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Investigating the extent of parallelism in morphological and genomic divergence among lake trout ecotypes in Lake Superior

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Abstract

Understanding the emergence of species through the process of ecological speciation is a central question in evolutionary biology which also has implications for conservation and management. Lake trout (*Salvelinus namaycush*) is renowned for the occurrence of different ecotypes linked to resource and habitat use throughout North America. We aimed to unravel the fine genetic structure of the four lake trout ecotypes in Lake Superior. A total of 486 individuals from four sites were genotyped at 6822 filtered SNPs using RADseq technology. Our results revealed different extent of morphological and genetic differentiation within the different sites. Overall, genetic differentiation was weak but significant and was on average three times higher between sites (mean F_{ST} = 0.016) than between ecotypes within sites (mean F_{ST} = 0.005) indicating higher level of gene flow or a more recent shared ancestor between ecotypes within each site than between populations of the same ecotype. Evidence of divergent selection was also found between ecotypes and/or in association with morphological variation. Outlier loci found in genes related to lipid metabolism and visual acuity were of particular interest in this context of ecotypic divergence. However, we did not find clear indication of parallelism at the genomic level, despite the presence of phenotypic parallelism among some ecotypes from different sampling sites. Overall, the occurrence of different levels of both genomic and phenotypic differentiation between ecotypes within each site with several differentiated loci linked to relevant biological functions supports the presence of a continuum of divergence in lake trout.

Keywords: ecological speciation, local adaptation, morphometrics, population genomics, RADseq, salmonid

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Introduction

The study of diversification and ultimately speciation is central to evolution and relevant for conservation biology (Weissing *et al.* 2011). The most common and established mechanism of speciation is divergence in allopatry, where spatial and geographical barriers prevent gene flow, thus allowing genetic incompatibilities to accumulate, subsequently resulting in reproductive isolation following secondary contact (Coyne & Orr 2004; Tittes & Kane 2014). Some examples of allopatric

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isolation mechanisms in fishes include the glacial cycles in North America responsible for the origin of many freshwater species (April *et al.* 2013), the rise and fall of Lake Tanganyika, and the barriers created by high water flow in large rivers such as the Amazon or Congo River (reviewed in Bernardi 2013). However, a geographic barrier is not always needed and speciation can emerge in sympatry, or in parapatry despite high gene flow, by divergent selection on ecologically important traits (Gavrilets *et al.* 2007; Tittes & Kane 2014). Divergent selection on ecological traits can be caused by biotic and abiotic influences where adaptations to different environments or ecological niches result in the emergence of reproductive incompatibilities (Nosil *et al.* 2009; Bernardi 2013). The latter may create a continuum of divergence from continuous variation within a single gene pool, to ecotype formation and finally to complete differentiation and reproductive isolation (Lu & Bernatchez 1999; Hendry 2009; Nosil *et al.* 2009; Gagnaire *et al.* 2013). Models and case studies have shown that sympatric speciation is possible under gene flow when few loci underlying the divergent trait undergo strong selection, whereas gene flow homogenizes the rest of the genome (Gavrilets *et al.* 2007; Franchini *et al.* 2013).

