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ORIGIN OF GREAT LAKES BROWN TROUT, Salmo trutta:
A PHYLOGEOGRAPHIC ANALYSIS USING mtDNA SEQUENCE VARIATION

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This is work is dedicated to Daniel and Annette Byers. Without their love and support, I would never have made it as far as I have today. Thank you.
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ABSTRACT

The brown trout, *Salmo trutta*, was first introduced to the Laurentian Great Lakes in 1887 from European broodstocks to found a recreational salmonid fishery; however, the origins of these progenitor lineages remain largely unknown. Trout from these regions are very specialized to their native habitats and matching North American stocks to similar watersheds may help increase survivability by introducing the stock to a more appropriate environment. The objective of this study was to determine the European origins of brown trout found in the Great Lakes. We analyzed 144 brown trout from ten watersheds across Michigan and Wisconsin and identified their strain assignment according to the MIDNR classification using their mtDNA ND-1 sequences. European progenitor lineages occurring within these strain assignments were then identified using the first 309 base pairs of the mtDNA control region. Nine ND-1 haplotypes were found in the four most recently stocked strains. A total of four different European lineages were identified by 5 SNPs in the mtDNA control region in the 144 brown trout samples. One unique control region haplotype which has not been described was observed and a phylogeny was constructed with known sequences. We found that the Sturgeon River strain largely shares the same progenitor lineage as Gilchrist Creek. Fishery managers can use this information to make informed decisions about stocking watersheds where certain strains might prosper or to choose to not stock strains due to poor performance and great dissimilarity between North American watersheds and the European progenitor’s native watershed.
TABLE OF CONTENTS

LIST OF TABLES.......................................................... vii

LIST OF FIGURES....................................................... viii

CHAPTER

I. INTRODUCTION....................................................... 1
   Origin and Taxonomy of Brown Trout....................... 1
   Life History of Brown Trout................................. 3
   Brown Trout Genetics........................................... 7
   Brown Trout Introductions................................... 12
   Michigan Strains ............................................... 13
   Genetic Strain Management and Project Goals............ 15

II. MATERIALS & METHODS........................................... 18
   Sample Collection............................................. 18
   DNA Amplification and Sequencing.......................... 21
   Sequence Analysis............................................. 23

III. RESULTS........................................................... 26
   Great Lakes Lineage Assignments.......................... 26
   Lineage Phylogenetic Analyses............................... 30
   Sturgeon River Strain.......................................... 32

IV. DISCUSSION......................................................... 33
   Great Lakes Lineage Assignments.......................... 33
   Sturgeon River Strain.......................................... 38

V. CONCLUSIONS....................................................... 43

LITERATURE CITED................................................... 44
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Watersheds, sample sizes and haplotype totals for 144 brown trout sampled in Michigan and Wisconsin. The superscript numbers refer to the genotype designation as defined in Johnson et al. 2009</td>
<td>20</td>
</tr>
<tr>
<td>2. Primer sequences (5’-3’) for mtDNA analysis of Great Lakes brown trout (Salmo trutta)</td>
<td>23</td>
</tr>
<tr>
<td>3. Identification of polymorphic sites observed in the first 313 base pairs of the mtDNA control region in brown trout samples from the Great Lakes. Dashes indicate sequence homology with AT-s1 while mutations are listed. Genotypes AT-s1 and DA-s1 in Bernatchez (2001); A* genotype in Apostolidis (1997); GC3 genotype has not yet been described in the literature</td>
<td>27</td>
</tr>
<tr>
<td>4. Strain assignments given by ND-1 sequences, sample sizes (n), and European lineage assignments given by control region sequences among 144 brown trout samples from 7 Michigan and 3 Wisconsin populations. Numbers in parenthesis refer to the strain haplotype designations in Johnson (2009). Lineage designations AT-s1 and DA-s1 are described in Bernatchez (2001). Asterisk indicates lineage assignment reported in Apostolidis et al. (1997) with original nomenclature. The GC3 genotype has not yet been described in the literature</td>
<td>29</td>
</tr>
<tr>
<td>5. Strain designation and lineage designation of 10 brown trout from the Sturgeon River strain. The strain designation was determined by using the entire mtDNA ND-1 region as described in Johnson et al. (2009). The lineage designation was determined by using the first 313 bp of the mtDNA control region as described in Bernatchez (2001). The designation GC3* indicates that a unique control region sequence was observed and a European match could not be designated</td>
<td>32</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Sequence of the 5'–end of 299 bp of the mtDNA control region including 10 base pairs of the tRNA gene proline for the AT-s1 genotype by Bernatchez (2001). Variable positions among brown trout samples here are underlined and positions numbered</td>
<td>27</td>
</tr>
<tr>
<td>3. Unrooted maximum likelihood phylogenetic tree of observed haplotypes and reference haplotypes of the first 313 base pairs of the mtDNA control region in major brown trout lineages. The reference bar is measured in substitutions/site. The labels in bold are haplotypes that are observed in Great Lakes populations</td>
<td>30</td>
</tr>
<tr>
<td>4. Unrooted maximum parsimony phylogenetic tree of observed haplotypes and reference haplotypes of the first 313 base pairs of the mtDNA control region in two major brown trout lineages. The labels in bold are haplotypes that are observed in Great Lakes populations</td>
<td>31</td>
</tr>
</tbody>
</table>
INTRODUCTION

Origin and Taxonomy of Brown Trout

The brown trout (*Salmo trutta*) is a member of the order Salmoniformes which belongs to the ray-finned fish superorder Protacanthopterygii. The origin of Salmoniformes can be traced 100 to 150 million years ago but the earliest known member of the family Salmonidae occurred 50 million years ago as the primitive salmonid known as *Eosalmo driftwoodensis* (Wilson and Li 1999). The origin of these early members of Salmonidae is thought to have occurred from a genome duplication event between 50 and 100 million years ago which resulted in a tetraploid genome (Allendorf and Thorgaard 1984, King et al. 2007). The cells of these salmonids behave as diploids, despite having two times as much DNA in each cell compared to individuals of related families. A recent mitogenomic study of Protacanthopterygii showed that the sister group of Salmonidae is Esociformes, which includes the freshwater resident pike, pickerels, and mudminnows (Ishiguro et al. 2003). This finding supports the hypothesis that salmonids are more closely related to esociform fishes than osmerid fishes, which includes smelts, galaxiids and icefishes. The oldest fossils relating salmonid and esociform fishes have been found to be 150 million years old (Arratia 1997), while molecular analysis performed by Johnson and Patterson (1996) estimated that these two lineages diverged approximately 95 to 100 million years ago.

Although the subject is heavily debated, the ancestor of salmonids likely had a freshwater origin (Hoar 1976, Holz 2005, Jonsson and Jonsson 2011). This freshwater origin
of salmonids is also supported by the structure of the glomerular kidney in freshwater fish as compared to marine fish (Hoar 1976, Tanaka 1985), the lack of marine parasites in salmonids (Ramsden et al. 2003), zinc: calcium ratios in the otoliths of Salmoniformes compared to sister orders (Limburg and Elfman 2010), among other studies. It should also be noted that there are no salmonids which are exclusively marine. Fyhn et al. (1999) postulated that early teleosts, or bony fishes, colonized marine systems via marine excursions for feeding and maturing and then returned to freshwater to spawn. The ancestors of these teleosts lived in freshwater for about 250 million years before returning to marine systems during the Jurassic period. Teleosts were not able to spawn in marine systems until an adaptation was developed which increased the water content in the yolk of their eggs. This adaptation, known as oocyte hydration, was key in permanently colonizing marine systems and is estimated to have been developed around 55 million years ago (Fyhn et al. 1999, Kristoffersen and Finn 2008). Salmonids did not develop this mechanism and are therefore unable to spawn in marine systems.

