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The Lake Charr *Salvelinus namaycush*: Biology, Ecology, Distribution, and Management

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Genetic Diversity

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Abstract The use of genetic information in fishery management has become increasingly valuable as input to decision making. The lake charr *Salvelinus namaycush* represents an important model species of management concern for studying ecological divergence. We compiled a comprehensive assessment of the knowledge of lake charr genetic diversity. The following topics were reviewed: spatial patterns of genetic diversity, the relationship between genetic and morphological distinctiveness of ecotypes, heritability of phenotypes, evidence for "reverse speciation," and genetic effects of hatchery stocking. Important patterns that emerged were: strong divergence and high genetic uniqueness for most inland lake populations; evidence for heritability of traits associated with lake charr ecotypes; inconsistent support for genetic differentiation of ecotypes; an emerging view that lake charr diversity is distributed along a depth gradient in large lakes rather than discrete ecotypes; and hatchery supplementation and stocking have had profound but highly variable impacts on genetic diversity of populations. Knowledge gaps were identified to guide future research and to assist lake charr management and include investigations into the molecular mechanisms and evolutionary processes generating phenotypic diversity.

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1 Introduction

Molecular ecology and its associated evolutionary concepts and principles (e.g., variation, genetic drift, inbreeding, selection, connectivity; see Box 1) are increasingly being integrated into sustainable fishery management and aquatic ecosystem conservation, with recent developments in genomics technologies giving rise to a set of powerful, precise, and cost-effective genetic tools (Hendry et al. 2011; Ovenden et al. 2015; Casey et al. 2016; Bernatchez et al. 2017). The potential for molecular ecology to provide significant added value to fishery stock assessments and species rehabilitation programs has been recognized by fishery scientists and managers internationally, including the bilateral United States–Canada Great Lakes Fishery Commission, since the 1980s (Billingsley 1981). The lake charr *Salvelinus namaycush* is a major resource for both recreational and commercial fisheries, and is a treasured subsistence fishery of Indigenous Nations, particularly in large northern lakes (e.g., Great Bear Lake, Great Slave Lake). As an ecologically, economically, and culturally important species throughout its range, effective conservation and management of the lake charr requires knowledge of the distribution of genetic diversity within and among populations. The lake charr is also a species of tremendous interest in applied and fundamental research, notably for understanding the genomic basis of phenotypic variation, local adaptation, and origins of species. Due to its propensity for forming multiple morphological and life history variations (hereafter referred to as ecotypes), the lake charr is an interesting and exceptional model for studying eco-evolutionary processes involved in phenotypic divergence.

The first lake charr genetic studies focused on comparisons of allozyme variation among lake charr populations (Dehring et al. 1981; Krueger et al. 1989). Subsequently, studies concerned with lake charr re-establishment investigated postglacial colonization lineages using mitochondrial DNA restriction fragment length polymorphisms (RFLP; Wilson and Hebert 1996 1998), ecotype distinction and genetic impacts of human activities and stocking using microsatellite DNA markers (e.g., Page et al. 2004; Piller et al. 2005; Halbisen and Wilson 2009; Guinand et al. 2012; Valiquette et al. 2014; Baillie et al. 2015, 2016b; Harris et al. 2015), adaptive immune gene diversity and copy number variation in major histocompatibility complex (MHC) genes (Dorschner et al. 2000; Noakes et al. 2003; Baillie et al. 2018), gene expression to identify traits underlying phenotype (Goetz et al. 2010), and genome-wide scans of single nucleotide polymorphisms (SNPs) to investigate population structure and genomic variation among ecotypes (Bernatchez et al. 2016; Perreault-Payette et al. 2017; Perrier et al. 2017; Ferchaud et al. 2018). As part of a long-term project, the lake charr genome is currently being sequenced and annotated

(L. Bernatchez, unpublished data). Such knowledge will permit a deeper understanding of the structure of the lake charr genome, the genetic basis of ecologically relevant traits, and the consequences of human activities on patterns of genomic diversity.

Lake charr phenotypic diversity has traditionally been conceptualized using discrete ecotypes (e.g., lean, humper, siscowet, butterfly, redfin). Recent studies by Baillie et al. (2016a) and Chavarie et al. (2018) generated an emerging view of lake charr genetic and phenotypic diversity where adaptive variation is distributed along ecological and environmental gradients, rather than as discrete ecotypes. This information alters the conceptual model that fishery managers have been working under where "ecotypes" follow a genetically pre-determined developmental plan. Phenotypic clines within each ecotype also suggest that environmental conditions (i.e., phenotypic plasticity) interacts with genetic variation to determine morphological and life-history traits (Chavarie et al. 2021).

The genetic diversity of lake charr populations has also been dramatically re-shaped by human activities over many decades. Lake charr were severely reduced in abundance in the Laurentian Great Lakes by the mid-twentieth century and became extirpated from all lakes except Lake Superior and one small region of Lake Huron (Zimmerman and Krueger 2009). The combined effects of overfishing, the introduction of invasive species, water quality degradation, and hatchery supplementation influenced the genetic diversity of lake charr populations. Genetics and genomics can now provide more than biological stock structure information (see Ovenden et al. 2015) and we feel that empirical, theoretical, and applied genomics research must be embraced together if we are to understand the adaptive capacity of the lake charr, and how to manage it, in the face of ongoing natural and human-induced environmental change (McMeans et al. 2016).

The objectives of this chapter are to review the accumulated knowledge of genetic variation within and among lake charr populations, review evidence for the genetic basis of phenotypic divergence of lake charr populations, and to synthesize knowledge relevant for management and conservation of lake charr genetic diversity. We address these objectives with a series of questions on the diversity of postglacial genetic lineages observed in lakes today, the relationship between genetic and morphological distinctiveness among ecotypes, ecological axes of genetic divergence among lake charr populations, the genetic basis of lake charr phenotypes, evidence for losses in genetic and morphological diversity in the human-altered Laurentian Great Lakes and potential for "reverse speciation," and effects of hatchery supplementation on lake charr genetic diversity. We first introduce and present literature from an ecotypic perspective, then consider recent evidence in support of ecological-gradient-based genetic divergence, followed by case examples of human impacts on lake charr. The final section highlights emerging (epi)genomics approaches and technologies that may help resolve questions regarding the relationship between phenotype and genotype in lake charr and their genetic associations with habitat and evolutionary trajectories.

Box 1: Population Genetics Principles and Glossary

For those unfamiliar with the terms or concepts discussed in this chapter, we provide a brief primer on molecular ecology and population genetics concepts relevant for understanding the content discussed herein. Molecular ecology is an interdisciplinary field of study that applies molecular genetic markers to ecological questions. The variation of molecular genetic markers (for the purposes of simplicity: genes) are governed by Mendel's principles of inheritance and various evolutionary concepts collectively under the umbrella of population genetics. For diploid organisms like humans, or functionally diploid organisms like lake charr,[1] individuals carry two copies of each gene that are called alleles. One copy is inherited from the organism's mother and the other from its father. Mendel's principles of inheritance state that alleles are inherited randomly with equal probability and alleles at different genes are inherited independently. In the context of a population of organisms, this generates predictions that link the frequency of an allele in the population with the expected frequencies of combinations of alleles (genotypes) carried by individuals. Deviations from these expectations in real samples can be used to make inferences about various evolutionary forces influencing populations.

Four major evolutionary forces generate, maintain, and shape molecular diversity in natural populations. They are: mutation, migration (or gene flow), genetic drift, and selection. Mutation is the source of all variation and occurs spontaneously through errors in DNA replication and the action of selfish DNA and RNA elements (e.g., viruses and transposable elements). The remaining forces simply shape the variation created by mutation. Directional and divergent selection and genetic drift tend to erode genetic diversity through either the stochastic loss of variation due to finite population sizes (drift) or selection for, or against, specific variants in certain environments. These forces result in the genetic subdivision or structuring of groups of organisms when interbreeding between these groups is low. Migration that results in interbreeding between the groups is known as gene flow and works in the opposite direction from selection to homogenize genetic variation among structured groups. Similarly, balancing selection will favor the maintenance of genetic variation within populations.

Glossary

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[1]All salmonid fishes, including the lake charr, are descended from an ancestral species that experienced a genome duplication at least 60 to 88 million years ago (Allendorf and Thorgaard 1984; Crête-Lafrenière et al. 2012; Macqueen and Johnston 2014). This duplication resulted in an organism whose cells had four copies of the genome rather than the normal two. Over time these duplicated genes may have become non-functional or evolved new functions such that most modern lake charr only carry two copies of each gene, although some regions (~15% of the genome) show residual tetraploidy.

Box 1 (continued)

Allele: One of the possible variant copies of a locus.

Allelic richness: The sample size corrected the number of unique alleles found in a group of samples.

Genetic divergence: Differences in allele frequencies among groups of organisms that are greater than those that could be explained by sampling artifacts.

Genotype: The combination of alleles that an organism carries at one or more loci.

Haplotype: Series of physically linked alleles belonging to the same strand of DNA.

Heterozygosity: The frequency of individuals in a sample that possess two different alleles at a specific locus.

Marker, Locus (singular), Loci (plural): Specific region or location in a species' genome.

Microsatellite: Type of variable genetic marker used for population genetic inference characterized by short (2–6 base pairs) tandemly repeated DNA sequences.

Mitochondrial DNA (mtDNA): DNA contained within the mitochondrial organelle that is maternally inherited.

SNP: Single Nucleotide Polymorphism, the most common class of genomic variation, characterized by the presence of two (or more) alternate base pairs at a specific genomic location.

2 Geospatial Patterns of Lake Charr Genetic Diversity

Contemporary geospatial patterns of lake charr genetic diversity are shaped by biogeographic history, landscape features, ecological opportunity, and the influence of human activities. Lake charr expanded their range from multiple refugia after the last glacial maximum and today are distributed across many thousands of North American lakes that formed as the ice retreated (Wilson and Hebert 1996; Wilson and Mandrak 2021). As a "nearly obligate" lake-dwelling species, except in the very northern part of its distribution, lake charr populations have experienced limited connectivity over the last few thousand years. This limited connectivity has led to spatial patterns of genetic diversity where local populations harbor subsets of a larger regional gene pool (Perrier et al. 2017).

Landscape genetics provides a framework of testable expectations or predictions (e.g., genetic diversity is positively correlated with lake size) often used in evolutionary and conservation biology to understand factors and mechanisms that affect the distribution of genetic variance, and hence local adaptation, in spatially fragmented and complex systems (Manel et al. 2003; Storfer et al. 2007). Basic assumptions of landscape genetics theory are that patterns of population genetic

structure should reflect geographical features, such as physical distance separating populations, lake surface area, and elevation. In this section, we briefly review relationships between lake size and lake charr genetic diversity, as well as elevation and dendritic patterns. As more sophisticated genetic marker panels are developed in the coming years, more detailed relationships between genomic and habitat variation will be resolvable.

2.1 Refugial Origins, Postglacial Dispersal, and Secondary Contact

Like many North American boreal freshwater species, the spatial genetic structure of the lake charr was profoundly shaped by Pleistocene glaciations (Bernatchez and Wilson 1998; Wilson and Mandrak 2021). Repeated glacial advances and retreats alternated between destroying and revealing suitable habitats and, when combined with dispersal largely restricted to freshwater habitats, impacts on species' ecology and genomes have been especially pronounced (Pielou 1991; Wilson and Mandrak 2021). In a comprehensive project to reveal historical biogeographic origins and patterns of re-distribution of the lake charr, Wilson and Hebert (1996, 1998) tested alternate dispersal hypotheses using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). The contemporary distribution of four major mtDNA lineages observed following screening of 1416 lake charr from 93 populations across the species' range showed that extant populations of lake charr originated from at least five glacial refugia (Fig. 1).

The work of Wilson and Hebert (1996, 1998) provided clarity to the multitude of glacial refuge hypotheses that had been previously proposed, including several
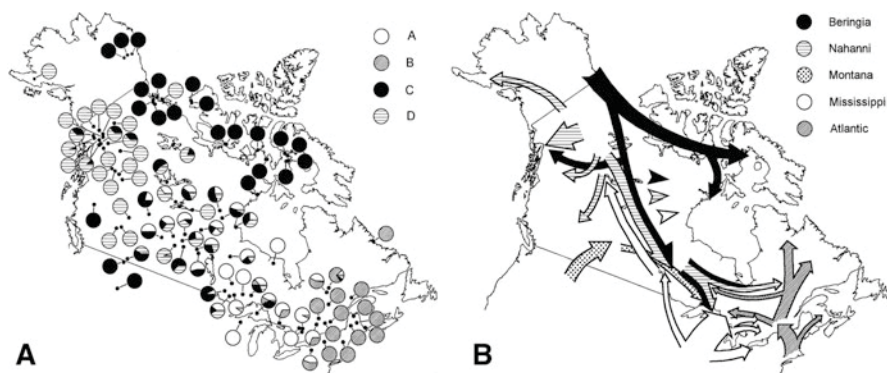
Fig. 1 Distribution of lake charr *Salvelinus namaycush* glacial refugia mtDNA RFLP lineages (**a**) and hypothesized refugial origins and patterns of dispersal for extant lake charr populations based on distributions of mtDNA haplotypes (**b**). From Wilson and Hebert (1998)

single refugia scenarios and various combinations of two or more (reviewed in Crossman and McAllister 1986). Current distributions of refugial lineages are best explained by the dynamics of proglacial lakes that formed along edges of the Wisconsinan ice sheet. Patterns and timing of connections between proglacial lakes changed dynamically as ice sheets receded to facilitate large-scale dispersal from multiple refugia. Long-distance dispersal is particularly evident from the Mississippian refuge in all directions and the eastward spread of lake charr from northwestern refuges. Dispersal through proglacial lakes also enabled extensive secondary contact among refugial groups.