Ecological speciation has been extensively documented in several geologically young fish species living in sympatry. For instance, sympatric speciation has occurred in Midas cichlids (*Amphilophus* spp.) (Franchini *et al.* 2013), Lake Victoria cichlids (Wagner *et al.* 2013) but more commonly in several temperate freshwater fishes such as stickleback (*Gasterosteus* spp.), smelt (*Osmerus* spp.) and especially in salmonids such as whitefish (*Coregonus* spp.), trout (*Salmo* spp.), Pacific salmon (*Oncorhynchus* spp.) and charrs (*Salvelinus* spp.) (Taylor 1999; Jonsson & Jonsson 2001). Sympatric speciation is usually linked to trophic polymorphism in which intraspecific ecotypes use different habitats and resources (Smith & Skúlason 1996; Blackie *et al.* 2003; Hansen *et al.* 2012). Trophic polymorphism is common in postglacial lakes where retreat of the ice sheet creates unoccupied niches and opportunities for intraspecific competition (Blackie *et al.* 2003; Zimmerman *et al.* 2009). These conditions are believed to be responsible for the extensive radiation in North American freshwater fishes where several species are adapted to different ecological niches (Schluter 2001). Parallel evolution of shared phenotypic traits linked to trophic resource use has been demonstrated in several postglacial systems. These shared morphological traits between populations can be accompanied by shared genetic architecture underlying the ecologically important traits or can arise from independent genetic processes (Ralph & Coop 2014). For example, the repeated divergence of marine and freshwater stickleback exhibiting similar phenotypic changes in body armour has been described and the repeated reduction in armour plates was found to be controlled by the same set of loci (Colosimo *et al.* 2005; Jones *et al.* 2012). On the other hand, convergent phenotypic traits may not always be controlled by similar developmental pathways as is the case for cavefish (*Astyanax* spp.), beach mice (*Peromyscus polionotus*) and fruit fly (*Drosophila* spp.) (reviewed in Arendt & Reznick 2008; Bernatchez 2016). For instance, the evolution of parallel phenotypic divergence between benthic normal and limnetic dwarf whitefish (*Coregonus* spp.) in several North American lakes was found to be only partially associated with parallelism at the genome level (Gagnaire *et al.* 2013; Laporte *et al.* 2015).

Lake trout (*Salvelinus namaycush*) are renowned for the occurrence of different ecotypes linked to resource and habitat use throughout North America. In small lakes, lake trout diverge mainly into a planktivorous and piscivorous ecotype (Vander Zanden *et al.* 2000; Bernatchez *et al.* 2016), whereas several large lakes harbour at least four ecotypes associated with differential resource partitioning (Muir *et al.* 2015). For instance, four different ecotypes occur in Great Bear Lake and Lake Superior, three in Great Slave Lake and two in Lake Mistassini and Rush Lake (Muir *et al.* 2015). In Lake Superior, four distinct ecotypes have been reported that are recognized based on differences in morphology and coloration but also in life history traits, physiology and ecology (Muir *et al.* 2015). For instance, they differ in traits such as growth rate, asymptotic length and weight, size at sexual maturity, as well as developmental rate of fertilized eggs or fry. They also differ in physiology such as buoyancy and swim bladder retention (Muir *et al.* 2015; Hansen *et al.* 2016). The 'lean' ecotype has a slender, streamlined body with low body lipid content, and occupies shallow waters where it preys upon pelagic fishes (Burnham-Curtis & Smith 1994; Moore & Bronte 2001; Bronte *et al.* 2003; Zimmerman *et al.* 2009; Goetz *et al.* 2011). The 'humper' ecotype inhabits offshore, mid-water shoals, feeds on small prey and is sexually mature at relatively smaller sizes (<500 mm) (Burnham-Curtis & Smith 1994; Stafford *et al.* 2014; Hansen *et al.* 2016). It also has a small head with moderately large eyes dorsally positioned and short-paired fins (Moore & Bronte 2001; Bronte *et al.* 2003; Zimmerman *et al.* 2009). The 'siscowet' ecotype is recognized by its sloping snout, moderately large eyes and high body fat content, which may facilitate diel vertical migration to follow the migration of ciscoes (Burnham-Curtis & Smith 1994; Bronte *et al.* 2003; Bronte & Sitar 2008; Ahrenstorff *et al.* 2011; Hansen *et al.* 2012; Hrabik *et al.* 2014). Lastly, the 'redfin' ecotype has a robust body, a large head, a long deep peduncle and large fins (Muir *et al.* 2014). Several

hypotheses have been proposed to explain the origin of these ecotypes (Wilson & Mandrak 2004; Eshenroder 2008). These could be the result of developmental plasticity in which a single genotype expresses different phenotypes matching selection optima or can be genetically based or a mix of both (Goetz *et al.* 2010). While this does not rule out a role for developmental plasticity, two lines of evidence suggest some genetic basis for the phenotypic differences observed between the ecotypes. First, progeny from wild lean and siscowet gametes have been raised in a common garden experiment and key phenotypic features that differentiate wild leans and siscowets such as condition factor, morphology and lipid content were maintained (Goetz *et al.* 2010). Furthermore, the same study uncovered transcriptional differences in lipid-related genes between the two ecotypes (Goetz *et al.* 2010). Second, morphological differences in the operculum and supraethmoid bones have been documented between leans, siscowets and humpers. Cranial bones are of taxonomic significance in salmonids and are unlikely affected by environmental conditions and ontogenic shifts (Burnham-Curtis & Smith 1994).