The family Salmonidae is split into three subfamilies: Coregoninae (whitefish and ciscoes), Thymallinae (grayling) and Salmoninae (salmon, trout and char) (Nelson 2006). Salmoninae is composed of five genera: Brachymystax (lenox), Hucho (huchen and taimen), Salvelinus (charrs), Onchorhynchus (Pacific salmon and trout) and Salmo (Atlantic salmon and trout) (Phillips et al. 2004, Nelson 2006, Esteve and McLennan 2007, Xia et al. 2007). The exact taxonomy of Salmo is still a subject of great debate and more than 60 varieties of brown trout have been described (McKeown et al. 2010, Jonsson and Jonsson 2011). Much of this confusion comes from the great amount of phenotypic plasticity expressed by brown trout, some of which can be accounted for by the significant amount of genetic diversity in the species, by the range of environments in which they inhabit, and by the interaction of
these genetic and environmental factors (Ferguson et al. 1995, Langerhans 2008). Primarily however, the brown trout (*Salmo trutta*) and the Atlantic salmon (*Salmo salar*) comprise the Salmo genus.

*Life History of Brown Trout*

Brown trout show a great deal of variation in their life history adaptations (Bernatchez et al. 1992). This is due in part to their great adaptability to many different environment types and due to the great geological changes which have taken place over their native range (Valiente et al. 2010). The last glaciation period commenced around 75,000 years ago, culminated around 18,000 years ago with the ice cap reaching as far south as modern France, and tapered around 10,000 years ago with the ice cap over northwestern Europe receding and disappearing (Denton and Hughes 1981). These post-glacial colonizations involved many different lineages of trout which became geographically isolated from each other and from other major bodies of water. In order to deal with this changing environment, brown trout have adapted three different basic life history strategies: a stream-resident form, a lake-resident form and a sea-run (anadromous) form. These various life history adaptations result in different growth rates, ages at maturity and reproductive efforts and success (Jonsson 1981, Jonsson and Hindar 1982).

Adult brown trout spawn in cold freshwater streams with coarse, stony bottoms which can vary in temperature from 0-25° C and contain dissolved oxygen amounts from 5.0-5.5 mg/L, with eggs needing intergravel dissolved oxygen levels to be at least 9.0 mg/L (Mills 1971, Forseth et al. 2009). After spawning, anadromous and resident adult brown trout travel to available lacustrine habitats to overwinter (Jonsson and Gravem 1985). This utilization of lacustrine habitats appears adaptive and can increase the production of juvenile
brown trout from river systems (Jonsson 1985, Klemetsen et al. 2003, Cote 2007). Here they segregate based on size and age, the smaller trout centering on the rocky areas near the shore to avoid predation and the larger trout utilizing available rocky and sandy areas (Hegge et al. 1993). As winter passes, stream-resident trout return to their natal streams, lake-resident trout remain in a lacustrine habitat and sea-run trout head to estuarine and marine habitats to feed and grow until the next spawning event. These sea-run brown trout do not exploit true marine habitats, unlike the related Atlantic salmon, since they cannot efficiently regulate their ionic concentrations in sea water and they are also not as physiologically adapted for continuous swimming in the open ocean (Hoar 1976, Webb 1988).

At the optimal embryo survival temperature of 8-10°C, brown trout eggs hatch between 40 and 70 days after fertilization (Crisp 1981, Ojanguren and Braña 2003). These eggs hatch into alevins, a very early life stage of brown trout which derives energy from the yolk sac which is still attached to their ventral surface. After salmonid alevins have hatched, they hide among the stones and crevices, in tree roots and undercut banks and in macro-vegetation in streams (Beland et al. 2004). As the alevins grow and uses all of the energy from the yolk it must start feeding in the water column and it enters the next stage of development, called the parr. These parr feed on drifting and epibenthic invertebrates, maximizing their net energy intake by utilizing shelter to decrease predation, balancing foraging opportunities and minimizing dangerous behaviors (Jenkins and Keeley 2010). This invertebrate drift is positively correlated with stream velocity and the density of the epibenthic zoobenthos is dependent on the stability and porosity of the substrate and water depth (Lancaster et al. 1996, Jowett 2003). These parr are usually 7 cm or less in length and prefer water depths between 5 and 30 cm, with the preferred depth increasing with fish length and presence of suitable shelter (Greenberg 1994, Riley et al. 2009). As the number of
shelters decrease, the influence of all other factors affecting carrying capacity become much stronger and the carrying capacity of the stream decreases (Finstad et al. 2007, 2009). As carrying capacity decreases and juvenile density increases, brown trout parr leave their natal streams and migrate to lacustrine systems to utilize these highly productive areas (Landergren 2004). In order to avoid predation and survive in this new ecosystem, migrant salmonids significantly adjust their diets and grow at rates much higher than their counterparts who stayed in the streams (Dempson 1996, Cote 2007). As the parr’s body mass increases, larger food items become preferred and these larger trout can eat things that the smaller trout cannot (Lillehamer 1973). These larger lake-feeding juvenile salmonids become piscivorous early on and have higher densities of protein and fat than those in the tributaries (Dempson 2004). Some parr who did not initially migrate head from tributaries to lakes between ages 1 and 3 and live in water between 0 and 10 m in depth to take advantage of increased resources and mature (Haraldstad and Jonsson 1983). The largest adult fish exploit the greater depths over the juveniles due to the lower optimal temperature for growth (Macpherson and Duarte 1991, Lafrance et al. 2005).

As some of the parr grow to 10-12 cm, they can undergo a transformative process called smoltification (McCormick et al. 2007). If a salmonid parr does not commence this process, then it will become a freshwater stream-resident individual (Hansen et al. 1989). Smoltification prepares the brown trout for life in the pelagic zone by altering its behavior, physiology and morphological characteristics in order to take advantage of resources which occur in open waters. The process is triggered from environmental cues like photoperiod and temperature (McCormick et al. 1998), with photoperiod determining what time of year this process occurs and temperature affecting the time of the beginning of the process and the rate at which these changes occur (McCormick et al. 2002, Zydlewski, et al. 2005).
During smoltification, the brown trout assumes a silvery color which masks parr marks on their sides. Thyroid hormones play a direct role in this color change and are responsible for the distribution of two purines, guanine and hypoxanthine, which cause this silvering (Hutchison and Iwata 1998). This color change is adapted for predator avoidance in pelagic habitats and may also be essential for water balance in a hyper-osmotic habitat (Hoar 1988). The body form of the trout changes as well, with the swim bladder increasing in relative size (Saunders 1965) and an increase in length relative to mass which results in a lower condition factor during the transformation process (Beeman et al 1995). The enzyme Na\(^+\)/K\(^+\)-ATPase increases in activity and production as smoltification occurs, with this enzyme being associated with increased secretory capacity of gills in salt and brackish waters (Spencer et al. 2010). In brown trout, there is a positive correlation between this enzyme’s increased activity and the tendency for trout to migrate downstream to lacustrine or marine habitats (Aarestrup et al. 2000). As smoltification concludes, the brown trout loses its positive rheotaxis and follows the current to the next large body of water. As the brown trout grow and sexually mature, they return to their natal streams to spawn. Brown trout regularly mature in the first autumn subsequent to smolting (Jonsson 1985, Jonsson et al. 2001). In order to survive, they go through a desmoltification process which results in a loss of their silvery color and a reduction of Na\(^+\)/K\(^+\)-ATPase activity (Jonsson 1989, Fängstam et al. 1993). After spawning and overwintering, the anadromous and lake-run trout go through the smoltification process again and return to their respective body of water. This process occurs faster and to a greater extent each year the fish returns for spawning (Wedemeyer et al. 1980).

Brown trout can reach sexual maturity as either a parr or a smolt. More females go through smoltification in anadromous populations and the resulting sex ratios in migrating
groups is typically 60% female and 40% male (Jonsson 1985, Dellefors and Faremo 1988). This female behavior is thought to be adaptive to taking advantage of more productive lacustrine and marine habitats in order to acquire enough energy for the production of eggs for spawning. Since there is increased mortality with spawning behaviors, many more male parr reach sexual maturity and stay in the stream instead of smolting and migrating to a larger body of water (Hansen et al. 1989, Jonsson 1989).