2.2 Genetic Diversity Patterns in Large Lakes, Small Lakes, and Streams

Low rates of dispersal and a lack of suitable connectivity between most inland lakes during the past 6000–9000 years has led to high levels of differentiation among populations that reflect strong effects of genetic drift and a lack of migration among populations (Fig. 2; Halbisen and Wilson 2009; Valiquette et al. 2014; Perrier et al. 2017). In an extensive study of small lakes in Québec, lake charr genetic diversity was positively correlated with lake size and likely reflected initial founding events for these populations and effects of genetic drift in years since population establishment (Perrier et al. 2017). In small lakes, within-population genetic diversity was typically low and populations harbored a restricted subset of regional allelic diversity (Ihssen et al. 1988; Halbisen and Wilson 2009; Valiquette et al. 2014; Perrier et al. 2017). Consistent with evidence for lower diversity in smaller lakes, inbreeding was higher in smaller lakes (Perrier et al. 2017). Under these conditions, populations might be expected to have a reduced ability to adapt to changing climate or other factors, such as anthropogenic impacts because of insufficient genetic variation.

While increased levels of inbreeding and reduced genetic diversity appeared to limit adaptation for the most highly inbred populations (Fig. 3; Perrier et al. 2017), inbreeding was negatively correlated with the probability of deleterious genetic variants in inland lake charr populations and predicted that inbreeding may facilitate purging of deleterious variants (Perrier et al. 2017). A similar pattern was observed in pristine lakes in Labrador, where lake charr genetic diversity was positively correlated with lake size (McCracken et al. 2013). Inbreeding can lead to genetic purging because it raises the likelihood of homozygous individuals for recessive deleterious mutations. This exposes deleterious variants to selection and they are rapidly removed from the population. While genetic purging can cause short-term benefits by reducing populations' genetic load from deleterious mutations, purging is expected to come with negative long-term fitness costs due to loss of linked adaptive variation during these selective sweeps. Thus, increasing inbreeding should not be considered an optimal strategy for reducing maladaptation in the lake charr.
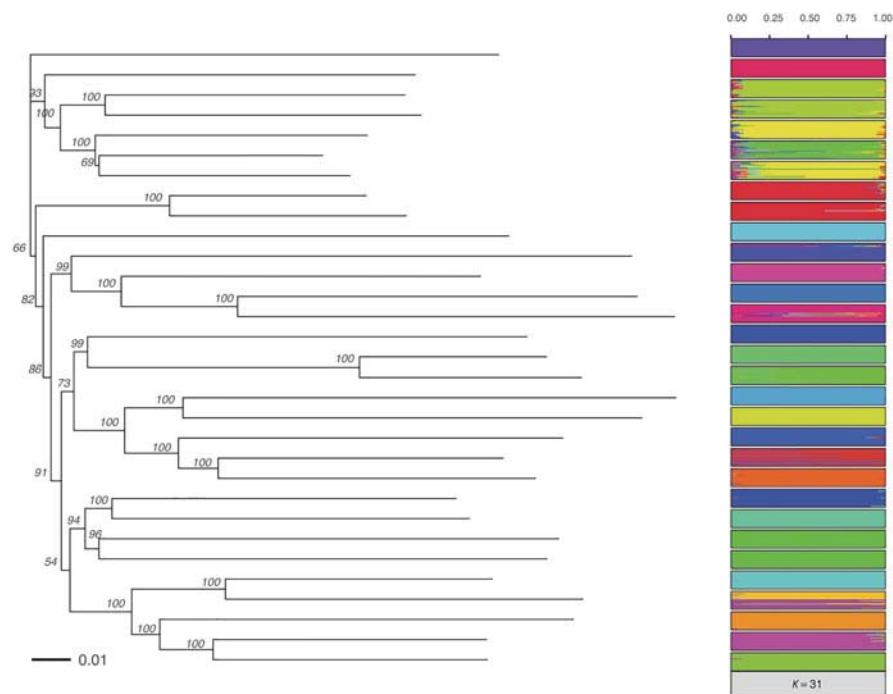
Fig. 2 Neighbor-joining tree based on Nei's genetic distance and structure plot for 31 populations of lake charr *Salvelinus namaycush* from Québec. Populations exhibit effects of strong genetic drift and limited gene flow as evidenced by long branch lengths and limited evidence for admixture in the structure plot. From Perrier et al. (2017)

As a consequence of pronounced genetic drift and limited connectivity, lake charr populations typically do not show a pattern of isolation by distance or regional hierarchical structuring according to hydrological connections (Halbisen and Wilson 2009; McCracken et al. 2013; Valiquette et al. 2014; Perrier et al. 2017), a pattern that may be unique to the lake charr among postglacial fishes. The exception to this lack of a spatial structuring pattern is for lakes at the southeastern edge of the distribution of lake charr in Vermont (Baillie et al. 2015). Here, variation is best explained by contemporary drainage basins, not proglacial drainages, and genetic differentiation increases with increasing elevation that suggests connectivity among these populations after postglacial colonization.

In large lakes, (>500 km^2), the situation is more complicated. Large lakes harbor higher levels of genetic diversity than small inland lakes (Ihssen et al. 1988; Halbisen and Wilson 2009), but landscape genetics patterns are not as clear due to the natural complexity of large lakes. Large lakes vary in both surface area and depth, which influences suitable habitat for and population sizes of lake charr. Lake
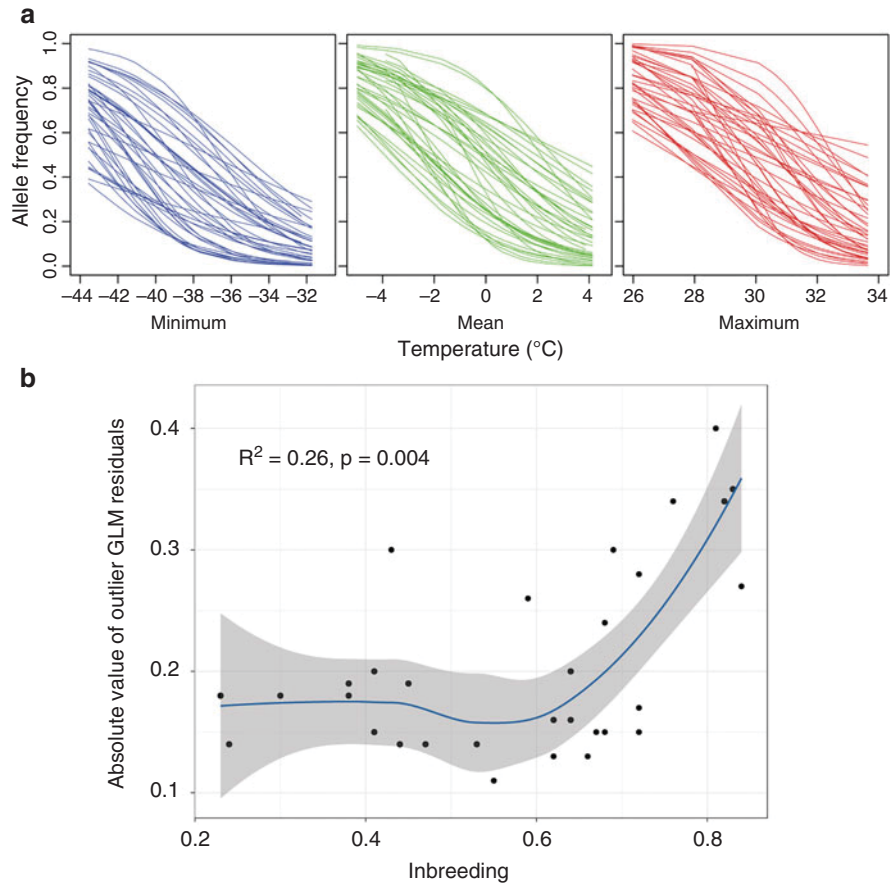
Fig. 3 Clinal association of allele frequency of 44 SNPs with minimum, mean, and maximum annual temperature for 31 populations of lake charr *Salvelinus namaycush* from Québec (**a**). Residuals from the GLM of outlier allele frequencies with temperature as a function of the population inbreeding coefficient (**b**). Higher levels of inbreeding increase the deviation of populations from the model fit suggesting increased inbreeding may be limiting adaptation. From Perrier et al. (2017)

charr populations in large lakes also tend to have within-lake geographical genetic structure (Guinand et al. 2003, 2012; Page et al. 2004; Harris et al. 2015; Baillie et al. 2016b; Marin et al. 2016; Perreault-Payette et al. 2017; Chavarie et al. 2018) that suggests the species may form a meta-population composed of multiple semi-independent gene pools in large lakes that confound whole-lake comparisons of diversity.

3   Comparative Genetic Diversity Among Ecotypes

The lake charr's extensive phenotypic diversity within- and among-lakes (Chavarie et al. 2021; Muir et al. 2016) has historically been characterized using a "discrete ecotype" conceptual model (e.g., lean [various lakes], humper, siscowet, redfin [Lake Superior], huronicus [Rush Lake, Michigan, U.S.A.], butterfly [Great Bear Lake, Northwest Territories, Canada], and many others). While Chavarie et al. (2021) contains a detailed treatment of the full range of morphological and ecological variation present in the lake charr, we provide a brief overview here to contextualize the patterns of genetic variation discussed below. Lean lake charr are the archetypal form found throughout the species' range and are characterized by long, fusiform bodies presumably adapted for a pelagic lifestyle. In large, deep lakes, lean lake charr are primarily found in shallow surface waters (<80 m depth) while several different forms (i.e., humpers, huronicus, siscowets) are more commonly found in deep waters (>80 m depth) and developed traits associated with life in deep water (e.g., short, deep bodies, and long paired fins).

   The origin of ecotype variation has been of great interest to fishery managers and evolutionary biologists alike. For managers, knowledge of whether ecotypes reflect unique stocks or populations with potentially different dynamics, habitat requirements, and susceptibility to stressors (e.g., fishing mortality, invasive species) is important for appropriate conservation, restoration, and management planning. For evolutionary biologists, the evolutionary forces and genetic mechanisms that generated and maintain divergent ecotypes are interesting for understanding of adaptive processes and speciation.

   Simple quantitative models describe phenotypes as resulting from the joint action of an individual's genotype and its environment. At the two extremes, lake charr ecotypes result entirely from exposure to different environmental conditions (e.g., prey communities, depth), or, alternatively, are completely genetically determined at birth. While neither of these extreme scenarios are likely, the relative contribution of genotype and environment to ecotype differentiation has important implications for stock delineation, fishery management, and prediction of evolutionary responses to changing environments. For example, conservation of biological (ecotype) diversity when a predominantly genetic basis exists for an ecotype might emphasize conservation of unique spawning populations of each ecotype, while a predominantly environmental basis for ecotype might emphasize maintenance of high-quality habitat types that give rise to each ecotype.

   Many lake charr ecotypes were initially described, and were once believed, to represent different species (Brown et al. 1981; Goodier 1981). Early molecular genetic work found that lake charr in Lake Superior were structured primarily based on ecotype and secondarily among geographically dispersed populations within each ecotype (Page et al. 2004). The genetic distinctiveness of ecotypes in the lake has consequential effects on management recommendations. For example, Page et al. (2004) stated that in light of the evidence of significant genetic differentiation among morphotypes, lake charr morphotypes should be managed as distinct

units in a manner like that advocated for imperiled Pacific salmon. This context of reproductively isolated ecotypes shaped much of the subsequent thinking about lake charr ecotype divergence.

For the purposes of discussing lake charr ecotypes, we will make use of a "biological species concept" which defines species based on the principle of reproductive isolation (Coyne and Orr 2004). Under this paradigm, ecotypes represent incipient species that fall somewhere along a continuum of reproductive isolation from complete panmixia to complete reproductive incompatibility (Hendry 2009; Chavarie et al. 2021). Two main possible evolutionary routes exist for the origin of ecotype variation: (1) allopatric divergence of ecotypes in geographic isolation (e.g., separate glacial refugia) where reproductive isolation developed and has subsequently been maintained following secondary contact of these diverged forms after dispersal from glacial refugia, or (2) multiple instances of sympatric divergence where divergence occurred along parallel environmental gradients found in multiple lakes throughout the species' range.