Lake trout (*Salvelinus namaycush*) were once the dominant predator in the Great Lakes. It historically supported one the most important freshwater commercial fisheries before being extirpated in the 1950s in all lakes except Lake Superior, where it is now considered restored and Lake Huron, where recruitment has been increasing (Riley *et al.* 2007), but it remains at relatively low abundance (Bronte *et al.* 2003; Zimmerman & Krueger 2009). The collapse of lake trout populations has been associated with anthropogenic factors, including habitat degradation, pollution and overfishing, as well as predation by invasive sea lamprey following the construction of navigation canals (Page *et al.* 2003, 2004; Bronte & Sitar 2008). A review by Zimmerman & Krueger (2009) examined impediments to its recovery or restoration and provided guidelines to maintain, increase or re-introduce lake trout populations in the Laurentian Great Lakes. Here, understanding and evaluating genetic structure and diversity of remaining lake trout population was identified as a key research topic.

The general goal of this study was to gain insight into the nature and origin of the different lake trout ecotypes in Lake Superior. More specifically, we aimed to (i) investigate the extent of both morphological and genomewide genetic differentiation and connectivity among the four lake trout ecotypes from different geographic locations within the lake, (ii) identify possible adaptive genetic differentiation among ecotypes by means of genome scans and genotype–phenotype associations and (iii) examine the degree of parallelism at the phenotypic and genotypic levels among ecotypes from the four sampling sites. To achieve this, we used RADseq to genotype lake trout from the four ecotypes and from four sites from Lake Superior. In parallel, geometric morphometric analyses were performed on head and body shapes.

Methods

Sampling

Fish from the four lake trout ecotypes were sampled in 2013–2014 from four sites in Lake Superior; Big Reef (2014), Stannard Rock (2013–2014), Superior Shoals (2013) and Isle Royale (2013) (Fig. 1, Table 1). For the first three sites, a nylon gill net, 183 m long by 1.8 m high, was used with 30.5-m-long panels of different mesh sizes (50.8–114.3 mm). Nets were deployed for 24 h at different depth ranges (0–50 m, 50–100 m and >100 m) approximately representing preferred depths of the different ecotypes. A picture of each fish was taken following the protocol in Muir *et al.* (2012), and a biopsy of either the adipose or pectoral fin was collected and preserved in 95% ethanol. The fourth site, Isle Royale, was sampled in 2013 using overnight sets of 274- to 823-m-long gill nets with nine panels (91.4 m long by 1.83 m high) of single mesh size (5.1, 6.4, 7.6, 8.9, 10.2, 11.4, 12.7, 14.0, 15.2 cm). Pictures were taken using the same protocol (Muir *et al.* 2012), and liver or gonads were conserved in RNA Later. Samples without pictures or genetic material were removed from subsequent analyses. Information about total length (mm), wet weight (g) and sex, and depth of capture was recorded for each sampled individual.

Ecotype assignment

Consensus of both morphometric analyses (body and head) and visual identification as visual interpretation of fish pictures by lake trout experts (see Acknowledgements) was used to assign an ecotype to each fish per Muir *et al.* (2014). Fish less than 430 mm long with the exception of humper-like fish, which are <430 mm when sexually mature, were excluded to remove the confounding effect of ontogenetic shifts in morphology (Zimmerman *et al.* 2009). Body and head were analysed separately to distinguish locomotion (body) from feeding habit (head). In addition, morphometric analyses were conducted separately for each site to investigate morphological variation among sites. Landmarks and semilandmarks were digitized and analysed with the Thin Plate Spline suite (TPS; State University of New York at Stony Brook; http://life.bio.sunysb.edu/morph). First, for each fish a rectangular grid was overlaid to identify belly curvature corresponding to