**Brown Trout Genetics**

The brown trout displays impressive adaptableness with its variation of life history adaptations, which can be attributed to its equally impressive genetic variation. It has been postulated that the brown trout may in fact be one of the most genetically diverse vertebrate species known (Ferguson 1989). By occupying many different types of ecosystems while playing many different roles in each ecosystem, salmonid species show evidence of adaptive variation (Hindar et al. 1991, Adkison 1995, Nislow et al. 2004, Garcia de Leaniz et al. 2007), although the exact loci which are under reputed selection are generally not known. However, research has shown that certain characteristics of distinct salmonid populations such as spawning time (Jonsson 1982, 1989, Quinn et al. 2000), migratory tendency (Brannon 1972, Northeote and Kelso 1981), embryonic development (Beacham 1989, Herbert et al. 1998), growth rates (Einum and Fleming 2000, Kavanagh et al. 2010), and others are under genetic control to some extent. Transplantation studies have shown that offspring of anadromous brown trout retain their ability to smolt and migrate to sea when released early on with non-anadromous brown trout above an impassable upstream waterfall (Jonsson 1982). A study with Chinook salmon showed that crosses between life history variants indicate that smolting in the first year of life was dominant and probably controlled
by few loci (Clarke et al. 1992, 1994). The amount of expression of these different
phenotypes which is under genetic control, environmental control or a combination of both
is not precisely determined however. The genes or genes, which control these quantitative
traits, are referred to as quantitative trait loci (QTL). These QTL regions are of great interest,
because the traits they control (growth, maximum adult size, fecundity) are often of great
economic and evolutionary interest. Methods which efficiently determine the exact location
of these genes are currently very expensive and time-consuming, so research efforts may be
better spent determining the structure of populations which display these desired traits. As
these technologies are developed and the regions discovered, specific behaviors and
physiological characteristics and their associated heritability will determine what forces
guided brown trout to become so genetically variable. For now, even if the loci are not
determined, it can be inferred that with the brown trout’s immense genetic variation comes
the ability to adapt to a variety of ecosystems.

Since brown trout are able to adapt to so many different types of ecosystems, a large
amount of their genetic divergence lies in the isolation of independent populations in various
types of watersheds. When looking at the brown trout’s native range, between 40 and 65%
of the genetic variation of the species is due to differences between populations (Ryman
1983, Ferguson 1989). With this range and genetic variation, the brown trout is one of the
most genetically substructured vertebrate species known (Allendorf and Leary 1988). This
dramatic substructure is due in part to a brown trout’s strong homing ability during
spawning to return to its natal stream and to the geographic barriers which separate these
watersheds. Studies have shown that even in the same river, different morphs of brown trout
can be significantly reproductively isolated from one another (Allendorf et al. 1976, Ryman
et al. 1979). Different populations of brown trout from tributaries of the same watershed
have been observed to vary in mitochondrial DNA (mtDNA) haplotypes (Ferguson et al. 1995, Hansen and Loeschcke 1996). Two brown trout of the same morph from different watersheds may appear morphologically similar, but are in fact very genetically dissimilar.

The mitochondrial genome has proved to be a key tool in phylogeographic analysis due to its haploid maternal inheritance, relatively small size, high mutation rate and lack of genetic recombination. New mitochondria are not made *de novo*, but rather originate from existing organelles (Jansen and de Boer 1998). Therefore, any changes in the mitochondrial genome are theoretically only due to mutation. A phylogeny, or a diagram which shows the evolutionary relationship between organisms, can be created using these mutations and shared nucleotides between organisms which have the sequence in common. By knowing the mutation rate and current mitochondrial sequence, comparative phylogenetic analyses can be performed to give a relatively detailed story of matrilineal genetic relationships (Cann et al. 1987, Hebert et al. 2003). These data, coupled with geographic information, can then be used to perform phylogeographic analyses which can track the physical maternal movements of ancestral populations over evolutionary time (Avise et al. 1987).

The mitochondria contains a 16,677 base pair circular genome found in the cytoplasm which codes for 22 tRNAs, 2 rRNAs and 13 subunits of the respiratory chain complexes so picking regions to compare between individuals can be difficult. Regions must be variable enough in order to show sufficient differences between many populations or individuals but must be similar enough so that related groups can be easily recognized. Ideally, the entire mtDNA genome from each individual would be sequenced so that every difference could be recorded but this is not financially feasible with current sequencing technologies. Brown trout become very genetically isolated quickly so many coding genes can be used for analysis (Ferguson 1989). For phylogenetic analysis, the mtDNA genes ND-
1, ND-5/6, cyt-b, and the control region have been shown in the literature to vary quite significantly over populations of brown trout and therefore serve as genetic indicators of the rest of the mtDNA genome (Bernatchez and Danzmann 1993; Bernatchez and Osinov 1995; Nielsen et al. 1998). The non-coding control region is putatively not under selection since the region does not code for an actual product but the region contains conserved sequence blocks which act as the origin for replication and binding sites for several proteins; therefore, considering this sequence as selectively neutral is under dispute (Lee et al. 1995, Clayton 2000, Moraes 2002). The region has still proved to be very useful in constructing accurate phylogenies however, since short lengths of this region provide enough mutations to construct an accurate phylogeny (Lee et al. 1995, Bernatchez 2001, Guo et al. 2003). In our study, we will be using the control region as well as the ND-1 region. These two regions differ in function, with the ND-1 region actually coding for a subunit of the oxidative phosphorylation pathway and the control region acting as some sort of regulating region for the replication of mtDNA, and therefore have different mutation rates. The control region seems to change at a reduced rate when compared to the ND-1 region, so using the two different regions will give a structure of the basic phylogeny as well as an increased resolution within some of the main groups by using the ND-1 sequences from the same individuals.

Recently, mtDNA sequences have been used to uncover the different evolutionary lineages which occur over the geographically diverse native range of brown trout (Bernatchez et al. 1992, Apostolidis et al. 1997, Bernatchez 2001, McKeown et al. 2010). Bernatchez (2001) determined that there are five major evolutionary lineages of brown trout based on mtDNA sequences of 1794 trout from 174 native populations: the Atlantic, Danubian, Adriatic, marmoratus and Mediterranean. These five main lineages are viewed as
the five main evolutionary significant units (ESU) of the species complex *Salmo trutta*, where an ESU is considered a population or group of populations which significantly reproductively isolated from other conspecifics and represent a key component of the evolutionary legacy of the species (Waples 1991, Bernatchez 2001). Based on the observed extent of the divergence, Bernatchez estimated that the lineages separated through allopatric fragmentation between 0.5 and 2.0 million years ago with the oldest divergences being between the lineages associated with the main watersheds of the Atlantic Ocean (Atlantic lineage), Ponto-Caspian Seas (Danubian lineage) and the Mediterranean Sea (Mediterranean, Adriatic, *marmoratus* lineages). The Atlantic lineage is described as being composed of largely anadromous populations which are based around northwestern European Atlantic watersheds, which provided the habitat for the lineage’s largest demographic expansion between 13,400 and 26,800 years ago as Pleistocene glaciers receded. Using nested clade analyses, Bernatchez (2001) extrapolated that the Danubian lineage’s center of origin is likely the Black Sea and its associated watersheds with the most significant demographic expansion taking place 270,000-290,000 years ago. The fact that this is the most genetically diverse lineage is tied into the complex geological history of the area which restricted gene flow and bottlenecked populations due to great changes in watershed connectivity over time. Bernatchez (2001) also describes the Mediterranean lineages as being very diverse due to the great amount of geographic variation of the west Mediterranean and the Balkan Peninsula with the largest demographic expansion of this group occurring between 67,000 and 134,000 years ago.

The process of these lineages differentiating was reliant heavily on geographic isolation and the accumulation of various ecological and genetic specializations due to the different selection regimes that each ecosystem imposed. Lu and Bernatchez (1998) reported
that the salmonid *Coregonus clupeafoemis* (lake whitefish) has shown similar geographic isolation and evolutionary lineage structure and when individuals were mated from different evolutionary lineages, the progeny showed much higher embryonic mortality thought to be due to partial genetic incompatibilities. Even when lineages occur in the same watershed differences in trophic niches, life history adaptations and genetic constraints greatly reduces the amount of gene flow between these distinct populations (Bernatchez et al. 1999, Lu and Bernatchez 1999).