Molecular genetic data have shed light on the debate between allopatric versus sympatric divergence of lake charr ecotypes. Different ecotypes share the same mitochondrial haplotypes within each of the Great Lakes across continental North America (Burnham 1993) and ecotypes are generally more genetically similar to one another within lakes than when populations of the same ecotype are compared among lakes (Dehring et al. 1981; Krueger et al. 1989; Guinand et al. 2012; Baillie et al. 2016a, b; Perreault-Payette et al. 2017; but see Page et al. 2004). Collectively, this pattern suggests that independent divergence of ecotypes multiple times since the last glaciation is a more plausible explanation for observed patterns of phenotypic diversity than allopatric divergence of lake charr ecotypes during previous glacial cycles.

Ecological speciation, where divergent selection on populations exploiting different habitats or resources leads to reproductive isolation and eventual reproductive incompatibility (Schluter 1996, 2001; Rundle and Nosil 2005), provides a useful framework to conceptualize parallel evolutionary divergence of lake charr ecotypes in multiple lakes. Putative examples of ecological speciation are common for fishes colonizing postglacial habitats (Behnke 1972; Taylor 1999; Noakes 2008; Bernatchez et al. 2010). For these species, divergence in parallel at multiple locations is driven by selection along similar environmental axes (e.g., benthic versus limnetic). Thus, conserved phenotypic parallelism of lake charr ecotypes (e.g., between deep-water versus shallow-water forms) in geographically distant lakes provides evidence for similar ecological conditions driving ecotype divergence.

While molecular genetic data has proven useful for addressing questions of allopatric versus sympatric divergence, teasing apart potential reproductive isolation of lake charr ecotypes in several lakes has been more difficult using neutral markers (Northrup et al. 2010; Marin et al. 2016; Chavarie et al. 2016, 2018). This difficulty raises important questions about the genetic basis of ecotype divergence and mechanisms responsible for driving and maintaining ecotype divergence. In the sections below, we review hypothesized axes of ecotype divergence, evidence for

reproductive isolation of ecotypes, and alternatives to the leading hypothesis of ecological speciation for explaining lake charr ecotypic differentiation.

3.1 Genetic Evidence for Ecotype Reproductive Isolation

Based on observed patterns of genetic variation among ecotypes and available data on the heritability of lake charr phenotypes (Sect. 5), Eshenroder (2008) developed an eco-evolutionary conceptual model to explain the evolutionary origins of lake charr ecotypes. The model proposed a resource-driven basis for ecotype polymorphism and thus divergent ecotypes only occur where an appropriate ecological opportunity exists. In particular, the model stipulates that divergence occurs in large, deep lakes (e.g., Great Slave Lake, Laurentian Great Lakes) with appropriate deep-water prey species to facilitate feeding resource polymorphism (Eshenroder 2008). The model postulates that a lean-like piscivorous form of lake charr is the ancestral form that survived glaciation and recolonized North America during the last glacial retreat. This form, driven by intense intraspecific competition, diversified into various deep-water forms specialized for feeding on deep-water invertebrates (e.g., humper-like) or deep-water fish (siscowet-like). While Eshenroder's (2008) model was largely influenced by observed genetic and heritable differences in phenotype between lean and siscowet ecotypes in Lake Superior, small inland lakes harbor phenotypic variation in the form of piscivorous and planktivorous ecotypes that are also compatible with this ecologically driven basis for evolved morphological divergence (Bernatchez et al. 2016).

Eshenroder's eco-evolutionary model drew heavily from some of the earliest studies of genetic variation in the lake charr. Surveys of allozyme variation among lean, humper, and siscowet lake charr forms from three locations in Lake Superior revealed significant divergence in allele frequencies among both ecotypes and sites (Dehring et al. 1981). Numerous subsequent studies also supported the partitioning of genetic variance among ecotypes in Lake Superior using allozymes (Krueger et al. 1989), microsatellites (Guinand et al. 2003, 2012; Page et al. 2004; Baillie et al. 2016a), and single nucleotide polymorphisms (Perreault-Payette et al. 2017), although the relative importance of sampling site over ecotype for partitioning variation varies among studies. Furthermore, genetic differences have also been demonstrated among multiple shallow-water ecotypes in Great Bear Lake (Harris et al. 2015) and between piscivorous and planktivorous lake charr in inland lakes of Québec (Bernatchez et al. 2016). The genetic differentiation among ecotypes in these studies rejected the null hypothesis of panmixia, but the generally weak genetic differentiation of ecotypes at neutral markers raises questions about the degree of reproductive isolation among lake charr ecotypes across the range.

Baillie et al. (Fig. 4; unpublished data) specifically tested hypotheses that lake charr ecotypes are reproductively isolated and comprise discrete genetic clusters. They sampled tissue from ecotypes within four large lakes, Great Bear Lake, Great Slave Lake, Lake Mistassini, and Lake Superior, and one small lake, Rush Lake.
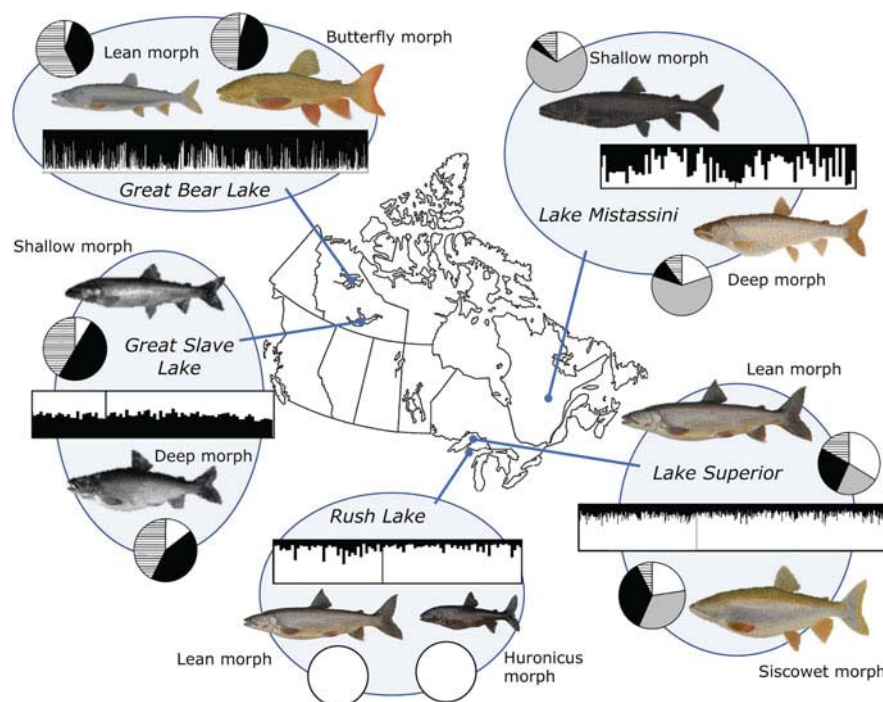
Fig. 4 Different lake charr *Salvelinus namaycush* ecotypes in the same lake share the same mitochondrial haplotypes (pie charts: white, gray, black, and horizontal lined slices represent Mississippian, Atlantic, Beringian, and Nahannian glacial refugia lineages, respectively) across continental North America and do not exhibit strong genetic distinction based on data from microsatellite markers (bar plots generated in Program STRUCTURE). The size of fish is to scale within but not among lakes. Baillie et al. unpublished data

Results from mitochondrial and microsatellite DNA analyses supported the hypothesis that ecotypes arose independently in lakes after postglacial colonization (Fig. 4). In contrast to previous studies, morphologically and ecologically differentiated lake charr ecotypes within lakes were largely genetically indistinguishable using neutral microsatellite markers and conventional population genetic analyses (Fig. 4). A lack of strong support for reproductive isolation among ecotypes was not likely due to a lack of power in genetic markers and statistical techniques previously used, but rather likely reflects ongoing gene flow between ecotypes. These results collectively suggested that lake charr ecotypes were at an early stage of divergence with incomplete reproductive isolation.

Genomic perspectives on ecological speciation with gene flow suggest that during the early stages of divergence, selection will heterogeneously influence divergence throughout the genome (Feder et al. 2012; Nosil and Feder 2012). Divergent selection on ecologically relevant genomic variation will be opposed by forces of gene flow and recombination that will break up ecologically favorable combinations of alleles. Where selection on genomic variation is stronger than these

opposing forces, "genomic islands of divergence" will develop to explain phenotypic polymorphism despite apparent panmixia in regions not influenced by selection (Feder et al. 2012; Nosil and Feder 2012). All studies that have failed to identify strong genetic divergence between ecotypes have employed microsatellite markers, and while effective and powerful markers for estimating gene flow, they offer a selectively neutral perspective of genome-wide patterns used to infer adaptive divergence between ecotypes. Given the lack of strong evidence for reproductive isolation among ecotypes, genomic data appear poised to make significant contributions to our understanding of lake charr ecotype divergence and ecological axes driving divergence (e.g., Larson et al. 2014).

3.2 Genomic Architecture of Parallel Divergence

Species that exhibit repeated parallel divergence in phenotypes across locations raise questions about whether the genetic basis of such divergence reflects the same, or different, genetic architectures (i.e., the same or different sets of genes). Where genomic data were available, the genetic basis of such parallel divergence resulted from both shared (Colosimo et al. 2004; Hohenlohe et al. 2012; Laporte et al. 2016) and unique genetic architectures (Gagnaire et al. 2013; Elmer et al. 2014; Laporte et al. 2016). Recent advances in next-generation sequencing techniques now provide genomic tools with the resolution required to investigate divergence at adaptive loci in the face of gene flow (Feder et al. 2012). Applications of genomic approaches to the question of lake charr ecotype divergence have the potential to provide insight into both ecotype divergence in the face of gene flow and potential genome-wide parallelism among lakes with similar ecotypes. Thus far, only two studies have investigated the genome-wide basis of ecotype divergence: one in Lake Superior, and one in inland lakes of Québec.

In Lake Superior, reduced representation genome sequencing (RADseq) of four ecotypes (lean, humper, siscowet, redfin) from four locations tested for parallelism at the genomic level (Perreault-Payette et al. 2017). Several outlier loci among ecotypes were consistent with known differences between Lake Superior ecotypes (e.g., lipid metabolism, visual acuity), although results did not support parallel genomic changes underlying repeated phenotypic divergence among sites (Fig. 5). Different genetic architectures may explain phenotypic parallelism among Lake Superior ecotypes at different sites. In support of this hypothesis, morphology could also be used to discriminate fish among sampling sites, which may reflect subtle differences in convergent evolution of forms at each site based on different genetic architectures (Perreault-Payette et al. 2017). Alternatively, reduced-representation sequencing used by Perreault-Payette et al. (2017) may have lacked the resolution to survey important areas of the genome involved in divergence among ecotypes. In the face of ongoing gene flow, the existence and size of islands of divergence for important loci underlying the ecotype will depend on the amount of gene flow, the strength of selection, and the frequency of recombination (Feder et al. 2012; Nosil and Feder
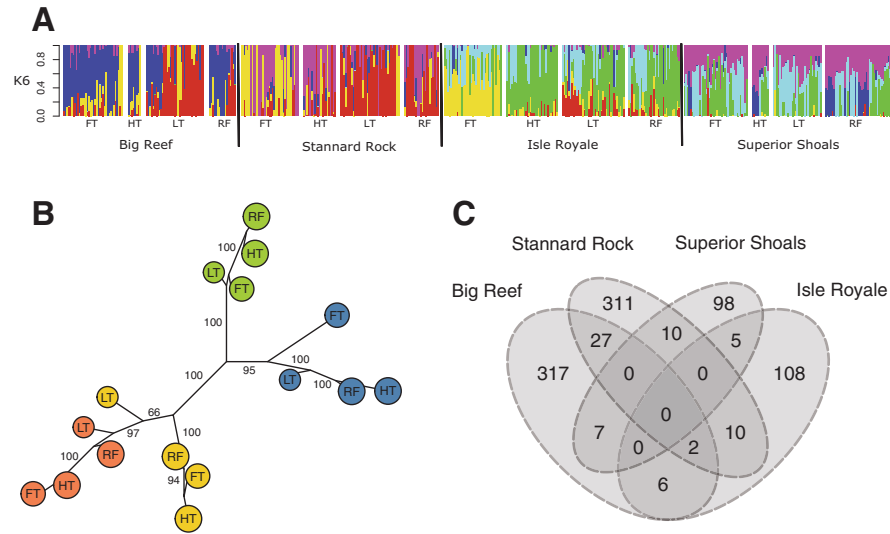
Fig. 5 Patterns of genomic variation in Lake Superior lake charr *Salvelinus namaycush*. (**a**) Admixture plot based on 486 individuals and 6822 SNPs (including outliers) for $K = 6$. Individuals are shown by sites and ecotypes. (**b**) Neighbor-joining tree based on 486 individuals and 6822 SNPs. Yellow circles represent Big Reef, orange circles Stannard Rock, blue circles Isle Royale and green circles Superior Shoals. Bootstrapping support is indicated on each branch. The four ecotypes are: lean (LT), humper (HT), redfin (RF), and siscowet (FT). (**c**) Venn diagram of the number of outlier SNPs among ecotypes that are shared among sites. From Perreault-Payette et al. (2017)

2012). Improved genetic resources, including a reference genome (currently in preparation; L. Bernatchez, unpublished data; see Sect. 8), will be essential for identifying the genomic architecture of adaptive traits of lake charr ecotypes. Finally, considering the likely polygenic basis for divergent adaptive traits, improved models describing the link between genotypes and phenotypes are required to properly address the mechanistic basis of phenotypic parallelism in the lake charr (Bernatchez 2016).