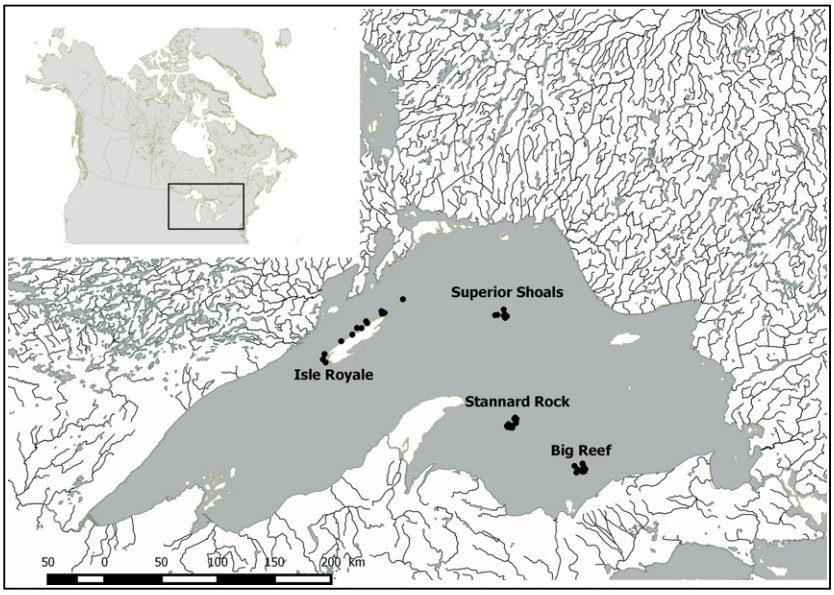

Fig. 1 Map of Lake Superior sampling sites; Isle Royale, Superior Shoals, Stannard Rock and Big Reef. Circles correspond to sampling locations for each site.

Table 1 Sampling site information and consensus analysis of body shape, head shape and visual identification

Sites	Year	Coordinates	*N*		Lean-like	Siscowet-like	Humper-like	Redfin-like	No consensus	Total
Big Reef	2014	46°46,545°N	132	Consensus	$39^{B,H,V}$	$46^{B,H,V}$	8^{V}	17^{V}	13	123
		86°28,378°W		Chosen	39	40	8	17		104
Stannard Rock	2013-2014	47°11,450°N	362	Consensus	$63^{B,H,V}$	$66^{B,H,V}$	24^{V}	24^{V}	40	217
		87°11,531°W		Chosen	40	40	24	24		128
Isle Royale	2013	47°21,550°N	214	Consensus	$55^{B,H,V}$	$37^{B,H,V}$	$35^{H,V}$	$33^{H,V}$	42	202
		88°30,497°W		Chosen	40	37	34	33		144
Superior Shoals	2013	48°02,464°N	394	Consensus	$35^{B,H,V}$	$133^{B,H,V}$	11^{V}	$62^{H,V}$	74	315
		87°07,536°W		Chosen	31	41	11	42		125

Number of fish sampled per sampling sites (*N*), year of collection and coordinates is provided. Ecotypes were identified by consensus analysis of body shape (B) and/or head shape (H) and/or visual identification (V). Fish for subsequent genetic analysis were chosen based on ecotypes consensus. Fish <430 mm long were removed prior to the analysis.

20–30–40–50% of body length using the program REVIT (Autodesk) (Fig. S1a, Supporting information). The body grid was anchored at the tip of the snout and the mid-point of the hypural plate. Second, 16 homologous landmarks and four semilandmarks were digitized on each fish with the program TPSDIG2 and semilandmarks were slid using TPSUTIL (Fig. S1a, Supporting information). Semilandmarks were used to represent belly curvature, which is known to be distinctive between the two major ecotypes, leans and siscowets (Muir *et al.* 2014). Similarly, a squared grid was overlaid on each fish head dividing it into 10 equally spaced sections using the program REVIT (Fig. S1b, Supporting information). The head grid was anchored at the tip of the snout and the posterior edge of the opercula. Eight homologous landmarks and 20 semilandmarks were digitized on each fish head with the program TPSDIG2 and semilandmarks were slid using TPSUTIL (Fig. S1b, Supporting information). Distortions from rotation and size were removed by the program TPSRELW producing partial warps scores which are size-free variables. A principal component analysis (PCA) was performed to reduce the number of morphometric variables or scores and extract divergent morphometric patterns. Subsequently, relevant axes were supplied to a Bayesian clustering analysis implemented in the R package MCLUST v.4. MCLUST is a normal mixture modelling for model-based cluster analysis, classification and density estimation that include the Bayesian information criterion (BIC) for model selection and that do not require