**Brown Trout Introductions**

The native brown trout range is primarily Europe, western parts of Asia and northern Africa. Due to the sport value of the brown trout, artificial introductions commenced in 1864 when trout were transferred from England to Tasmania (Frost and Brown 1967, MacCrimmon and Marshall 1968). After this introduction, additional plantings were made in South Africa and Asia from similar stock. Brown trout were first brought to North America from German hatcheries in 1882 under the name “common trout” (Borne 1885, Mather 1900) and Scottish hatcheries in 1883 under the name “Loch Leven trout” (Smiley 1889). The brown trout from the German hatcheries were first planted on April 11, 1884 in the Pere Marquette River, Michigan (O’Keefe 2009). In 1885 the brown trout from the Howietown Scottish Hatcheries were planted across many streams and lakes across Michigan and New York, most of which were not exactly recorded (Smiley 1889). Both shipments were imported by the New York State hatchery superintendent Richard Mather, who split the shipments into similar shares for New York and Michigan fish culture programs (Mather 1900). From these initial stocks, nearly all states and Canadian provinces have had brown trout introduced and many have naturally reproducing populations.
(MacCrimmon and Marshall 1968). In the twentieth century, brown trout were successfully introduced into South America from both European and American stocks.

The brown trout readily invades and establishes new populations when introduced due to its wide habitat tolerance and adaptability in life history traits, making it one of the 100 most dangerous invasive species (Lowe et al. 2000). Since brown trout are predacious, they can severely impact native fish species through direct predation or through competition for invertebrates. This pressure can restructure ecological trophic interactions by resulting in a strong top-down control of community structure (Jonsson 2011). The majority of brown trout introductions were carried out in the late 1800s, before the true ecological implications were thoroughly understood (Laikre 1999).

**Michigan Strains**

Today the Michigan Department of Natural Resources (MDNR) maintains four brown trout strains in its hatcheries: Gilchrist Creek (GC), Seeforellen (SF), Wild Rose (WR), and, since 2010, Sturgeon River (SR) (MDNR 2007, 2011).

The Seeforellen strain (SF) was imported by the Caledonia State Fish Hatchery of New York from 1979-1986 from German hatcheries which provided the fish from the alpine lakes of West Germany (Johnson and Rakoczy 2004). The SF strain was reported to be a lacustrine morph which were piscivorous and attained great sizes in appropriate systems (Garrell and Strait 1982). The SF strain was first brought to Michigan in 1989 to the Oden State Fish Hatchery. This strain was founded with 1,326 fish with an unknown sex ration which were the direct progeny of the lot received from the German hatchery (D. Sampson, Oden State Fish Hatchery, personal communication). SF trout are slower to grow from ages 0-2 when compared to the other strains, weighing less and being shorter, but reach a
significantly larger length and weight later on while also living longer (Johnson and Rakoczy 2004, Wills 2006). These trout are more temperamental in a hatchery setting, inhabiting the lower portion of the water column and not being aggressive top-feeders, but show promise to produce large lake run trout (D. Sampson, Oden State Fish Hatchery, personal communication).

The Wild Rose (WR) strain is a previously domesticated strain which was brought to Michigan in 1987 from the Wild Rose State Fish Hatchery in Wisconsin. The records held by the Wisconsin Department of Natural Resources give no indication as to the origin of this strain or its broodstock. The original shipment of eggs sent to the Oden State Fish Hatchery totaled 506,730 eyed eggs which were the progeny of 202 female brood fish and 1,262 fish from this shipment of eggs were used to found a broodstock (D. Sampson, Oden State Fish Hatchery, personal communication). The WR strain brown trout perform very well in a hatchery setting, accepting their artificial diets and growing very quickly in the raceway setting. A significantly larger amount of WR trout reach sexual maturity before SF at age 2 but by age 3 nearly 100% of the trout are sexually mature; however, Wisconsin studies suggest that mature WR trout have an annual mortality rate of 85% and higher (Johnson and Rakoczy 2004).

The Gilchrist Creek (GC) strain is a wild strain which was founded in 1995-1996 from 353 females and 328 males which were taken from a wild population of naturally reproducing brown trout in Gilchrist Creek, Montmorency County, Michigan (D. Sampson, Oden State Fish Hatchery, personal communication). There is no record of Gilchrist Creek being stocked, so these nonnative fish must have originated from a historical or undocumented planting somewhere in the watershed. The GC brown trout are also more temperamental in a hatchery setting, showing a strong avoidance response to overhead
moment and not acting as aggressive feeders with their artificial diets (D. Sampson, Oden State Fish Hatchery, personal communication). The GC brown trout grow slower than their domesticated strain counterparts but outperform them by showing survival rates 100x that of SF and 6x of WR (Wills 2006). Also, the GC brown trout live past ages 3 and 4 at much higher rates than those of the SF and WR strains (Wills 2005, 2006).

The Sturgeon River (SR) strain was founded in 2010 from a wild population of naturally reproducing trout in the Sturgeon River, Cheboygan County, Michigan. Like Gilchrist Creek, there is a lack of a record of the Sturgeon River being stocked, so these nonnative fish must have originated from a historical or undocumented planting somewhere in the watershed. There are sizeable amounts of fish which occur naturally in the Sturgeon river which also utilize Burt Lake during certain parts of their life; these are shown to genetically belong to the same population (MDNR 2008). Comparative survival and growth studies are currently ongoing for the SR strain, but initial estimates have shown that SR brown trout may be 5x more abundant than WR after two years in paired planting experiments (MDNR 2011).

Genetic Strain Management and Project Goals

Recently, major advances have been made in the application of molecular methods in population and fisheries management. These tools give managers the ability to quantitatively track survival and fecundity of stocked fish as well as the interactions which occur between wild and stocked fish (Ferguson 1989, Hansen et al. 2000, references therein). Since we know very little about the actual evolutionary or economic value of specific genes in populations, the main goal of fisheries managers should be to conserve genetic variation and
maintain as much genetic diversity in stocks and wild populations as possible (Ryman 1991, Laikre 1999).

Despite major molecular work being done with brown trout in European populations, very little has been done, comparatively, on North American stocks. Genetic analyses performed by Tiano et al. (2007) showed, through restriction fragment length polymorphism (RFLP) digests, that several of these different strains were genetically differentiated at the mtDNA loci ND-5/6 and ND-1, producing 2 haplotypes for the GC strain, 2 for the WR strain and 2 previously unknown haplotypes from wild brown trout which were sampled from the Rogue River, Kent County, Michigan. Johnson et al. (2009) then showed through direct sequencing of the entire mtDNA ND-1 region that the three strains and wild individuals could be identified through nine haplotypes which are comprised of 25 different single nucleotide polymorphisms (SNP).

Despite this knowledge of genetic differentiation and identification, the origins of these European progenitor lineages for the currently stocked strains in Michigan remain largely unknown. These lineages are well known to be specialized in their life history adaptations, behaviors, genetic composition and physiological attributes which are largely determined by their natural environments (Bernatchez et al 1992; McKeown et al. 2010; Jonsson & Jonsson 2011 and others). Although trout from different populations become generally indistinguishable when introduced to the same watershed, a process called phenotypic plasticity, they still carry their genetic signature as well as possible adaptations from their native habitat. By identifying the origins of these strains, fishery managers can make informed decisions about which strains could be stocked to possibly increase survival or growth, based on the strain’s progenitor origins and the strain’s overall genetic diversity.