Small Canadian Shield lakes support ecologically divergent populations of lake charr (Wilson and Mandrak 2021; Bernatchez et al. 2016). In Québec, striking phenotypic parallelism in traits was associated with the foraging strategy of piscivorous and planktivorous ecotypes that occurred in both sympatry and allopatry (Bernatchez et al. 2016). Where these ecotypes occurred in sympatry, genetic divergence was consistently observed between ecotypes within lakes (Fig. 6). Furthermore, ecotype discriminating loci identified by means of a multivariate (polygenic) statistical framework correctly predicted ecotype assignment for seven of nine allopatric populations when they were not included in the training set (Bernatchez et al. 2016). These results provided the first evidence of a parallel genomic basis for parallel ecotypic divergence of lake charr. The inconsistent assignment results for two of the sympatric populations most likely reflected ongoing gene flow between
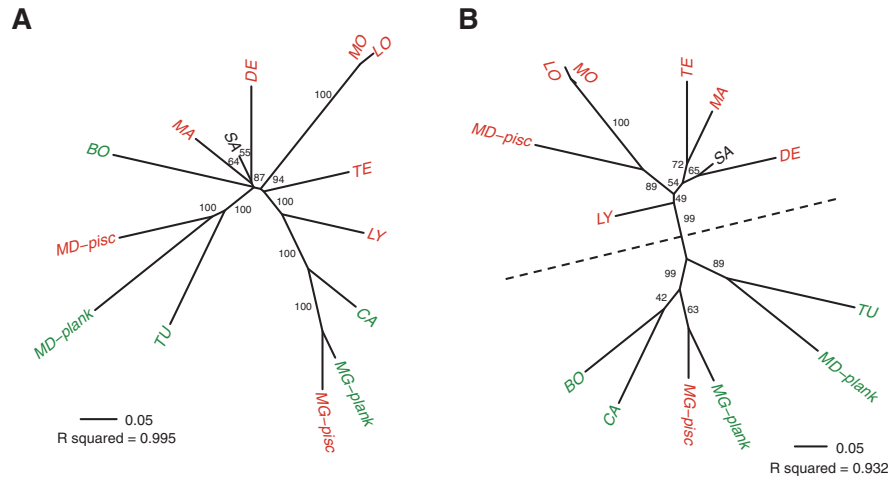
Fig. 6 Genomic parallelism underlying piscivorous (red) and planktivorous (green) ecotypes of lake charr *Salvelinus namaycush* in Québec, Canada. (**a**) Neighbor-joining tree constructed with pairwise F_{ST} values among the 14 lake charr groups based on 3925 SNPs. (**b**) Neighbor-joining tree constructed with pairwise F_{ST} values among the 14 lake charr groups based on 48 outlier SNPs between piscivorous (red) and planktivorous (green) ecotypes. Bootstrap values are indicated on the branches based on 1000 replicates. The dashed line shows the major split between planktivorous and piscivorous ecotypes. From Bernatchez et al. (2016)

ecotypes that reduced the strength of differentiation within lakes but could also reflect plasticity or different genetic architectures for ecotypes in lakes. Much remains to be discovered about the maintenance of lake charr ecotypic diversity in the face of gene flow.

3.3 Functional Inferences from the Major Histocompatibility Complex (MHC) Genes

In addition to genomic techniques, other "functional" genetic markers show promise for improving genetic discrimination of lake charr ecotypes. Major histocompatibility genes (MHC) critical for vertebrates' (including fish) adaptive immune systems involved in pathogen detection are known to experience positive selection (Bernatchez and Landry 2003). MHC genes are also known to be involved in mate choice through disassortative mating in salmonids (Landry et al. 2001). Functional loci, such as this, that have direct sequence-specific effects on organismal survival can provide complementary information to neutral markers for delineating important groups of organisms and environmental forces driving divergence. Studies of the lake charr investigated variation in the peptide-binding region (PBR) of the Major Histocompatibility Class IIβ (MHCIIβ) gene (Dorschner et al. 2000; Noakes et al.

2003; Baillie et al. 2018). Diversity at the MHCIIβ locus is higher for lake charr than other salmonids and a large proportion of private, ecotype-specific alleles exist (Dorschner et al. 2000; Baillie et al. 2018). Results suggest that different ecotypes may experience different pathogen communities and that the MHCIIβ locus may provide higher resolution for differentiating lake charr ecotypes than neutral markers.

The parallel sequencing technique recently used by Baillie et al. (2018) provided greater quantitative power of MHCIIβ variation than in past studies and suggested the presence of up to four alleles within individuals. This observation is the first time that putative copy number variation had been detected in the lake charr. Phylogenetic and principal component analyses on MHCIIβ sequences clearly showed two major groups of lake charr MHC alleles and were supported by the degree of change (DOC) statistical method used to call individuals' genotypes (Baillie et al. 2018). Taken together, the results of Baillie et al. (2018) indicate that lake charr may have a duplicated MHCIIβ locus. However, 96% of successfully genotyped lake charr showed evidence of only one copy of the MHC locus (one or two alleles). The number of alleles per individual was scored conservatively and the number of individuals with three or more alleles was likely underestimated. Further work is required across lakes within the species range to test the hypothesis of lake charr MHCIIβ copy number variation and its relevance for ecotype differentiation in other lakes.

3.4 Ecotype Divergence and a Role for Phenotypic Plasticity

In addition to weak differentiation of lake charr ecotypes throughout the range, no genetic divergence was found between lean and humper-like ecotypes from Lake Mistassini (Marin et al. 2016), Rush Lake (Chavarie et al. 2016), and a recently introduced population in Flathead Lake (Stafford et al. 2014). Genetic divergence was also lacking between a lean and an undefined deep-water form of lake charr in Atlin Lake (Northrup et al. 2010). Phenotypic plasticity has been proposed by many of these authors as a possible mechanism to explain the presence of divergent ecotypes in these lakes. Phenotypic plasticity, the ability of a genotype to be expressed in different phenotypes due to different environmental stimuli, has been hypothesized to play a role in driving population establishment, local adaptation, and speciation (West-Eberhard 2003; Pfennig et al. 2010). Surprisingly, experimental evidence directly testing the role of plasticity in explaining differences between ecotypes of lake charr is lacking. As such, the relative importance of divergent natural selection versus phenotypic plasticity in lake charr ecotype divergence remains unclear, as for many systems (Perry et al. 2018). Salmonids are thought to be highly phenotypically plastic and responsive to environmental variables (Hutchings 2011). Furthermore, in a closely related species, the Arctic charr *Salvelinus alpinus*, a substantial amount of plastic variation contributed to ecotype differences (reviewed in Klemetsen 2010). The role of plasticity in generating and

maintaining lake charr ecotype variation is an under-developed area of research that could benefit from further investigation using controlled mating and common-garden rearing experiments (but see Goetz et al. 2010, 2014 discussed below).

4 Genetic Evidence for Ecological Axes of Adaptive Divergence

Recent genetic data have challenged the discrete ecotype conceptual model (Baillie et al. 2016a, 2018) and incited a view of lake charr genetic and phenotypic diversity where diversity is distributed along environmental gradients, rather than by reproductively isolated divergent ecotypes. On the basis of genetic evidence, water depth is an important ecological variable that promotes and maintains lake charr diversity (Baillie et al. 2016a, 2018). Genetic variation was more strongly partitioned among depth strata than among ecotypes. These genetic differences correlated with morphological and life-history traits and also varied along a depth gradient. Additionally, the immunogenetic diversity of lake charr was examined to ascertain whether immune genes would be useful for current and future lake charr habitat-genetic studies (Baillie et al. 2018). Immune genes are often closely tied to habitat because pathogen diversity varies greatly among local environments. Consequently, major histocompatibility complex (MHCIIβ) gene diversity better reflected habitat (water depth) differences than microsatellite markers (Baillie et al. 2018).

4.1 Parallel Patterns of Divergence in Lake Charr

To understand mechanisms and potential for future divergence within any species, adaptive diversification should be examined in parallel among independent populations in similar environments and ecological niche axes. Independent populations that colonize similar environments and evolve similar traits provide evidence for nonrandom processes responsible for divergence driven by changes in gene frequencies (Endler 1977; Schluter 2001; Kaeuffer et al. 2012). Evidence required to conclude adaptive diversification involves detection of sources of divergent selection, such as competition for habitat or food, ultimately leading to reproductive isolation (e.g., partially restricted gene flow, complete isolation), and correlations between sources of divergent selection and reproductive isolation (Rundle and Nosil 2005; Østbye et al. 2006). Simple habitat category contrasts, such as lake versus stream, benthic versus limnetic, or high predation versus low predation, provide important starting points for investigations of more nuanced ecological axes of divergent selection (Kaeuffer et al. 2012; Stuart et al. 2017).

Lake charr water-depth clinal patterns were consistent in replicate at multiple spatial scales within a sampling site (e.g., Isle Royale; Baillie et al. 2016a) and

among multiple sampling sites within a lake (e.g., Lake Superior; Perreault-Payette et al. 2017). Phenotypic clines present within each ecotype clearly suggest that environmental conditions influenced genetic control of morphological and life-history traits. Local adaptation and maintenance of intraspecific genetic diversity are important to a species' persistence in the face of rapidly changing environmental conditions. Where human activities impede the conservation of genetic diversity, habitat heterogeneity is thought to play a major role in the maintenance and promotion of diversity (Larkin et al. 2016). This work provides a framework for understanding the evolutionary potential of lake charr for managers and conservation programs seeking to re-establish declining or extirpated populations.

4.2 Nonparallel Patterns of Divergence

Where traits diverge in different directions along similar niche axes for different populations, the niche axes in question do not fully explain variation or intraspecific biological diversity, and traits are considered nonparallel traits. Therefore, nonparallel and parallel patterns of divergence across populations are important to recognize and better understand the conservation and restoration of biodiversity (Arendt and Reznick 2008; Kaeuffer et al. 2012). In Lake Superior, despite generally high parallelism for locomotive traits, several patterns were not parallel among geographically disparate populations. Morphological variables related to feeding traits showed low to no parallelism along a depth gradient across shoals and this non-convergence could be explained principally by differences among ecotype (Baillie et al. 2016a). Key differences in trait variation with depth occurred among ecotypes. For instance, the lean ecotype had longer and leaner bodies than humper, siscowet, and redfin ecotypes. The fusiform body of the lean ecotype may be more constrained than deep-water ecotypes with depth variation. This result is supported by previous studies of lake charr depth distribution data, which show that siscowets are often observed in shallow water during summer while leans are rarely found in deep water (Moore and Bronte 2001). The large amount of unexplained variation in morphology may be attributable to prey assemblage overlap within depth strata, which maintains variation in foraging traits through disruptive selection even within ecotypes (Kaeuffer et al. 2012; Chavarie et al. 2018).

4.3 Conclusions

Phenotypic clines in morphology within ecotypes followed a depth gradient, despite ecotypes having morphological traits best suited to shallow or deep depths (Baillie et al. 2016a). This observation suggests that a degree of phenotypic plasticity likely operates in the lake charr system as a result of resource plasticity. Furthermore, awareness that environmental conditions can influence genetic control of life-history

traits has increased over the last few decades (Gutteling et al. 2007). Environmental variables may affect ontological processes after egg fertilization, which subsequently may influence phenotypic expression (Stearns 1976; Moran 1994; Sinervo and Svensson 1998; Bailey et al. 2015). Growth rates and age-at-maturity are correlated with temperature, with northern populations growing slower and maturing later than southern populations (Redick 1967; Allen et al. 2005). Therefore, variation in water temperature at different depths may affect growth. Based on available data, we hypothesize that lake charr variation is shaped initially by phenotypic plasticity and genotype-by-environment effects, and second, divergent natural selection operated on plastic phenotypes, thereby leading to adaptive divergence and partially restricted gene flow.

5 Heritable Basis of Phenotype

An alternative way of interrogating the evolutionary basis of lake charr phenotypic diversity is to assess the heritability of traits associated with major axes of variation among ecotypes. The strongest evidence for a heritable basis of traits associated with lake charr ecotypes comes from quantitative genetic experiments where divergent lake charr ecotypes were bred and reared in common environments. Beginning in the mid-1900s, the culturing of lake charr for stocking provided opportunities to investigate the heritability of lake charr phenotypes. Rearing offspring of artificial breeding crosses, where the parental ecotypes were known, under the same environmental conditions allowed investigators to rule out effects of phenotypic plasticity due to differences in environmental exposure and thus isolate genetic contributions to the variance in phenotype between ecotypes. These experiments broadly support a heritable basis of many important phenotypic differences among ecotypes and populations of lake charr.

