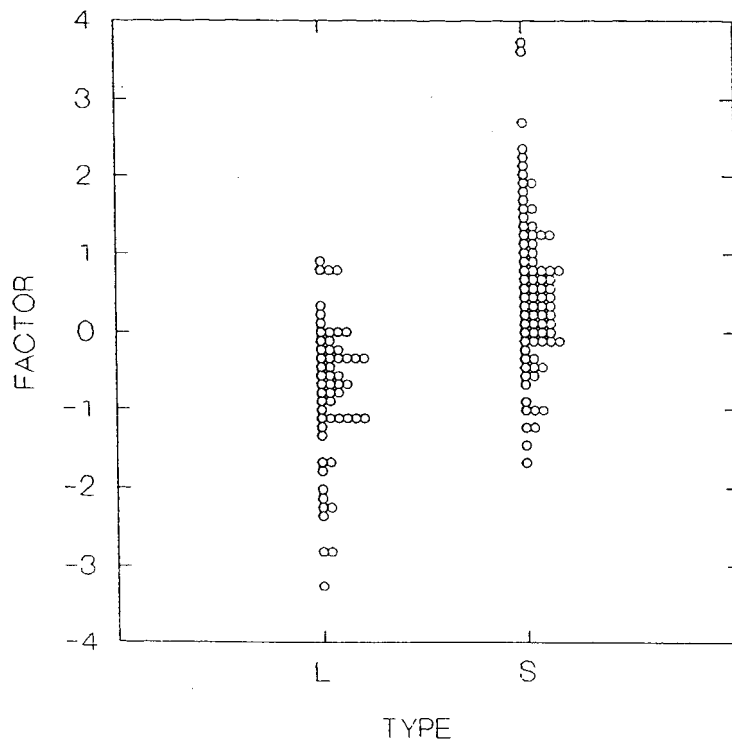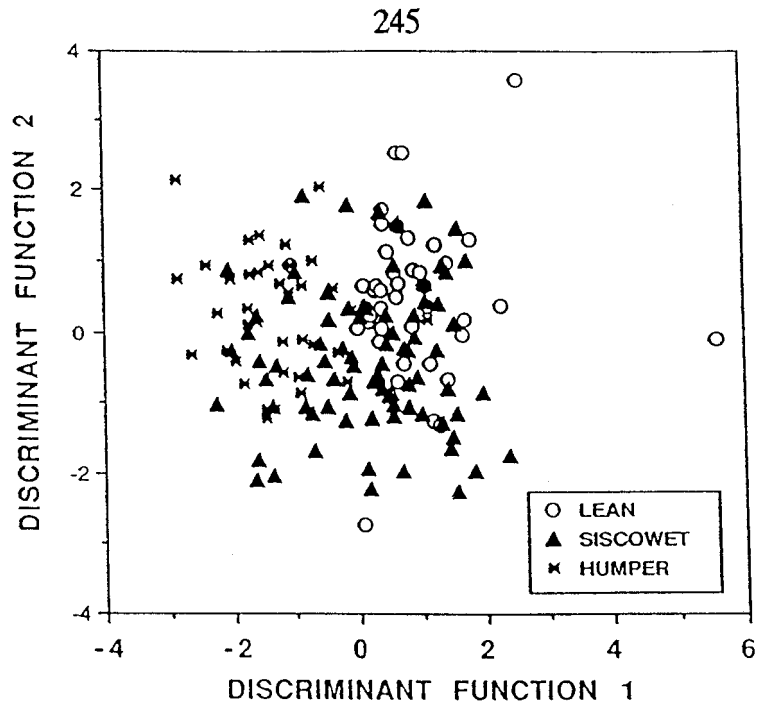
Figure C.7. Discriminant analysis results. (a) Analysis of all data for lean, siscowet, and humper phenotypes in a correlation matrix. Leans and humpers can be distinguished, but siscowets overlap in both dimensions. (b) Analysis of lean and siscowet phenotypes in a correlation matrix shows that leans and siscowets cannot be discriminated, even with *a priori* classification.