The objective of this study was to determine the European origins of brown trout strains
found in the Laurentian Great Lakes. To accomplish this goal, we identified their brown trout strain assignment using their mtDNA ND-1 sequences (Johnson et al. 2009) and then identified the European progenitor lineages using the first 309 bp of the mtDNA control region (Bernatchez 2001).
MATERIALS & METHODS

Sample Collection

Brown trout samples were archived from six different rivers and two Great Lakes over the course of 2009, 2010 and 2011. The sample ‘Rogue River 26’ was previously obtained from another study in 2003; the original nomenclature for this haplotype was ‘BB’ (Tiano 2007).

The Wisconsin Department of Natural Resources (WDNR) provided samples from the Root River, Kewaunee River and Menomonee River. Fifteen samples were sequenced from the Root River which were collected by electrofishing during October 2009. Seventeen samples from the Kewaunee River were sequenced and were collected by electrofishing during October 2009. Thirty three samples were sequenced from the Menomonee River and were collected by electrofishing during October and November of 2009.

Samples from the Platte River, Little Manistee River and Grand River were provided by the Michigan Department of Natural Resources (MDNR). Eight samples were sequenced from the Platte River and were collected by electrofishing during September and October 2009. Fifty two samples were sequenced from the Manistee River and were collected by electrofishing during October 2009. Three samples were sequenced from the Grand River and were collected by electrofishing during October of 2009. These river systems were chosen because they represent systems which are targeted by the WDNR and MDNR for salmonid production and are stocked heavily as a result.
Additional samples were also collected from Lake Michigan and Lake Superior by local anglers from 2009-2011. These were included in order to provide a broader representation of the trout which are targeted by local anglers. A sample from the 41 lb. 7 oz. Michigan State Record trout which was caught by Mr. Tom Healy on September 11, 2009 in the Manistee River was provided by Mr. Healy for analysis. The Wisconsin DNR provided a sample from the 41 lb. 8 oz. world record brown trout which was caught in Racine, Wisconsin by Mr. Roger Hellen on July 16, 2010. This allowed for analysis of a total of 144 brown trout (Table 1).
Table 1. Watersheds, sample sizes and haplotype totals for 144 brown trout sampled in Michigan and Wisconsin. The superscript numbers refer to the genotype designation as defined in Johnson et al. 2009.

<table>
<thead>
<tr>
<th>Michigan</th>
<th>Total</th>
<th>Gilchrist Creek</th>
<th>Wild Rose</th>
<th>Seeforellen</th>
<th>Rogue River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little Manistee River</td>
<td>52</td>
<td>35 G(^1)</td>
<td>12 WR</td>
<td>4 S(^1), 1 S(^2)</td>
<td></td>
</tr>
<tr>
<td>Platte River</td>
<td>8</td>
<td>6 G(^1)</td>
<td>1 WR</td>
<td>1 S(^1)</td>
<td></td>
</tr>
<tr>
<td>Grand River</td>
<td>3</td>
<td>1 G(^1), 1 G(^2)</td>
<td>1 S(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rogue River</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1 RR</td>
</tr>
<tr>
<td>Sturgeon River*</td>
<td>10</td>
<td>7 G(^1), 1 G(^3)</td>
<td>1 S(^1)</td>
<td>1 RR</td>
<td></td>
</tr>
</tbody>
</table>

**Wisconsin**

| Menomonee River  | 33    | 11 G\(^1\)      | 9 WR      | 5 S\(^1\), 1 S\(^2\), 4 S\(^3\) | 3 RR        |
| Kewaunee River   | 17    | 5 G\(^1\)       | 4 WR      | 3 S\(^1\), 1 S\(^2\), 1 S\(^3\), 1 S\(^4\) | 1 RR        |
| Root River       | 15    | 5 G\(^1\)       | 2 WR      | 2 S\(^1\), 2 S\(^4\)   | 4 RR        |
| Lake Michigan    | 1     |                 |           |             | 1 RR        |
| Lake Superior    | 5     | 3 G\(^1\)       |           | 1 S\(^1\), 1 S\(^3\)   |             |
| **Total**        | **144**| **73 G\(^1\), 1 G\(^2\), 1 G\(^3\)** | **28 WR** | **17 S\(^1\), 3 S\(^2\), 7 S\(^3\), 3 S\(^4\)** | **11 RR**   |
Figure 1. Sampling sites for brown trout population analysis. The letters correspond to the following watersheds: A- Lake Superior, B- Lake Michigan, C- Menomonee River, Wisconsin, D- Kewaunee River, Wisconsin, E- Root River, Wisconsin, F- Platte River, Michigan, Michigan, G- Manistee River, Michigan, H- Rogue River, Michigan, I- Grand River, Michigan.

**DNA amplification and sequence analysis**

Total genomic DNA was extracted individually from approximately 25 μg of caudal fin tissue using QIAGEN DNeasy® tissue kits and was eluted in a final volume of 50 μl of sterile water. To identify the strain (WR, SF, GC) of the individual, a 1.2 kilobase region of
the ND-1 mtDNA gene was isolated by a polymerase chain reaction (PCR) using custom primers and categorized as described in Johnson et al. (2009). To identify the evolutionary lineage of the individual, the first 313 bp of the mtDNA control region (D-loop) were isolated by PCR as describe in Bernatchez (2001) using custom primers. This amplification was performed using an Eppendorf Mastercycler (Westbury, NY). The PCR reaction volume totaled 50 μl containing the following for individual samples: 5 μl 10x Thermopol buffer (New England Biolabs (NEB, Ipswich, MA)), 100 picomole of each primer, 2.5 Units (U) of Taq DNA polymerase (NEB), 20 mM of each deoxynucleotide dATP, dCTP, dGTP, and gTTP, and 2 μl of DNA sample template. The PCR was run under the following conditions for both reactions: 5 minutes at 94 °C, followed by 30 cycles at 94 °C for 30 seconds, 64 °C annealing for 45 seconds, and 72 °C extension, ending with the termination step of 7 minutes at 72 °C and a 4 °C holding step (Johnson et al. 2009). Sterile technique was performed during the processing of all samples. To confirm the presence of PCR products in our samples, we performed gel electrophoresis using a 1% agarose gel containing ethidium bromide and then photographed the gels under a UV light. The remaining PCR products were then purified with the QIAGEN QIAquick PCR purification kit. Samples were then sent for direct sequencing at the Annis Water Resources Institute, Muskegon, Michigan.

Custom primers were designed for the PCR and sequence analysis reactions of the ND-1 and control regions. The primers mtCR1-Rev and mtCR1-For were both used in the PCR reaction while only the forward primer mtCR1-For was used for sequence analysis.
Table 2. Primer sequences (5'-3') for mtDNA analysis of Great Lakes brown trout (*Salmo trutta*).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtCR1-Rev</td>
<td>CCGGGCAGGGGATTAAGGGC</td>
</tr>
<tr>
<td>mtCR1-For</td>
<td>CCGGAGGTCGGAGGTTAAAAACCC</td>
</tr>
<tr>
<td>BTND1-3861R</td>
<td>TGAACCCCTATCATGCGCCAGCT</td>
</tr>
<tr>
<td>BTND1-2767F</td>
<td>GCGCTAAGGTGGCCAGAGCCC</td>
</tr>
</tbody>
</table>

**Sequence analysis**

The DNA sequence analysis was performed using the entire ND-1 region (975 bp) and the first 313 bp of the control region (Figure 2). Applied Biosystems Sequence Scanner (v1.0) was used for analyzing sequences and determining SNPs. After the sequence of the ND-1 region was determined, it was entered into the National Center for Biotechnology Information (NCBI) search query BLAST. This compared the ND-1 sequence to those sequences described in Johnson et al. (2009) as well as all other highly similar ND-1 sequences stored. After the first 313 base pairs of the control region were determined, they were also entered into NCBI and were run through a BLAST search.