priori information about groups such as discriminant function analysis (Fraley & Raftery 2012). Components accounting for more than 65% of the variance were supplied to the MCLUST algorithm. The best model (with highest BIC) was the one able to separate leans from siscowets as they are the most morphologically differentiated ecotypes (Fraley & Raftery 2012; Muir *et al.* 2014). Group classification resulting from the chosen model was retrieved for each individual. The visual identification of each collected fish from Big Reef, Stannard Rock and Superior Shoals was conducted by visual consensus of three trained biologists. Visual identification of Isle Royale fish was provided by an experienced biologist. An ecotype was assigned to each fish based on the consensus from body shape, head shape and visual identification. Two of three similar ecotype assignments were needed to assign to each fish a particular ecotype. In the case of different head, body and visual assignments, the fish were assigned 'no consensus' and removed from subsequent analyses. Fish for subsequent genetic analyses were chosen as follows: (i) fish with 100% consensus having the lowest group uncertainty and (ii) fish with 2/3 consensus having the lowest group uncertainty. However, if no individual of a given ecotype was identified based on morphometric analysis, the visual identification only was used and taken into account in subsequent analyses as ecotypes differ in several life history traits (e.g. size and age at maturity, colour) that are not taken into account in morphometric analyses but that are still used commonly by local expert fishery biologists to distinguish ecotypes.

Morphometric analysis

Two multivariate analyses were used to test for morphological differences between the four ecotypes at the four sites and to investigate among site differences for the same ecotype. First, a principal component analysis (PCA) was performed to reduce variable dimensionality, and components explaining most of the variance were selected based on the broken stick method. Then, a multivariate analysis of variance (MANOVA) was conducted in R (package STATS) on the selected components. As partial scores derived from a configuration that included semilandmarks do not have the same number of free variables as degrees of freedom, a requisite of MANOVA, a between-group analysis (group-PCA) implemented in the R package MORPHO was conducted on partial warps (Webster & Sheets 2010). This analysis takes into account uneven group size and does not require normality or homogeneity of variance (Mitteroecker & Bookstein 2011). The Euclidean distance between group mean was tested using 10 000 permutations. For both analyses, the effects of the sampling site, sex and ecotype were tested.

Sample DNA extraction and sequencing

Genomic DNA was extracted from individuals representing each ecotype at the four sites using a salt-extraction protocol adapted from Aljanabi & Martinez (1997). Sample quality and concentration were checked on 1% agarose gels and using the NanoDrop 2000 spectrophotometer (Thermo Scientific). Each individual's genomic DNA was normalized to 20 ng/μl of 10 μl (200 ng total) using PicoGreen (Fluoroskan Ascent FL, Thermo Labsystems) in 96-well plates. The ddRAD libraries were constructed and sequenced on the Ion Torrent Proton platform (IBIS, Laval University) following the protocol in Mascher *et al.* (2013). Briefly, restriction digest buffer (NEB4) and two restriction enzymes (*PstI* and *MspI*) were added to each sample. Digestion was completed by incubation at 37°C for two hours, and enzymes were inactivated by incubation at 65°C for 20 min. Two adaptors (one unique to each sample and the second common) were added to each sample, and ligation was performed using a ligation master mix followed by the addition of T4 ligase. The ligation reaction was completed at 22°C for 2 h followed by 65°C for 20 min to inactivate the enzymes. Samples were pooled in 48-plex and cleaned up using QIAquick PCR purification kits. The library was then amplified by PCR and sequenced on the Ion Torrent Proton P1v2 chip. The detailed methods for SNP identification, SNP filtering and genotyping using STACKS v.1.32 (Catchen *et al.* 2011) are presented in Supporting information. Resulting VCF was converted to various formats necessary for other programs using PGDSPIDER 2.0.7.2 (Lischer & Excoffier 2012) and VCFTOOLS (Danecek *et al.* 2011).