5.1 Common Garden Experiments with Reared Lake Charr

Morphology has been the primary axis on which forms of lake charr have been identified (Chavarie et al. 2021). First-generation crosses between wild-caught lean and siscowet lake charr reared in a common environment exhibited heritable growth and morphological differences (Goetz et al. 2010). Morphological differences were detectable as early as age-1 (Goetz et al. 2010) and trajectories of divergence in head shape and caudal peduncle length were consistent with morphological divergence observed between wild adult forms (Khan and Qadri 1970; Moore and Bronte 2001). These results indicated the morphological differences between lean and siscowet were both heritable and likely maintained throughout ontogeny. These results confirmed that phenotypic variation among lake charr ecotypes was not purely plastic, but also has an additive genetic component.

Heritable differences in morphology, in particular paired fin size, are of important consequence because of hydrodynamic and hydrostatic swimming modes employed by different lake charr ecotypes (Eshenroder et al. 1999; Muir et al. 2014). Hydrodynamic swimming achieves greater speed and horizontal acceleration, whereas hydrostatic swimming provides greater maneuverability vertically through the water column. Two ecotypes (siscowet and redfin) of Lake Superior have long gliding fins, and increased fat content (discussed below), that promote buoyancy primarily through hydrostatic swimming. In contrast, the streamlined lean ecotype has hydrodynamic adaptations for fluvial and pelagic swimming and is not often found in deep water. Humpers inhabit moderate depths at offshore shoals with strong current and show characteristics adaptive for hydrodynamic lift (Muir et al. 2014).

Other common garden experiments have revealed heritable differences in developmental rate among different hatchery and wild populations of lake charr reared under similar laboratory conditions (Horns 1985) and a heritable basis for both growth rate and age-at-maturity for purebred crosses of fish from Lake Opeongo and Lake Louisa in Ontario reared in a common environment (McDermid et al. 2007). Hybrid crosses of males from Lake Louisa with females from Lake Opeongo produced offspring that were intermediate to parental purebreds (McDermid et al. 2007), which suggests phenotypes result from additive inheritance of these traits (where the effect of each allele contributes additively to the inheritance of phenotypic traits as opposed to the influence of dominance or epistatic effects). Collectively, heritability of developmental rate and age-at-maturation are consistent with observations of population-specific differences in age-at-maturation that have been reported (Krueger and Ihssen 1995).

Fat content has long been recognized as an important and defining characteristic that distinguishes lean and siscowet charr ecotypes in Lake Superior (Goodier 1981). Eschmeyer and Phillips (1965) were the first to quantitatively demonstrate that muscle fat content was consistently higher for wild-caught siscowet charr compared to lean charr. They further demonstrated that humpers had intermediate fat content closer to lean ecotypes than siscowet. These differences between lean and siscowet were subsequently shown to be maintained when pure-type breeding crosses of lean and siscowet ecotypes were raised in a common hatchery environment (Eschmeyer and Phillips 1965). The offspring of hybrid lean x siscowet breeding crosses exhibited intermediate fat contents, and for the single size category where comparison was possible, fat content appeared to be inherited in an additive manner.

The early work of Eschmeyer and Phillips (1965) on body fat differences was corroborated and expanded upon by recent studies of Goetz et al. (2010, 2014). Siscowet were again found to have higher lipids in whole body analyses (Goetz et al. 2010), and in a more detailed tissue-level analysis, siscowet had higher lipids in both muscle and liver tissues than lean lake charr (Goetz et al. 2014). Hybrid crosses of lean and siscowet showed intermediate lipid contents between the two ecotypes (Goetz et al. 2014), which further suggested that lipid content is inherited through the additive effects of alleles. The composition of lipid differences between ecotypes are characterized by a higher proportion of polyunsaturated fatty acids (PUFAs) in the lipid profile of siscowet that could not be explained by differences in diet (Goetz

et al. 2014). Increased levels of PUFAs may reflect an adaptation for maintaining membrane fluidity at lower temperatures for siscowet but the significance of these differences for wild populations is uncertain (Goetz et al. 2014). In contrast to results for muscle and liver tissues, leans have higher circulating levels of lipids in their blood plasma, higher glycogen levels in muscle and liver tissues, and higher blood glucose than siscowet. Goetz et al. (2014) concluded that lean and siscowet represented different "metabolotypes" that differed considerably in the way they process and store energy.

Metabolic differences between siscowet and lean charr are likely mediated through differential regulation of gene expression. Genes related to lipid processing and transport and immune response were differentially expressed in livers of siscowet and lean charr reared in the same environment (Goetz et al. 2010). Transcriptional patterns suggested that leans maintained greater lipid storage in liver tissue while siscowet exhibited greater transport and deposition of lipids in peripheral tissues (Goetz et al. 2010). These gene expression patterns were partially congruent with physiological differences (Goetz et al. 2014). In particular, higher expression of myostatin proteins was demonstrated in siscowet muscles (Goetz et al. 2014). These proteins play a role in the positive regulation of adipose tissue mass and were consistent with higher levels of lipids in muscle tissue of siscowet than lean ecotypes. The two results differed, however, in predictions of lipid levels in livers of leans. Transcriptome data suggested that leans were storing fat in this tissue, but physiological data indicated that siscowets had fattier livers. The RNA sequencing work of Goetz et al. (2010) represented a very early application of this technique to characterize gene expression. Improved sequencing technologies and analysis methods for this type of data are now available that would facilitate a more complete characterization of lake charr transcriptomes and provide further insight into differential metabolic regulation exhibited by these ecotypes.

High lipid levels in siscowet (Eschmeyer and Phillips 1965; Goetz et al. 2010, 2014) and differences in buoyancy between ecotypes in Lake Superior (Muir et al. 2014) and other lakes (Zimmerman et al. 2006, 2007) have been speculated to represent adaptations for maintaining buoyancy at different preferred depths (Henderson and Anderson 2002). In support of this hypothesis, swim bladder gas retention has a heritable basis for lake charr from inland Ontario lakes (Ihssen and Tait 1974). Fish from Lake Simcoe that evolved with access to deep habitats retained their buoyancy better than fish from shallow Lake Louisa when reared in a common environment. Reciprocal hybrid crosses between the two populations showed intermediate buoyancy to further support a heritable basis for this trait.

Finally, other physiological differences with a heritable basis have been observed between siscowet and lean ecotypes in Lake Superior. Despite sharing the same tanks as siscowet, lean charr showed higher constitutive expression of certain immune system transcripts (complement proteins; Goetz et al. 2010). These differences may reflect adaptation to a greater pathogen diversity in warmer surface waters for the lean ecotype than the siscowet ecotype, which spend most of their time in deeper, colder water (Goetz et al. 2010). While this hypothesis remains to be tested, studies of MHC loci also support differentiation of Lake Superior lake charr along a

depth gradient (Baillie et al. 2018; see Sect. 3.3) that may also be indicative of adaption to alternative pathogen pools.

5.2   Experimental Stocking of Lake Charr

Further indirect evidence of heritable differences for phenotypes relevant to the survival of lake charr is based on the assessment of stocking efforts. Locally sourced strains out-perform non-local strains in experimental stocking of inland lake systems (Plosila 1977; Siesennop 1992). Mechanisms that underlie differential survival of strains are unclear but may be related to behavioral differences or competitive abilities that are as yet uncharacterized (Siesennop 1992). In the Great Lakes, recapture data and genetic stock assignment of wild-spawned progeny have been used to assess the performance of various hatchery strains. In lakes Huron, Michigan, and Ontario, the Seneca strain, derived from lake charr from Seneca Lake in New York, has consistently been represented at higher proportions than expected among wild produced progeny of stocked fish (Marsden et al. 1993; Grewe et al. 1994; Eshenroder et al. 1995; Perkins et al. 1995; Page et al. 2003; Roseman et al. 2009; Scribner et al. 2018). The specific mechanism for this performance difference has never been elucidated, although Seneca strain fish suffered lower sea lamprey *Petromyzon marinus* wounding rates in Lakes Ontario (Schneider et al. 1996) and Huron (Madenjian et al. 2006) than other strains. The progenitors of the Seneca hatchery strain are known to have co-existed with sea lamprey for over a century and may have evolved an adaptation that allows them to escape lamprey predation, possibly by suspending off the bottom, thereby reducing vulnerability to sea lamprey depredation. Mortality after lamprey attacks in the laboratory that are similar for Seneca and Lake Superior strains suggest that behavioral differences or environmental preferences may explain this strain's reduced incidence of sea lamprey wounding in the wild (Swink and Hanson 1986).

5.3   Transgenerational Plasticity

While the kinds of common garden experiments described above provided strong evidence that divergence of traits associated with lake charr ecotypes is heritable and not strongly influenced by phenotypic plasticity in the F1 generation, a potential role for transgenerational plasticity exists for determining ecotypic differences in the F1 generation. Adults used to produce breeding crosses in many studies were sourced from wild populations that may have passed on heritable but nongenetic markers to influence their offspring's development. For example, short-term heritable epigenetic marks (e.g., DNA methylation) can produce heritable phenotypes in offspring without affecting the DNA sequence (Verhoeven et al. 2016). The role of epigenetic modifications in producing adaptive (or mal-adaptive) phenotypes is an emerging

area of research that may have important relevance for understanding lake charr ecotypic divergence. For instance, migratory (steelhead) and nonmigratory rainbow trout *Oncorhynchus mykiss* differed in many methylated regions (DMRs), which is the first evidence of a relationship between epigenetic variation and life history divergence in salmonids (Baerwald et al. 2016). Recent studies of sticklebacks Gasterosteidae spp. have also provided clear evidence for DMRs as a result of developmental acclimation to different temperatures (Metzger and Schulte 2017). These differences were stable through ontogeny (larvae to adult) and had corresponding effects on gene expression (Metzger and Schulte 2018). Furthermore, studies of coral reef fishes have demonstrated that transgenerational acclimation to temperature was mediated by the transmission of parental methylation profiles (Ryu et al. 2018). If ecotypic differences initially arise or are maintained as a result of phenotypic plasticity as some authors have speculated (Marin et al. 2016; Chavarie et al. 2016; Chavarie et al. 2021), heritable epigenetic variation may initiate the divergence process. Multigenerational common garden experiments are needed to investigate potential transgenerational plasticity, and epigenetic studies are needed to provide important insight into fundamental questions on the origin and maintenance of ecotype diversity.

6   Temporal Trends in Laurentian Great Lakes Lake Charr Population Genetic Diversity and Structure After the Fishery Collapse

After the collapse of lake charr populations in the Laurentian Great Lakes during the 1950s, the species was extirpated from lakes Michigan, Ontario, and Erie, only remnant populations survived in Lake Huron, and population sizes were depressed in Lake Superior. To investigate temporal changes in genetic variation in the Laurentian Great Lakes over the span of this event, "ancient" DNA extracted from 40-year-old lake charr scale samples collected during years before, during, and after the collapse (c. 1940–1959) in lakes Michigan, Huron, and Superior was genotyped using five microsatellite loci and then compared to samples collected during 1995–1999 from lakes Huron and Superior where lake charr populations had persisted (Guinand et al. 2003). Extinct Lake Michigan lineages harbored most of the Laurentian Great Lakes meta-population genetic diversity and gene diversity decreased in populations from lakes Superior and Huron over time (Guinand et al. 2003). Naturally reproducing populations from Lake Superior, believed responsible for the resurgence of lake charr abundance and distribution, were probably affected by hatchery supplementation (Guinand et al. 2003; see Sect. 7). Thus, past demographic declines in abundance and the extirpation of native lake charr populations between 1954 and 1999 appeared to have resulted in a dramatic decline in the amount of standing genetic variation in the lake charr (Guinand et al. 2003).

6.1 Reductions in Genetic Diversity Within Ecotypes in Lake Superior

Temporal changes in genetic diversity and structure of three sympatric lake charr ecotypes (lean, siscowet, and humper) in Lake Superior at three points in time (1948 = pre-collapse period of intensive fishing, 1959 = collapse due to sea lamprey, and 1990s = recovery) confirmed that lean and siscowet ecomorphs lost substantial genetic diversity (23 and 21%, respectively) from pre-collapse levels (Guinand et al. 2012). Lean charr were affected more heavily and declined quicker after the collapse than siscowet charr (Guinand et al. 2012). Significant genetic differentiation was also evident among ecotypes historically, prior to declines in abundance, and among contemporary populations, which suggests that periods of population decline and resurgence in abundance and distribution did not result in loss of genetic distinctiveness among morphs (Guinand et al. 2012).

Recent work using samples and genotypes from these previous studies, while adding contemporary samples across Lake Superior and additional microsatellites, indicated that lake charr may still be losing allelic richness today (Baillie et al. 2016b), in spite of the tremendous recovery efforts where Lake Superior lake charr were declared restored by the 1990s (Muir et al. 2012). Although both the census size of lake charr populations and the effective number of breeders (a population genetic estimate of the number of reproductive adults efficiently contributing to a cohort) in Lake Superior have made spectacular recoveries, lake charr in Lake Superior have lost 6% allelic richness and 41% private allelic richness since the 1990s, and possibly 30% of overall neutral genetic diversity since the 1950s (Fig. 7; Baillie et al. 2016b).