The control region sequences observed, as well as representative reference sequences listed in Bernatchez (2001), were used to construct a more complete phylogenetic relationship of the Atlantic and Danubian lineages of brown trout. All alignments were performed using CLUSTALX 2.1 (Larkin et al. 2007). Sequences obtained from the Atlantic salmon *Salmo salar*, NCBI accession number AF133701, and the Arctic char *Salvelinus alpinus*, NCBI accession number AF545048, were used as outgroups. The Atlantic salmon was chosen because it is the only other member of the genus *Salmo* and the Arctic char was
chosen because it is another member of the family Salmonidae. A maximum-likelihood phylogenetic tree was constructed in MEGA 5.0 (Tamura et al. 2011) using the first 313 base pairs of the mtDNA control region. For the likelihood trees, models of DNA evolution were selected using likelihood ratio tests as implemented in jMODELTEST (Guindon and Gascuel 2003). Bootstrapping was used to gauge support of the branches (Felsenstein 1985; 10,000 pseudoreplicates). A maximum-parsimony phylogenetic tree was also constructed in the PHYLIP v3.69 program suite with 1,000 pseudoreplicates. These trees were then loaded and scaled with the program Dendroscope 3.2.2 (Huson et al. 2007).

Maximum likelihood and maximum parsimony analyses both operate under similar mathematical mechanics, both analyzing a data set by producing trees with scores and picking the tree with the best score which represents the tree which was most likely to represent the data set with the most reasonable amount of changes. A maximum likelihood analysis uses a fixed model of genetic evolution in a parametric method which produces a phylogram, or a tree which has meaningful branch lengths and structures which show the actual amount of site variation between characters or individuals in a data set. The model of evolution which is used is typically user determined and can be chosen to reflect the type of sequence that is provided, some models having an equal chance for all types of mutations or other models which favor certain nucleotides or types of mutations over others. The model chosen depends on what type of sequence is being observed, whether it be a non-coding region, a region under a certain type of selection, or a coding region under an unknown type of selection. Typically, the best model is the simplest model with the fewest input parameters which most accurately reflects the type of data. A maximum parsimony analysis is a non-parametric method which creates a tree using common characters or sites and models trees after this which are scored by showing the least amount of evolutionary change to describe
the data set. This method produces a cladogram, or a tree which shows relative relation between individuals by displaying an equal branching pattern but does not reflect time or amount of genetic distance. Both types of analyses were performed on this data set in order to show the relation between the groups of brown trout under different lights. The maximum likelihood analysis shows how divergent the groups are by showing genetic distances between the haplotypes and also shows where a new Atlantic haplotype lies in relation to the other sub-types of the Atlantic lineage (Bernatchez 2001).
RESULTS

*Great Lakes Lineage Assignments*

The 144 brown trout samples produced 9 mtDNA *ND-1* haplotypes that were used to assign each sample to strain (Table 1) and four control region haplotypes were identified in the same set (Table 4). All 144 samples which were processed returned readable samples. A sample was considered readable if approximately 90% of the sequence had ‘Very good’ base identification, as defined in the program Applied Biosystems Sequence Scanner (v1.0). All samples had a 100% base identification on the variable position sites which determined clade identification.
Figure 2. Sequence of the 5′–end of 299 bp of the mtDNA control region including 10 base pairs of the tRNA gene proline for the AT-s1 genotype by Bernatchez (2001). Variable positions among brown trout samples here are underlined and positions numbered.

Table 3. Identification of polymorphic sites observed in the first 313 base pairs of the mtDNA control region in brown trout samples from the Great Lakes. Dashes indicate sequence homology with AT-s1 while mutations are listed. Genotypes AT-s1 and DA-s1 in Bernatchez (2001); A* genotype in Apostolidis (1997); GC3 genotype has not yet been described in the literature.
There was no variation between ND-1 assignment and the identification of the associated progenitor lineage, for example, all 75 fish which were identified as having a maternal line from the Gilchrist Creek strain were identified as belonging to the AT-s1 haplogroup. Five SNPs were observed in the control region (Table 3) which differentiated between 4 haplotypes. The Rogue River haplotype had one unique ND-1 polymorphism which corresponded to the AT-s1 haplogroup. Four ND-1 haplotypes were observed in the SF broodstock in Johnson et al. (2009), and three of four match an AT-s1 group except the third SF haplotype, which matches a DA-s1 haplogroup (Bernatchez 2001). The WR strain had one characteristic ND-1 haplotype which corresponded to one characteristic control region haplotype. The GC strain had three distinctive ND-1 haplotypes. The GC1 and GC2 haplotypes corresponded to the AT-s1 haplogroup (Bernatchez 2001). The GC3 haplotype corresponded to a control region haplotype which has yet to be described or reported in the literature.
Table 4. Strain assignments given by ND-1 sequences, sample sizes ($n$), and European lineage assignments given by control region sequences among 144 brown trout samples from 7 Michigan and 3 Wisconsin populations. Numbers in parenthesis refer to the strain haplotype designations in Johnson (2009). Lineage designations AT-s1 and DA-s1 are described in Bernatchez (2001). Asterisk indicates lineage assignment reported in Apostolidis et al. (1997) with original nomenclature. The GC3 genotype has not yet been described in the literature.

<table>
<thead>
<tr>
<th>Strain Designation</th>
<th>$n$</th>
<th>Lineage Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeforellen (1)</td>
<td>17</td>
<td>AT-s1</td>
</tr>
<tr>
<td>Seeforellen (2)</td>
<td>3</td>
<td>AT-s1</td>
</tr>
<tr>
<td>Seeforellen (3)</td>
<td>7</td>
<td>DA-s1</td>
</tr>
<tr>
<td>Seeforellen (4)</td>
<td>3</td>
<td>AT-s1</td>
</tr>
<tr>
<td>Gilchrist Creek (1)</td>
<td>73</td>
<td>AT-s1</td>
</tr>
<tr>
<td>Gilchrist Creek (2)</td>
<td>1</td>
<td>AT-s1</td>
</tr>
<tr>
<td>Gilchrist Creek (3)</td>
<td>1</td>
<td>GC3**</td>
</tr>
<tr>
<td>Wild Rose</td>
<td>28</td>
<td>A*</td>
</tr>
<tr>
<td>Rogue River</td>
<td>11</td>
<td>AT-s1</td>
</tr>
</tbody>
</table>
Figure 3. Unrooted maximum likelihood phylogenetic tree of observed haplotypes and reference haplotypes of the first 313 base pairs of the mtDNA control region in major brown trout lineages. The reference bar is measured in substitutions/site. The labels in bold are haplotypes that are observed in Great Lakes populations. Node values equal 100 unless otherwise stated.

Phylogenetic analysis of Michigan brown trout lineages and complete representative sequences from the two major phylogenetic clades resulted in two distinct clusters, similar to what is seen in Bernatchez (2001). The new GC3 sequence and the AT-s1 sequence only differ by one base pair, so they were grouped together. The WR1 sequence shared a
mutation with the DA group and two with the AT, so it seems to show an intermediate form between the two groups. The Da-s1 group is clustered well within the other DA sequences, showing high similarity with the other DA sequences.

Figure 4. Unrooted maximum parsimony phylogenetic tree of observed haplotypes and reference haplotypes of the first 313 base pairs of the mtDNA control region in two major brown trout lineages. The labels in bold are haplotypes that are observed in Great Lakes populations.

The maximum parsimony analysis shows a similar structure, with the DA and AT clades grouping together. Also, the WR haplotype is located in the same position being sister to the AT cluster and still close to the DA clade. The ATs1 and GC3 are also placed in the same positions. In the maximum parsimony analysis the AT-s3 haplotype is not placed in the polytomy which contained AT-s1 and GC3 in the maximum likelihood but rather is placed being sister to the whole group.
Ten broodstock were sequenced from the new Sturgeon River strain which were provided by the Michigan Department of Natural Resources in the fall of 2011. Four \textit{ND-1} haplotypes and three control region haplotypes were observed within this stock. Eight of these broodstock were identified as GC since they shared the same \textit{ND-1} genotypes. The other two fish were identified as a SF haplotype and as a RR haplotype. The control regions were also sequenced to identify the associated lineage. The GC1, SF1 and RR individuals were identified as belonging to the AT-s1 clade but the GC3 haplotype yielded a unique control region sequence which has yet to be described in the literature. This control region haplotype matched the AT-s1 in all positions except at position 202 (Figure 2, Table 3), where a T-C mutation occurred.