Genetic diversity and differentiation

We first estimated pairwise population differentiation using Weir's and Cockerham's estimator of pairwise F_{ST} (Weir and Cockerman 1984) in GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004) with 10 000 permutations. Similarly, measures of observed (H_o) and expected heterozygosity (H_e) and inbreeding (F_{IS}) were estimated using GENODIVE 2.0b23b. Effective population size (N_e) and number of polymorphic loci (N) for each sampling site were estimated using the program NEESTIMATOR v.2.01 (Do *et al.* 2014). Briefly, the program was run with the linkage disequilibrium model, the random mating system and a critical value of 0.05 (Pcrit) to exclude alleles that occur in only a single copy in the sample. Genomewide diversity (π) and the increase in individual homozygosity relative to mean Hardy–

Weinberg expected homozygosity (F_h) were estimated for each site with the data set prior to filtration using the R package *stackr* (Gosselin & Bernatchez 2016) (https://github.com/thierrygosselin/stackr). Lastly, an analysis of molecular variance (AMOVA) was conducted to quantify the proportion of genetic variance explained by sites relative to that explained by variation among the four ecotypes (Meirmans & Van Tienderen 2004). The AMOVA was run with two different levels of hierarchical subdivision: first with sites nested within ecotypes and then ecotypes nested within sites. A total of 10 000 permutations were used to access significance and an infinite allele model was chosen. Because AMOVA does not allow missing data, missing values were replaced by randomly selecting alleles proportional to total allele frequency in GENODIVE 2.0b23. A Mantel test between genetic divergence (F_{ST} matrix) and phenotypic divergence (head and body Euclidean distances matrices) was conducted using the R package VEGAN (Oksanen *et al.* 2016) to assess the extent of association/parallelism in morphology and genetic among ecotypes and sampling sites.

Population clustering

Population clustering and connectivity were estimated with the program ADMIXTURE 1.23 (Alexander *et al.* 2009). This program estimates ancestry in a model-based manner where individuals are considered unrelated and allows choosing the best number of possible genetic groups present in the data based on a cross-validation procedure. The program was run with values of K ranging from 1 to 20. A population tree was built using the program TREEFIT (Kalinowski 2009) and visualized with the program FIGTREE v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Genetic distances were calculated using θ (Weir & Cockerham 1984) between each pair of population and the neighbour-joining (NJ) distance-based method was used for tree construction. Support for each branch was assessed by bootstrapping using 1000 permutations (Kalinowski 2009).

Population assignment

Population assignment was conducted to investigate the power to classify an unknown individual to either a sampling site or an ecotype. This analysis was run using GENODIVE 2.0b23 with the home likelihood criteria (the likelihood that an individual comes from the population where it was sampled), which is more appropriate when only part of all possible source population have been sampled (Meirmans & Van Tienderen 2004). A significance threshold of 0.05 was applied and zero frequencies were replaced by 0.005 as suggested by Meirmans & Van Tienderen (2004). To avoid bias due to the calculation of allele frequencies from the same individuals which are subsequently assigned, the program uses the leave-one-out (LOO) validation procedure in which a targeted individual is removed from its source population before calculation of the allele frequency. For this analysis, missing values were replaced by randomly picking alleles from the global allele pool. All loci were used for this analysis such that no correction was necessary to avoid high grading bias associated with using a subset of markers based on their ranking of level of differentiation (Anderson 2010).

Outlier detection and phenotype–genotype associations

We used two different types of approaches to detect outlier SNPs potentially under divergent selection between ecotypes and sites: (i) genome scans performed among the different ecotypes and/or sites; and (ii) association tests between genotypes and continuous sphenotypic values.