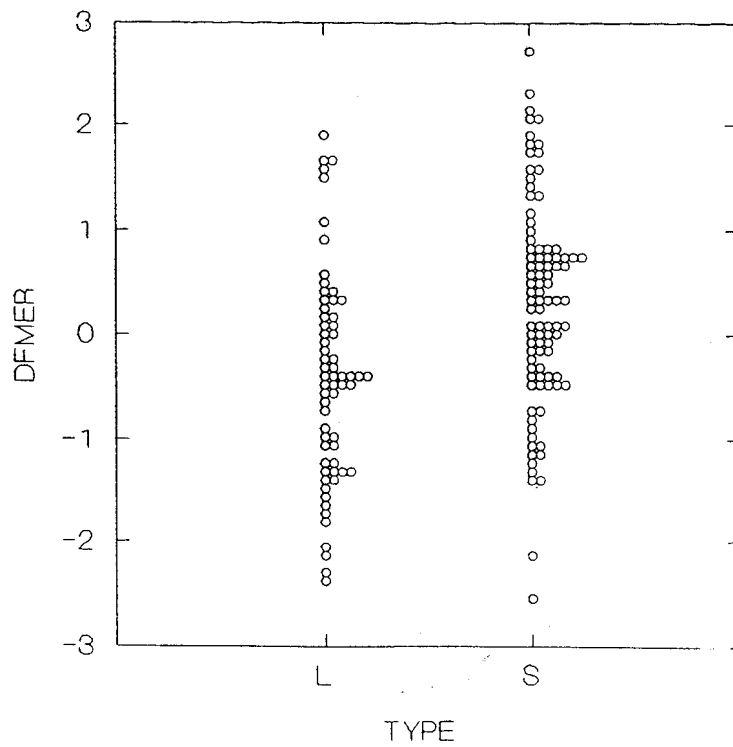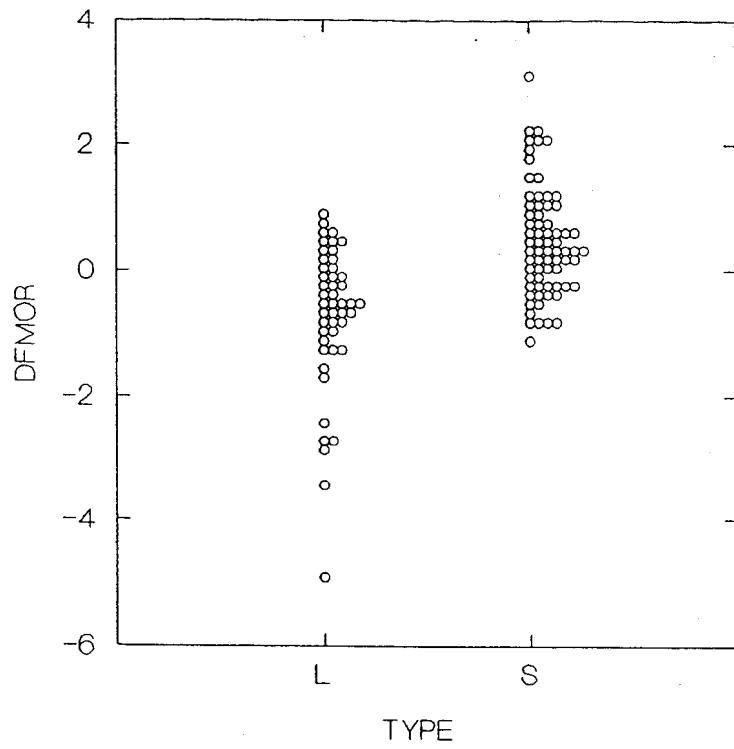
Figure C.8. Results of discriminant analysis of (a) morphometric, and (b) meristic characters for lean and siscowet lake trout. Morphometric data shows less overlap between leans and siscowets, but does not allow discrimination.

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