Functional genetic data from Major Histocompatibility Complex class IIβ gene (MHCIIβ) diversity provided similar evidence of genetic diversity loss. Data from three studies of lake charr MHCIIβ variation in Lake Superior on samples from different periods provide insight into temporal trends in allelic variation at this important immune locus (Dorschner et al. 2000; Noakes et al. 2003; Baillie et al. 2018). While these three studies used different approaches to genotyping, thereby preventing direct comparison of specific allele identities, the number of MHCIIβ alleles detected per individual in contemporary samples (2006–2007) was lower (0.41 alleles/ind; Baillie et al. 2018) than from the 1990s (0.58 alleles/ind; Dorschner et al. 2000; Noakes et al. 2003). Next-generation amplicon sequencing methodology is likely more sensitive than previous electrophoretic (e.g., single-strand conformational polymorphism analysis) and cloning methods, which suggests this difference may be an underestimate of the MHCIIβ allelic diversity lost since the 1990s (Baillie et al. 2018). Erosion of MHC diversity can represent a serious risk to populations by increasing disease susceptibility (Garrigan and Hedrick 2003; Goyette et al. 2015; Rico et al. 2016), which may be particularly important considering the influx of invasive species in recent decades (Holeck et al. 2004). More high-resolution genomic studies (i.e., whole-genome resequencing) will allow the investigation of patterns of heterozygosity across the genome to give more comprehensive insights into the loss of genetic diversity in functional genomic regions.
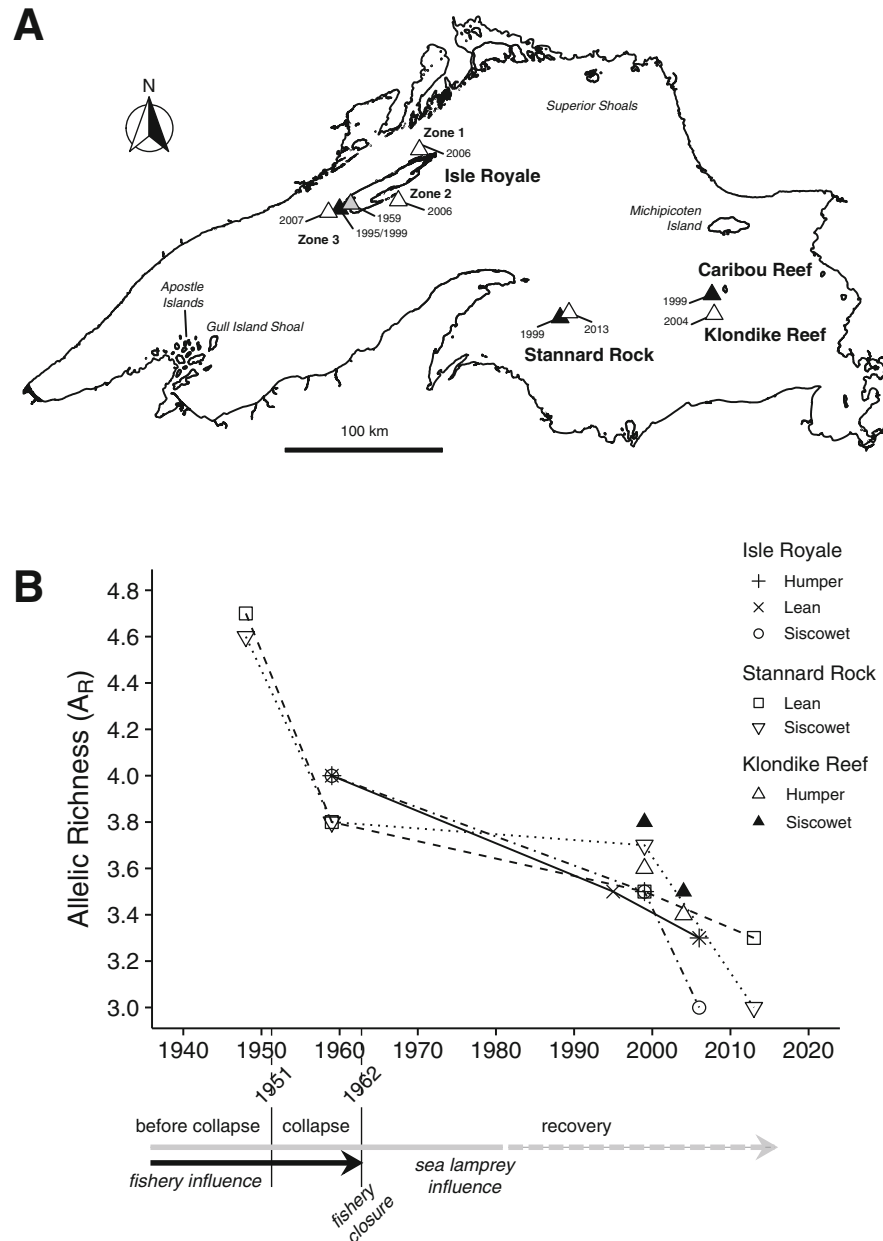
Fig. 7 Lake charr *Salvelinus namaycush* genetic data collection sites within Lake Superior representing three main locations, Isle Royale, Stannard Rock, and the Caribou Reef/Klondike Reef area (**a**) and temporal trends in allelic richness (**b**) of lake charr ecotypes: lean, humper, and siscowet from 1948 to 2013. Open, black, and dark gray triangles on the map represent contemporary, recovery, and historical samples, respectively. Points represent previously published data from Guinand et al. (2012) collected during 1948, 1959, and 1995–1999 as well as data collected during 2004–2013 by Baillie et al. (2016b). Arrows below the graph indicate three management periods (in bold) and represent dominant influences during pre-collapse and recovery management (gray arrow is dotted to symbolize reduced sea lamprey influence after 1980). From Baillie et al. (2016b)

6.2   Reductions in Genetic Distance Among Ecotypes in Lake Superior

The loss of allelic diversity through time within lake charr populations in Lake Superior has been paralleled by changes in among-population variation. As detailed in Sect. 3, genetic differentiation of ecotypes had been reported in early molecular genetic work conducted on lake charr from Lake Superior. Microsatellite genotypes from lake charr samples collected during the 1990s indicated that lake charr in Superior were structured primarily based on ecotype and secondarily among spatial sampling sites (Page et al. 2004; Guinand et al. 2012). While ecotypes could be differentiated genetically (Guinand et al. 2012), genetic distinctiveness among ecotypes apparently collapsed (Baillie et al. 2016b). Lake charr clustered together by ecotype across geographic locations during the recovery period (1990s) but clustered together by location in the contemporary period (2000s) (Fig. 8). Re-analysis of data from Guinand et al. (2012) and Page et al. (2004) in direct comparison with contemporary data using multiple population genetic methods (e.g., tree clustering, AMOVA, hierarchical FST tests, multiple co-inertia ordination analyses) provided strong corroborative proof that ecotypes could be discriminated genetically during 1995–1999, but not during 2004–2013 (Baillie et al. 2016b). The five-locus data set reproduced the same population genetic structure as when 18 loci were used. The overall reduction in genetic distance among ecotypes was ~60% (averaged among all pairwise ecotype comparisons at Isle Royale).
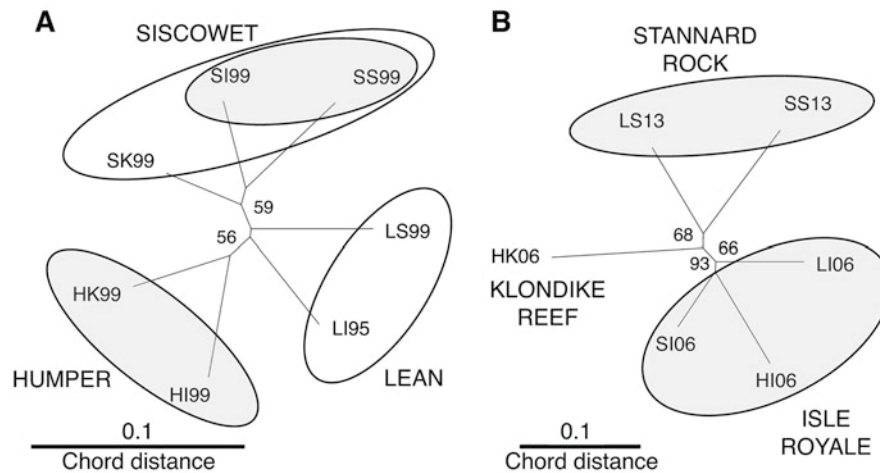
Fig. 8 Neighbor-joining tree of Cavalli-Sforza and Edward's chord distance based on Lake Superior lake charr *Salvelinus namaycush* recovery period samples genotyped at five microsatellite loci (**a**) and contemporary period samples genotyped by 18 loci (**b**). Sample codes indicate ecotype (L, lean; H, humper; S, siscowet), sampling location (I, Isle Royale; S, Stannard Rock; K, Klondike Reef), and year, e.g.,99 = 1999, 13 = 2013. Numbers on branches represent bootstrap values greater than 50% (100 replicates). From Baillie et al. (2016b)

The MHCIIβ data from Lake Superior reinforced findings based on microsatellite markers that differentiation among Lake Superior ecotypes decreased greatly since the 1990s. The number of MHCIIβ alleles shared by both lean and siscowet increased over time (Baillie et al. 2018). The number of shared alleles increased from 20% overlap in the 1990s (Noakes et al. 2003) to 35% overlap in contemporary samples (Baillie et al. 2018). Despite the overlap between lean and siscowet, almost half of MHC alleles were unique to ecotypes. Thus, Lake Superior lake charr ecotypes, except for redfins, can still be distinguished by immunogenetic differences despite no longer being distinguishable with microsatellites. These results are generally consistent with the hypothesized collapse of lake charr ecotypes based on morphological and microsatellite studies (Muir et al. 2014; Baillie et al. 2016b) and may reflect a reversal of the speciation process.

6.3   Reverse Speciation

Striking instances of collapse or homogenization of diverging ecotypes in fishes are numerous, especially during the early stages of diversification—this process is referred to as speciation reversal (Taylor et al. 2006; Seehausen 2006). Speciation reversals can occur when gene flow, which was previously restricted, increases among ecological variants. In some cases, phenotypic plasticity, or environmental responsiveness of traits, can increase susceptibility to reversals of divergence (Taylor et al. 2006; Seehausen 2006). For example, in once-heterogeneous environments that become homogenized, speciation reversal can occur through the adaptive convergence of genes under selection. Hybridization of formerly reproductively isolated populations represents another mechanism potentially driving speciation reversals (Ribeiro and Caticha 2009). In particular, hybridization may contribute to speciation reversal when ecotypes cross habitat "barriers" (Camacho et al. 2016) or results from dramatic changes in abundance or population dynamics (Bhat et al. 2014) as hypothesized for bloater *Coregonus hoyi* in Lake Huron (Eshenroder et al. 2016). A successful "re-speciation," after a collapse of diverging ecotypes, is thought to involve restoration of the selection regime that historically allowed for diversification in heterogeneous habitats and along environmental gradients (e.g., habitat restoration, translocation of former species assemblages; Hirsch et al. 2013; Jacobs et al. 2019). A growing body of research indicates that the ebb and flow of speciation, and its reversal, may naturally characterize evolutionary dynamics of adaptive radiations and may increase the adaptability of genomes (Turner 2002; Taylor et al. 2006; Seehausen 2006).

Based on previously observed losses in neutral genetic diversity and increased overlap among ecotypes in MHCIIβ alleles, a genetic collapse in differentiation among ecotypes may be occurring in lake charr from Lake Superior. The apparent homogenization of genetic and morphological variation indicates a possible reversal in the lake charr evolutionary trajectory of sympatric adaptive diversification in Lake Superior. Speciation reversal and loss of biodiversity are often difficult to detect

because ecotypes can homogenize rapidly and without major changes in the species distribution (Vonlanthen et al. 2012). In principle, divergent natural selection could maintain ecotype differences despite low genetic diversity at neutral genes (Feder et al. 2012). Data for Lake Superior suggests, however, that reproductive and ecological niche spaces have been altered to a degree that selection may not be able to counteract the homogenizing effects of gene flow. Already extirpated from lakes Michigan, Erie, and Ontario by the combined effects of overfishing and sea lamprey predation, the lake charr of Lake Superior are a considerable conservation and management concern as the last remaining stronghold for the species in the Laurentian Great Lakes. Understanding lake charr diversity and niches that ecotypes occupy is a top priority for rehabilitation and re-establishment of deep-water food webs (Zimmerman and Krueger 2009). Conservation approaches for this species could focus on managing ecological habitats by depth, in addition to regulating fisheries specific to ecotypes. Preserving ecosystem function requires maintaining the selective environment offered by functional ecosystems, which in turn require protection of ecological conditions and evolutionary mechanisms that generate and maintain species diversity (Vonlanthen et al. 2012).