Table 5. Strain designation and lineage designation of 10 brown trout from the Sturgeon River strain. The strain designation was determined by using the entire mtDNA \textit{ND-1} region as described in Johnson et al. (2009). The lineage designation was determined by using the first 313 bp of the mtDNA control region as described in Bernatchez (2001). The designation GC3* indicates that a unique control region sequence was observed and a European match could not be designated.

<table>
<thead>
<tr>
<th>n</th>
<th>Strain Designation</th>
<th>Lineage Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Gilchrist Creek (1)</td>
<td>AT-s1</td>
</tr>
<tr>
<td>1</td>
<td>Gilchrist Creek (3)</td>
<td>GC3*</td>
</tr>
<tr>
<td>1</td>
<td>Seeforellen (1)</td>
<td>AT-s1</td>
</tr>
<tr>
<td>1</td>
<td>Rogue River</td>
<td>AT-s1</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Worldwide, salmonid fisheries have become a significant economic interest and therefore the preservation and supplementation of these populations has become a major focus of legislation and conservation. Through targeted genetic studies outlined by the EU Fisheries and Agriculture Research (FAIR) project, native brown trout populations have been sampled and evaluated in order to gain an understanding of the evolutionary history of the brown trout and a current state of their genetic diversity (Laikre 1999). By taking a genetic management approach, brown trout populations are being broken down in to identifiable units which can conserve at the gene level. In the United States, even more care must be taken with genetic management since the entire stock, which is of comparable size to European stocks, was founded on just a minuscule fraction of the possible genetic diversity.

Great Lakes Lineage Assignments

Historically, North American and Great Lakes brown trout stocks have been portrayed as being of German origin. The 144 samples collected from Michigan and Wisconsin contained four different haplotypes in the mtDNA control region, showing that Michigan's stock is founded from several different populations found across Europe (Apostolidis et al. 1999; Bernatchez 2001).
The SF stock was shown to largely belong to the Atlantic clade, having the SF 1, SF 2 and SF 4 ND-1 haplotypes correlate to this group. Historically the SF strain has been stocked as a large, lake-run trout which matures sexually later in development than other strains but grows to be a larger size. The AT clade has been seen as being largely sea-run trout which survived in the northwestern European Atlantic coast during the last glacial retreat of the Pleistocene era (Bernatchez 2001). Michigan’s SF stock was started from eggs provided by New York’s Caledonia State Fish Hatchery in the late 1980s (Wills 2006) and their stock dates back to the first shipment of brown trout eggs brought to North America from Scottish and German hatcheries (Smiley 1889; Mather 1900). It was specifically noted by Mather (1900) that the eggs of the strain ‘seeforelle’ came from two different variants of brown trout: lake-resident and river-resident individuals. These two types of brown trout had variable egg sized, with the stream-run eggs being noticeably smaller than the lake-run eggs. The identification of the SF 3 strain haplotype as being a DA-s1 haplotype supports this, since the DA lineage is primarily observed as being river-resident individuals (Bernatchez 2001). The progenitor source population for the DA-s1 stock was likely not from a German stream, but rather from southeastern Europe or southern watersheds of the Black Sea (Bernatchez 2001). The presence of this group is noteworthy because previous research by Johnson et al. (2009) showed that the SF3 haplotype had two non-synonymous mutations in the mtDNA ND-1 sequence. It is possible that these non-synonymous mutations are the result of the group’s specialization to riverine systems, since changes occur in a subunit of a very significant metabolic pathway. The SF strain has been bred to perform as a large, lake-run trout but the presence of the river-resident haplotype could alter the stock’s performance in any given year.
The GC stock yielded three ND-1 haplotypes which corresponded to two different control region haplotypes which both belong to the Atlantic clade. The GC strain shares the AT-s1 European lineage with three of the four SF strain control region haplotypes and the Rogue River control region haplotype. However, it is considered unique because it is the only broodstock which has recently been established from a wild population. The GC strain survives at rates 100 times that of the SF strain and six times that of the WR strain (Wills 2006). The Gilchrist Creek strain also grew twice as fast as both the SF and WR strains from ages 0-2 (Wills 2005). These wild type strains far out perform domesticated stocks, and their source rivers should be managed accordingly.

The GC 3 haplotype expressed a unique control region sequence which has not yet been described in the literature. This sequence was added to the other Atlantic lineage sequences in the phylogenetic analyses described in Figures 3 and 4, making it the ninth Atlantic lineage haplotype. This group was placed sister to the AT-s1 group, as it only differed by one mutation.

It is possible that this group represents a strain introduced from Scotland. It was noted in Smiley (1889) that over 10,000 fry were given to the Flint and Pere Marquette Railroad in Grand Rapids, Michigan who planted them in rivers and tributaries in northern Michigan. Unfortunately, it was not recorded exactly which watersheds were stocked. These fry were from hatcheries in Loch Leven, Scotland which do share the AT-s1 haplotype (Bernatchez 2001). It will not be possible to differentiate between these Scottish trout and the trout from German hatcheries which were used to historically stock Michigan watersheds until additional genetic comparison is performed (see: Duguid et al. 2006; Hansen et al. 2010; McKeown et al. 2010).
This study further clarified the origins of the Rogue River haplotype described in Johnson et al. (2009) and Tiano et al. (2007). It was reported in Tiano et al. (2007) that the Rogue River strain was assigned as haplotype E after RFLP digestions, which contains characteristics of the SF strain. The SF ND-1 haplotypes 1, 2 and 4 and Rogue River strains were both identified as being AT-s1 haplotypes but the Rogue River strain has a unique mtDNA ND-1 haplotype (Johnson 2009). The upper Rogue River is separated from the lower reaches by a dam, which makes it impossible for fish to swim from Lake Michigan upstream to these portions. According to the MDNR Fish Stocking Database, the SF strain has never been stocked in the upper reaches of the river before Tiano et al. (2007) collected samples in 2002 and 2003. The river had been stocked before 2003 with WR strain, so the presence of the Rogue River haplotype either occurred in the WR broodstock but has since been lost or the Rogue River haplotype is the result of stocking efforts before 1995 and has been naturally reproducing since. It was reported in Johnson et al. (2009) that the Rogue River mtDNA ND-1 haplotype was nearly identical to a Danish hatchery brown trout sample. It should also be noted that the vast majority of native Danish brown trout are from the AT-s1 haplogroup, so it is possible that the progenitor lineage of the Rogue River haplotype is of Danish origin. This haplotype has become more interesting because it was observed in watersheds across Michigan and Wisconsin, but was not found in the broodstock which the same rivers were stocked with. Also, the world record brown trout from Racine, Wisconsin belonged to this RR haplotype.

Twenty-eight samples were identified by their mtDNA ND-1 haplotype to originate from the Wild Rose strain and all twenty-eight expressed the A* control region haplotype which is described in Apostolidis et al. (1997). This haplotype is expressed by a specific population of brown trout from the Garonne River in the Pyrenees Mountains in
southwestern France (Apostolidis et al. 1997). The brown trout from this watershed are accustomed to cold waters in high altitudes and have slow growth rates and reach sexual maturity at a later time, but have a longer life span (Gouraud et al. 2001). The densities of trout in this watershed are most positively affected by increased water depth since they typically spawn in the main channel of the river (Reyjol et al. 2001; Gouraud et al. 2001).