## 7   Genetic Effects of Hatchery Stocking

Lake charr populations have experienced variable intensities of stocking throughout North America with the most severe and extensive stocking efforts concentrated in lakes of the eastern half of the continent. Stocking has the potential to alter the genetic composition of populations when individuals have been sourced from genetically divergent populations or when source populations for stocking have experienced domestication selection. For much of the twentieth century, lake charr were stocked indiscriminately with little apparent consideration of the origins of source populations. Negative effects of stocking on wild salmonid populations have been demonstrated in recent decades (Araki et al. 2007; Frankham 2008; Christie et al. 2014) and drove interest in understanding the influence of stocking history on the genetic integrity of extant native populations of lake charr.

In most cases, early stocking (pre-1950s) of lake charr was too poorly documented to enable assessment of stocking intensity and its effects on native population genetic diversity. For lakes where records of the number of fish stocked and their origins exist, a more controlled assessment of genetic effects of stocking on native populations has been possible (Halbisen and Wilson 2009; Valiquette et al. 2014). Spatial coverage of studies investigating genetic effects of stocking on natural lake charr populations is widespread for inland lakes (Wisconsin: Piller et al. 2005; Saskatchewan: Giroux et al. 2009; Ontario: Halbisen and Wilson 2009; Quebec: Valiquette et al. 2014; Vermont: Baillie et al. 2015), while less is known about genetic effects of stocking in the Laurentian Great Lakes (Grewe et al. 1994; Page et al. 2004). Phylogenetic and population genetic approaches have both been used to characterize influences of stocking on extant lake charr populations. Mitochondrial

DNA (mtDNA) combined with biogeographical expectations of the postglacial distribution of mtDNA lineages have been used to identify the presence of putatively stocked fish or their descendants (e.g., Giroux et al. 2009) and mtDNA combined with population genetic analyses based on microsatellite markers have been used to investigate patterns of genetic diversity within and among un-stocked, stocked, and putative source populations.

Typical lake charr populations of inland lakes are characterized by low within-population genetic diversity and high genetic divergence from other populations that reflect isolation after postglacial dispersal (Ihssen et al. 1988; Halbisen and Wilson 2009; Valiquette et al. 2014; Perrier et al. 2017). In contrast to expected native patterns, lake charr populations with a strong stocking history show elevated levels of within-population genetic diversity (in particular allelic richness), reduced genetic differentiation from other stocked lakes, reduced genetic differentiation from source populations used for stocking, and evidence of admixture between hatchery and native gene pools (Fig. 9; Halbisen and Wilson 2009; Valiquette et al. 2014; Baillie et al. 2015). Despite differences in stocking histories and source populations, these patterns have been consistently observed in populations from Québec, Ontario, and Vermont. For heavily influenced populations in lakes surveyed by these studies, the effects of stocking have acted to homogenize genetic diversity and erode distinct genetic signatures of original native populations compared to un-stocked populations.

Further effects of stocking have been revealed through more detailed genomic analyses of stocked populations by using genotype-by-sequencing to characterize deleterious variation (SNPs) in stocked and un-stocked lake charr populations in
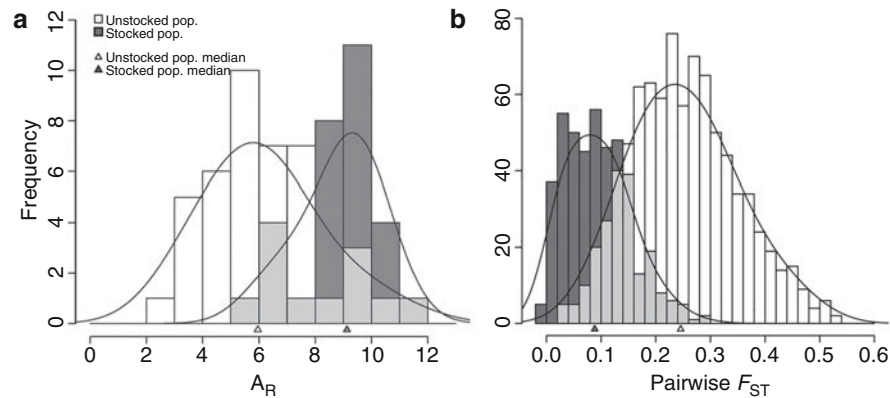
Fig. 9 Distribution of allelic richness (A_R) for un-stocked populations ($N = 42$; white bars) and stocked populations ($N = 30$; dark gray bars) of lake charr *Salvelinus namaycush* where triangles indicate median A_R value for each distribution and light gray is the overlap between the two distributions (**a**). Distribution of pairwise genetic divergence (F_{ST}) among un-stocked (N comparisons $= 861$; white bars) and among stocked populations (N comparisons $= 435$; dark gray bars) where triangles indicate median F_{ST} for each distribution and light gray is the overlapping between the two distributions (**b**). From Valiquette et al. (2014)

Québec. Deleterious variation is genetic variation that is predicted to cause an amino acid change in a functional protein that will adversely affect its function (Ferchaud et al. 2018). Negative effects of deleterious variation on populations would generally be expected to be counter-acted by stocking because stocking should reduce the effects of genetic drift as described above. Remarkably though, one source population for stocking in Québec had a high proportion of deleterious variation compared to most populations and had certain deleterious sites that were fixed (invariant) (Ferchaud et al. 2018). Thus, not only is stocking possibly eroding local adaptation through outbreeding depression, stocking is actually introducing deleterious variation into populations that previously did not exist. Future management of stocking activities should take note of this fact and screen potential source populations carefully for their suitability.

Despite the influence of stocking on genetic diversity in some lakes, variable effects of stocking on genetic diversity of recipient lake charr populations have been reported by multiple studies (Piller et al. 2005; Giroux et al. 2009; Halbisen and Wilson 2009; Valiquette et al. 2014). In some cases, heavily stocked lakes in Ontario (>100,000 released fish) appear to have resisted the effects of stocking and retained their historical genetic distinctness and lower diversity (Halbisen and Wilson 2009). In Québec, higher stocking densities and greater numbers of releases are associated with increased genetic impact but genetic effects on recipient populations are highly variable below a stocking threshold of approximately 45 fish/hectare (Valiquette et al. 2014). Populations in Saskatchewan (Giroux et al. 2009) and Wisconsin (Piller et al. 2005) have retained historical genetic signatures despite large introductions and only show residual evidence of stocking in the form of non-native mtDNA haplotypes or individuals with genotype assignment to stocking source populations. These examples represent different management jurisdictions, geographic locations, and stocking source populations suggesting variable effects of stocking on genetic diversity of lake charr populations are the norm rather than the exception.

Failure of stocking to have universal effects on the genetic diversity of recipient populations is undoubtedly linked to the adaptive suitability of stocking source populations and ecological and environmental conditions of a particular recipient lake (Halbisen and Wilson 2009; Valiquette et al. 2014). Even regionally or locally sourced populations for stocking fail to leave a signature in the genetic diversity of recipient populations in some lakes, which suggests either stocked fish do not survive or they do not contribute to reproduction (Valiquette et al. 2014). These observations may reflect important local adaptions of lake charr populations that are unique to each lake. Inland lakes represent genetically distinct systems that have been isolated since the last glacial period, and while populations variably experience negative effects of drift and inbreeding, much adaptive variation is retained (Perrier et al. 2017). Alternatively, just one generation of captive rearing is associated with reductions in fitness for other salmonids due to exposure to artificial hatchery environments (Araki et al. 2007; Christie et al. 2012). Lower fitness of stocked lake charr may thus result from either adaptive mismatch or from domestication effects associated with artificial rearing. Stocked fish generally grow faster to larger sizes than native wild fish (Morissette et al. 2018). Lakes with a native planktivorous

ecotype that are stocked with a piscivorous ecotype exhibit hybrids that outgrow either pure-type but have reduced body condition, which suggests they are mal-adapted to planktivorous type lake prey communities (Morissette et al. 2018). The results discussed above highlight the risks of outcrossing different lake charr ecotypes.

Encouragingly, preliminary evidence suggests that native populations in inland lakes can recover local genetic signatures over time after cessation of stocking. The average proportion of non-local genetic ancestry decreased from 95% in lakes with recent stocking to 20% in lakes with no stocking inputs for 15 years (Valiquette et al. 2014). These findings suggested that, once stocking has been stopped, the "wild" genetic makeup of populations that had been stocked could be reestablished, pre-sumably by purging alleles from populations contained within fish used for stocking. Longitudinal studies of these lakes are needed to confirm reductions occur, but this observation supports the interpretation that non-local genotypes have reduced fitness and provides hope for recovering local native gene pools of inland lake charr populations.

In the Laurentian Great Lakes, the effects of stocking on native genetic diversity are less clear. Human exploitation and sea lamprey predation severely reduced or eliminated native populations from the majority of the Great Lakes prior to the development of molecular genetic tools (Hansen et al. 1995). Archival samples allowed assessment of some historical genetic diversity and comparisons demon-strated that populations today have less genetic diversity than those prior to the Lake Superior population crash during the 1950s (Guinand et al. 2003, 2012; Baillie et al. 2016b). Hatchery supplementation played an important role in the recovery of these populations (Hansen et al. 1995). Only a fraction of the total lake charr genetic diversity of the Great Lakes is represented in current hatchery broodstocks (Page et al. 2004) where progenitors of stocked hatchery strains are largely derived from collections of the lean ecotype (Krueger et al. 1983).

In Lakes Michigan, Erie, and Ontario, where lake charr populations were completely extirpated, all wild production, and thus genetic diversity, is derived from previously stocked hatchery sources. In these lakes, as well as Lake Huron, proportions of wild-spawned juvenile fish attributable to hatchery strains by genetic assignment do not conform to expected proportions based on stocking rates and estimates of known-hatchery-origin adult fish present on spawning reefs (Marsden et al. 1989; Grewe et al. 1994; Page et al. 2003; Roseman et al. 2009; Scribner et al. 2018). The Seneca Lake strain out-performs other hatchery strains in these lakes (see Sect. 5.2 for a discussion of the reasons). Thus, genetic diversity in all Great Lakes, except Superior, likely reflects only a fraction of the genetic diversity that has been stocked due to the disproportionate contribution of certain strains to natural reproduction.

Lake Huron is the only lake, other than Superior, to possess remnant native populations of lake charr. These remnant native populations are restricted to two areas of Georgian Bay and are believed to have largely retained their distinct genetic signature (Guinand et al. 2003). Recent supplementation used hatchery stocks derived from these remnant populations (Iroquois Bay and Big Sound). These

locally sourced stocks appear to contribute disproportionately more to natural production than non-native stocks that were released (Stott et al. 2004; Scribner et al. 2018). Unfortunately, extensive evidence of interstrain hybridization exists in naturally produced fish in Georgian Bay, and potential fitness consequences of outbreeding depression that may result from these crosses are unknown.

Splake (F1 hybrid of lake charr and brook charr, *Salvelinus fontinalis*) were stocked in Lake Huron during the 1960s and 1970s and splake backcrossed with lake charr comprised the majority of stocking in Ontario waters of Lake Huron until the early 1990s (Eshenroder et al. 1995). The splake initially used for stocking was the result of a broodstock highly selected for several traits (e.g., rapid growth, early-maturation, and deep-swimming ability) to provide increased chances of reproducing before being killed by sea lamprey or fisheries (Tait 1970). This high level of artificial selection undoubtedly altered the genetic composition of these fish and although splake backcross fish had lower survival in matched plantings (Anderson and Collins 1995) splake backcrosses interbreeding with stocked fish from other sources and remnant wild populations comprised up to 30% of fish from certain sites in Lake Huron (Stott et al. 2004). A large proportion of lake charr in Lake Huron could have one-eighth brook charr ancestry. Consequences of this ancestry for the genetic diversity of extant lake charr are unclear, although the proportion is likely to diminish over time because these hybrids do not naturally occur and stocking of splake and their backcrosses ceased in the early 1990s, in part due to concerns about negative genetic effects (Krueger and May 1991). In contrast, splake continue to be stocked in Lake Superior, where they pose risks to both brook charr and lake charr populations (Feringa et al. 2016).

In Lake Superior, ongoing declines in genetic diversity have been observed despite population census sizes that have recovered to near pre-crash levels (see Sect. 6; Guinand et al. 2012; Baillie et al. 2016b). These declines are inconsistent with ongoing genetic drift and sampling effects and may reflect the effects of stocking (Baillie et al. 2016b). Of lean-type fish collected at two Lake Superior sites in 1995, 50–70% traced ancestry to a hatchery population rather than wild samples collected from the same sites in 1959 (Guinand et al. 2003). This result is not surprising given that most fish stocked into Lake Superior from the 1950s to the 1990s were of the lean ecotype (Krueger et al. 1983). Gene flow between ecotypes within sites and decreasing genetic differentiation between ecotypes (see Sect. 6.3; Baillie et al. 2016b) suggest that stocking may have eroded not only the native diversity of lean ecotypes but may also be affecting that of other ecotypes as well.