In Michigan, the WR strain is considered to be domesticated and has less than a 4% survival from fry to age 2 (Wills 2006). Despite these low returns, the WR strain accounted for the largest percentage of stocked brown trout in the past ten years at nearly 40% in Michigan streams, according to the MDNR Fish Stocking Database. In general, this stock is used for put-and-take fisheries. This low survival rate is logical considering the specialization of the progenitor population to its native high altitude, cold water streams and the fact that the WR strain has become very inbred due to years of hatchery containment, which can be inferred from the recorded exchanges of the MDNR and the WDNR that show a diminished group used to found the broodstock group in Michigan. Every year, the Oden State Fish Hatchery meets a demand of 1.22 million eyed eggs with age classes 3-7 producing 1.28 million eggs at various success rates with the age 7 females showing a fecundity value around 5,000 (D. Sampson, Oden State Fish Hatchery, personal communication). Research performed by Gouraud et al. (2001) showed that female trout from the Garonne River only showed a fecundity value of 2150 at age 7. Even with the original population in its native stream, this group of trout does not perform exceedingly well and its viability as a source for a major strain should be evaluated. This information suggests that this strain does not perform well in Great Lake streams and stocking efforts might be better spent supporting other strains.
The two world record trout were sequenced and were found to belong to the Atlantic lineage but to different stock strains. The Michigan record trout caught by Mr. Tom Healy was found have a maternal line from the Gilchrist Creek strain. Previous analysis using microsatellites done by Homola et al. (2012) found that this same fish originated from the Seeforellen strain and was not an interspecific hybrid of any type. These two findings raise the question if this record fish originated from a hatchery and the Seeforellen strain is contaminated with individuals from another strain or if this fish originated through natural reproduction in the Manistee River. Hybridization has long been used in fisheries to increase genetic diversity, resulting in increased reproductive potential and decreased mortality (Ihssen 1976; Halliburton et al. 1983; Billington et al. 1988; Epifanio and Nielsen 2001). Perhaps if strains are collapsing due to genetic homology, then crossbreeding methods could be employed to cross the most genetically dissimilar broodstock in order to produce offspring which are of sufficient diversity. The fact that this fish was not only large, but of a world record size, may support this notion. The record fish caught by Mr. Hellen in Racine, Wisconsin was found to be a Rogue River haplotype fish of the Atlantic lineage. This certain haplotype may be an untapped resource in the brown trout population in the Great Lakes since it is not seen in hatchery stock. If this haplotype can reproduce naturally and also produce fish of trophy size, then its integration into the hatchery system should be welcomed but also monitored so that the stock stays genetically diverse.

**Sturgeon River Strain**

The Sturgeon River strain was founded in 2010 from a population of naturally reproducing fish in the Sturgeon River, Cheboygan County, Michigan in order to offset the poor returns which had been seen in other strains in recent years. A 5 year paired planting
study involving 13 watersheds, 125,000 SR strain brown trout and 125,000 WR strain brown trout was started in 2010 to estimate the performance of the new strain and initial findings have shown that this strain does indeed outperform the WR strain significantly (MDNR 2011).

Ten SR broodstock were sampled and sequenced for this study. Using mtDNA ND-1 and control region sequences it was shown that this SR strain sample is in fact made up of two types of GC trout, one type of SF trout and one type of trout which has not been observed in Michigan hatcheries to date, the Rogue River haplotype (Johnson 2009). Eight of the ten trout were GC type trout, making this strain very similar to the GC strain with smaller genetic inputs from two other types. It is worth noting that of the two strains founded from naturally reproducing populations in Michigan, both are largely or completely composed of trout of the GC type. This type then could be more suited to Michigan rivers and lakes since the ability to found stable, naturally reproducing populations is not seen in other strains as strongly. This is also the first time that the RR haplotype is found in a hatching broodstock. This lineage of fish was likely found in a historical strain but has since been lost from the hatchery system. The fact that this fish still persists is notable, since it suggests that it has been naturally reproducing for some time.

The foundation of this strain marks the first time that Michigan’s strain types will not be phylogenetically distinct. Work done by Tiano et al (2005) and Johnson et al (2009) have shown that the broodstock which supplements all of the strains in Michigan are from different historical populations which could be differentiated by their ND-1 and ND-5/6 regions. Strains are often reported as ‘genetic strains’ in publications and articles to the public, but there is little agreement on what genetically defines a strain. If a strain is considered a representative, albeit artificial, sample of a historical population then
phylogenetic separations might be a more concrete method to differentiate populations of brown trout. In the future, phylogenetic identifications of populations may serve to found strains which would perform well in ecosystems which are similar to the systems that those specific fish have evolved and adapted in. Although the amount that these certain traits are passed on by hereditary means is under debate, any small adaptation that leads to even a fraction of a percent higher survival rate translates into significant monetary amounts when considering the size of aquaculture programs (Nislow et al. 2004, Garcia de Leaniz et al. 2007). A study by Ryman et al. (1999) showed that when two phenotypically distinct stocks were released into an area previously devoid of brown trout, they produced progeny which expressed behavioral and phenotypic differences which were measured and shown to be under genetic control. The study performed by Ryman et al. (1999), among others, shows that distinct populations have become adapted to certain environmental regimes and these physical traits and behavioral tendencies are indeed under a certain amount of genetic control.

If a strain is considered a structured population that performs well with high recruitment and acceptable trout length, which may be composed of many different individuals from a variety of populations which may have never encountered one another in a natural setting, then finer scale genetic measurements must be used to define this population’s founding members and to measure the amount of diversity in the offspring. In Michigan there is a unique opportunity to utilize the genetic diversity of brown trout stocks to create synthetic strains or phylogenetic groups which could not have occurred naturally. If a main goal of a fishery is to provide stocks which are genetically diverse, then we can utilize the various ESU which are present in North America to hybridize groups of fish in order to maximize genetic diversity and therefore maximize the health of the populations.
In order to preserve genetic diversity on all levels, mtDNA and nuclear DNA measurements must be considered in order to preserve diversity and maximize performance. Increased inbreeding and the resulting lack of genetic diversity are most often cited as the reason for poor returns from stocked brown trout since these individuals are typically less healthy and more susceptible to temperature spikes and diseases (Wills 2005, 2006; MDNR 2007, 2011). Mitochondrial haplotypes must be inventoried among stocks in order to preserve mtDNA diversity. The effects of genetic drift are much stronger when dealing with mtDNA because these haplotypes are effectively haploid in populations since only one copy is present in individuals. That is to say, the population can ‘fix’ or become homogenous with a single sequence because there are less sequences available and the mode of transmission is through a single maternal line instead of two copies of each gene being passed on. Wisconsin streams had a relatively higher diversity of mtDNA haplotypes in their streams than Michigan (Table 1). Wisconsin differs from Michigan in hatchery practices by rather than keeping a set number of broodstock fish, a sufficient sample of eggs is taken from each river and crossed with typically two male’s gametes. This allows for genetic diversity in the form of new mtDNA haplotypes being introduced into different stocks as well as selecting fish which have actually returned to the river to spawn. This method can help ensure that a population of naturally reproducing brown trout return to their natal rivers with a sufficient amount of genetic diversity. The effective population size, or the number of breeding individuals in an ideal population who would show the same allelic frequencies as a population under genetic drift or under the same amount of inbreeding as the observed population, is a sustained method which is used to measure and compare the amount of effective genetic transmission which is taking place. Research has shown that healthy, wild brown trout populations in Europe have effective population sizes anywhere from 70- >500
individuals in fjords (Jorde and Ryman 1996, Hansen et al. 2001) and large lentic systems to 55- >300 (Hansen et al. 2001; Aho et al. 2006) individuals in stream and river systems. If fish are being introduced into a system which already has a resident population, then the introduced fish must at least have diversity measures equal to the residents if the diversity and resulting health is to be maintained in the population. Research has also shown that the absolute minimum effective population size of broodstock is somewhere around fifty individuals if the health of the broodstock, their progeny and the wild populations is to be maintained. (Laikre et al. 1998; Aho et al. 2006). The SR population has proven to be healthy and self-sustaining but in order to preserve this group a detailed genetic profile must be maintained in order to monitor the diversity and reproductive success expressed by each generation. This information can then be used to adjust the SR broodstock which is being used to create offspring which carry desired traits of the SR population with similar measures of genetic diversity and effective population size.

If diversity is the goal of state hatcheries, then it can be reached by maintaining diversity with phylogenetic groups being the foundation of strain types or by creating synthetic strains by selectively breeding genetically unique individuals of various phylogenetic backgrounds in sufficient numbers. A high genetic diversity allows for a higher adaptive potential by maintaining a high number of alleles and associated phenotypes.
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