Despite the utility and widespread use of genotype assignment approaches for characterizing stocking influences on genetic diversity in inland lakes, Guinand et al. (2003) and Scribner et al. (2018) are the only published studies to have explicitly investigated hatchery influences in this way for any of the Great Lakes (but see Stott et al. 2004). This may be, in part due, to the low resolution among several genetically similar stocking source populations used in the Great Lakes. Improved genetic and genomic tools that provide greater resolution among hatchery stocks and interstrain hybrids as is currently being done for Lake Michigan (W.Larson, NOAA, personal communication) will improve the assessment of stocking contributions to recovering

lake charr populations. An opportunity also exists for more extensive sampling of archival samples to better characterize historical patterns of native lake charr genetic diversity and the contributions of hatchery stocks to genetic diversity of extant lake charr populations in the Great Lakes.

8   Future Directions

Throughout this chapter, we have provided suggestions for future studies to improve knowledge of various aspects of lake charr biodiversity. Ultimately, a critical need exists for improved genomic resources to support future lake charr genetic work. Here, we summarize future research and monitoring directions and possibilities contingent on rapidly emerging genomics and bioinformatics technologies of today to provide a roadmap for future genetic studies of the lake charr.

8.1   Whole-Genome Sequencing

A chromosome-level reference genome is currently being produced by an international collaboration (L. Bernatchez, unpublished data). A double haploid lake charr was generated and its genome sequenced to a coverage of approximately 90X using Pacific Biosciences (PacBio) Sequel long-read sequencing. Initial assembly of the PacBio reads generated a genome of approximately 2.3 billion base pairs (Gb) in length organized into 340 continuous sequences, which were then assembled into scaffolds using a recently published high-density linkage map (Smith et al. 2020). The current assembly is near chromosome-level, with more than 77% of the genome anchored to chromosomes and has a scaffold N50 of 39.7 million base pairs (i.e., more than half the genome is organized into pieces longer than ~40 Mb), making it a very high-quality fish genome. As of publication of this volume, polishing and annotation of the genome are in progress with its publication anticipated in 2021.

This reference genome will provide numerous opportunities to investigate the genomic and epigenomic basis of lake charr phenotypes, clarify the major ecological axes driving phenotypic and genomic divergence, and the extent to which parallel genomic changes underlie convergent phenotypes both with and among lakes. A high-quality reference genome will create possibilities for use of low-coverage whole-genome sequencing for population genomic studies. Low coverage sequencing (1–2X) provides a cost-effective solution for obtaining whole-genome data that will be especially suited to addressing many unresolved population genomic questions for the lake charr (Therkildsen and Palumbi 2017) and development of analytic approaches and software tools specifically designed for low coverage data will open new possibilities for understanding patterns of connectivity and reproductive isolation (Korneliussen et al. 2014). Additionally, low coverage sequencing is effective with small amounts of DNA (Therkildsen and Palumbi 2017) that will make it

suitable for use with archival samples to improve the resolution of temporal losses of lake charr genetic diversity and its consequences for populations in the Great Lakes.

An interesting possibility exists that large structural rearrangements (e.g., chromosomal inversions) are partially responsible for determining lake charr ecotypes. Structural variation of this kind suppresses recombination within the inverted region of a chromosome and causes the inverted region to be inherited in a large chunk that can function as a kind of "super-gene" (Wellenreuther and Bernatchez 2018). Chromosomal inversions are known to underlie important adaptive phenotypes in a wide range of organisms including some well-known ecotypic differences such as migratory and nonmigratory forms of rainbow trout and Atlantic cod *Gadus morhua* (see review by Wellenreuther and Bernatchez 2018). Currently available genomic datasets have an estimated resolution of one SNP per 450–700 Kb (i.e., ~3 Gb genome size / 4000–7000 SNPs), which suggests even reasonably sized inversions could have been missed. A reference genome and whole-genome sequencing data will allow for the characterization of structural variation and an assessment of its relevance for lake charr ecotype differentiation.

A high-quality reference genome will also support the fine-scale genomic assessment of the effects of stocking introgression on wild populations. New tools and analytic approaches based on the length and frequency of hatchery origin haplotypes have the power to improve the resolution of ancestry inference and resolve complex patterns of stocking influences (e.g., Leitwein et al. 2018). Further knowledge of deleterious genetic variation provided by a reference genome will improve management by selecting, where necessary, appropriate populations as stocking sources that closely match genetic-environmental signatures of recipient populations, while minimizing risks of introducing deleterious variation (Ferchaud et al. 2018).

8.2 Common Garden Experiments and Transgenerational Plasticity

The mechanistic basis of lake charr phenotypic diversity remains unresolved. While compelling evidence exists for a heritable basis for certain phenotypic traits, a role for transgenerational plasticity has not been ruled out. Multigenerational breeding experiments with controlled environmental conditions will be essential to assess the influence of transgenerational plasticity on lake charr phenotypes. These experiments would simultaneously provide an opportunity to conduct genome-wide association studies for important traits. Divergence at multiple sites within Lake Superior (Perreault-Payette et al. 2017) and throughout inland lakes (Bernatchez et al. 2016) allows replication across sites when investigating a shared genomic basis for convergent phenotypes.

The role of epigenetic mechanisms (e.g., DNA methylation, histone modifications) in facilitating transgenerational plasticity is an emerging area of research that holds great promise for understanding the mechanistic basis of lake charr ecotypes.

Epigenetic mechanisms play important roles in regulating gene expression and are especially important during development and tissue differentiation (Jones 2012). While many epigenetic changes are genetically encoded, they can also be altered in response to different environmental conditions and persist through an organism's life (Metzger and Schulte 2017). In exceptional cases, epigenetic variation has been shown to be stably transmitted over multiple generations and provides a mechanism that environmental influences can be transmitted across generations (Klosin et al. 2017). Epigenetic variation thus has the capacity to regulate environmentally-induced adaptive phenotypic variation and meets requirements to be acted on by natural selection, thereby suggesting it could facilitate rapid adaptation to environmental change (Rey et al. 2016). Growing evidence shows that epigenetic mechanisms are involved in the adaptive phenotypic variation of fishes. Recent work has identified epigenetic differences between migratory ecotypes of rainbow trout (Baerwald et al. 2016) and epigenetic divergence was found even when no divergence was identified at genetic markers between hatchery and wild populations of both coho salmon *Oncorhynchus kisutch* and rainbow trout (Le Luyer et al. 2017; Gavery et al. 2018).

A conceptual model for the origin of lake charr ecotypes could reasonably involve both genomic and epigenomic mechanisms. Given differential gene expression between lake charr ecotypes (Goetz et al. 2010) and within-ecotype morphological trait variation along depth gradients (Baillie et al. 2016a), divergence of lake charr ecotypes may first occur as a result of epigenetic changes caused by different developmental or rearing environments. For example, pressure or temperature effects during incubation (Ryu et al. 2018), or morphologically plastic responses due to different diets like those observed in cichlid fishes (Gunter et al. 2013) could be maintained by transgenerational plasticity or result from natural selection acting on genomic variation to fix these traits through a process called "genetic accommodation" (West-Eberhard 2003). The relative importance and interactions between each of these mechanisms will be of great interest in decoding the origins of lake charr ecotypes.

A reference genome for lake charr will facilitate whole-genome sequencing techniques to characterize epigenetic variation (e.g., bisulfite sequencing for methylation, chromatin-immunoprecipitation sequencing for histone modifications) and assess their contribution to ecotype divergence. Further expanding this work to include convergent phenotypes from other systems will enhance the ability to characterize the extent of genomic or epigenomic parallelism across the geographic range of the lake charr. Integrative work that compares the mechanistic basis of phenotypic parallelism across systems is sorely lacking from the lake charr literature. In particular, recently diverged phenotypes of lake charr (e.g., Flathead Lake, Montana) and lakes where divergent phenotypes exist but genetic differentiation is lacking (e.g., Rush Lake, Lake Mistassini) will be particularly important for contrasting the relative importance of selection, gene flow, and plasticity to phenotype divergence.

8.3 Archival Samples as Baseline for Contemporary Levels of Genetic Diversity

Investigations into the loss of genetic diversity in lake charr would not have been possible without archived scale collections (e.g., United States Geological Survey, United States Fish and Wildlife Service, and Ontario Ministry of Natural Resources and Forestry). Such archives remain a valuable resource for additional samples (Baillie et al. 2016b). Historical samples provide a reference point from which contemporary levels of genetic diversity can be interpreted (Bouzat 2001; Matocq and Villablanca 2001). In 2016, Baillie et al. (unpublished) were successful in genotyping MHC (using next-generation Illumina sequencing technologies) from archival scale samples dating back to 1948, which demonstrates the use of archival samples as a plausible tool for genetics studies that aim to investigate historical genetic patterns in the lake charr.

One potential limitation is that certain SNPs are more sensitive to DNA degradation and thus older samples may have lower genotyping success (Johnston et al. 2013). For example, accurate allele frequency estimation at these loci may require a greater number of individuals (Johnston et al. 2013). This could pose a problem if archival collections have limited numbers of samples. Nevertheless, historical samples have enormous potential to provide insight into the dynamics of lake charr genetic diversity in the Great Lakes. Genomic characterization of lake charr archival samples has yet to be done but will provide a clearer picture of historical patterns of native lake charr genetic diversity within and among sampling locations and depths.

Studies that use archival samples of lake charr will allow assessment of the contributions of hatchery stocks to the genetic diversity of extant lake charr populations and a more detailed assessment of demographic changes through time for lake charr populations in Lake Superior. Knowledge of historical patterns of genomic diversity throughout the Great Lakes will be important for informing recovery and re-introduction of lake charr throughout the Great Lakes.

8.4 eDNA

The environmental DNA (eDNA) field is a revolutionary cross-disciplinary area of biological science that uses genetic material, shed by living organisms, extracted from environmental samples, such as water, to determine organism presence and other population parameters (Lodge et al. 2012). The rapid expansion of eDNA technologies has generated an unprecedented ability to detect species and conduct genetic analyses. Therefore, careful inclusion of eDNA in studies of fishery resources can enhance the understanding and sustainability of aquatic resources and ecosystems. Exciting developments have occurred in the use of eDNA technology to assess lake charr populations. Lake charr abundance could be predicted simply by quantifying lake charr DNA from a water sample, which opens up

possibilities to assess and monitor population status in more lakes with less effort than conventional methods (Lacoursière-Roussel et al. 2016). Further development and validation of these kinds of approaches have the potential to revolutionize lake charr management.

9 Conclusions

The past, present, and future research discussed in this chapter contributes to an evolving framework that integrates molecular ecology studies into fishery management practices and permits insight into the evolutionary potential of the lake charr for managers and conservation programs seeking to re-establish declining or extirpated populations. Lake charr survived the last glacial maximum in at least five separate refugia and dispersed north and east through large proglacial lakes that followed the melting ice (Wilson and Mandrak 2021). As levels of these large proglacial lakes dropped, lake charr became distributed across smaller lakes that remained. With some exceptions, most of these populations have since been isolated for thousands of years. Finite population sizes in postglacial lakes and a lack of connectivity led to significant genetic drift and deep neutral divergence among inland lake charr populations. Low genetic diversity, and for some populations elevated inbreeding, suggests many populations may have difficulty adapting to changing climate.

Many inland populations, especially those near human habitation and recreation areas, have been stocked. For these populations, the genetic legacy of past stocking is obvious, and studies have indicated stocking negatively affected native populations, particularly where one ecotype (e.g., piscivorous) was stocked into a lake with only the opposite ecotype (e.g., planktivorous). However, genetic evidence also suggests that stocking effects are probably transient and populations are resilient to genetic changes in most lakes. Studies reviewed in this chapter highlight the importance of using molecular genetic tools to screen potential source populations for future supplementation or enhancement stocking. The choice of source populations should prioritize local sources that experience similar climatic and ecological variation, have similar patterns of genetic variation, and low levels of deleterious genetic variation.

In large lakes (e.g., Laurentian Great Lakes, Great Bear and Great Slave Lakes, Lake Mistassini), inconsistent support occurs for genetic differentiation of lake charr ecotypes. Furthermore, studies reviewed in this chapter challenged the traditional view of lake charr ecotypes and demonstrated that divergence along ecological gradients better explains contemporary patterns of genetic diversity. Open and important questions for understanding ecotypic variation in lake charr include identifying ecological forces responsible for generating within and among ecotype phenotypic diversity and genomic mechanisms that facilitate this divergence. Despite inconsistent support for genetic differentiation of ecotypes, strong evidence exists for heritability of many ecotypic traits. Emerging whole-genome sequencing

datasets and experimental evidence for the role of plasticity in ecotype defining traits will reveal important information about the origins of lake charr ecotypes that will improve the management of ecotype diversity.

Temporal loss of genetic diversity from Lake Superior populations, combined with reduced genetic differentiation among ecotypes, suggests that fisheries and invasive species (sea lamprey predation) reduced population sizes that led to a breakdown of reproductive barriers among ecotypes. Populations sizes have since stabilized, stocking has ceased, and sea lamprey is well controlled, so conditions for re-diversification now exist. Ongoing efforts to restore lake charr throughout the rest of the Great Lakes basin should focus on introducing deep-water ecotypes, consistent with those that are known from historical records, to speed recolonization of these habitats and realize the full range of lake charr diversity in these lakes.

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