For the first approach, two different methods were used to detect outlier SNPs potentially under divergent selection (i) among the four sites (individuals from the different ecotypes were pooled), (ii) among the four ecotypes (individuals from the different sites were pooled) and (ii) among the four ecotypes within each site, independently. First, the program BAYESCAN v1.2 was used to detect outliers based on locus-specific F_{ST} with a prior odd of 10 000 and a false discovery rate (FDR) of 0.05. BAYESCAN was run with 5000 iterations and a burn-in length of 100 000 as recommended by Foll and Gaggiotti (2008). Second, the program LFMM (Latent Factor Mixed Models) from the R package LEA was used to detect outliers based on allele frequencies exhibiting significant statistical association with selected phenotypes. Categorical variables were coded as orthogonal matrices on which a principal component analysis was applied and resulting scores were supplied to the LFMM analysis. LFMM was run with five repetitions, 10 000 cycles and 5000 burn-in as recommended by Frichot & François (2015). *P*-values were adjusted from their distribution and possible associations corrected for population structure detected from the admixture analysis as suggested by Frichot & François (2015).

For the second approach, phenotype–genotype associations were analysed with LFMM. This technique can uncover subtle changes in allele frequencies (such as expected in polygenic selection) that are not detected in traditional outlier analyses (Rellstab *et al.* 2015). LFMM was run with the same parameters stated in the previous paragraph with ten repetitions including the *P*-value adjustment, an FDR of 0.05 and a correction for

population structure based on the admixture analyses. The phenotypic variables were the principal components scores for each individual that explain most of the variation for head and body shapes based on the broken stick method.

Gene ontology

Loci potentially under selection detected by either of the different approaches (BAYESCAN and LFMM) were blasted against the rainbow trout genome (*Oncorhynchus mykiss*) (Berthelot *et al.* 2014) to determine possible functions with the following parameters: an e-value threshold of 1e-6, a word size of 11 bp and a max target of 100 bp. Resulting loci were filtered based upon three criteria: the number of similar hits, the bit score and sequence length. First, loci with only one hit and having ≥50 bp long were kept. Second, loci with multiple hits having the first best hit ≥20 bit score higher than the second best hit with sequence length ≥50 bp were kept.

Results

Ecotype identification and morphometric analyses

Based upon consensus analysis between head, body and visual identification, an ecotype was assigned to each fish. First, the best model for each site that distinguished, at least, between leans and siscowets with BIC values and mean uncertainties was used for ecotype assignment (Table S1, Supporting information). For each site, fish to be genotyped were chosen from the consensus identification (Table 1). If some ecotypes were not distinguished by the morphometric analysis from either the head or body shape, expert visual identification from these fish was used based upon the presence of life history traits divergence as stated in the Methods section (Muir *et al.* 2015). Based on the broken stick method, the first four PCs were retained for body shape and the first six PCs were retained for head shape corresponding to 70% and 81% of total variance, respectively, to conduct the multivariate analysis of variance (MANOVA). First, the overall shape difference between ecotypes was assessed by pooling similar ecotypes from the four sites. For the head shape, the ecotypes, the sites and the sex were significantly different ($P < 0.001$). Interactions between ecotypes and sex ($P < 0.01$) or sites ($P < 0.001$) were also significant. Similar results were observed for body shape ($P < 0.001$) (Table 2). The group-PCA revealed the same pattern for the head and body shapes except that no difference between sexes was detected (Table S2, Supporting information). The first axes of the group-PCA for head shape explained 56.2% of the variance and discriminated siscowets from leans, whereas the second axis explaining 15.9% of the variance discriminated humpers from redfins (Fig. 2a). For body shape, the first two axes of the group-PCA explained 65.5% and 14.5% of the variance and mainly distinguished leans from siscowets (Fig. 2b). In both head and body analyses, the third and fourth axes discriminated lake trout more by sampling sites than ecotypes (Fig. 2c,d). Ecotypes were not significantly different within all sites, either based on morphometric analyses of head or body shape, but yet could be differentiated by visual inspection (Fig. 2a,b and 3b, Table S3, Supporting information). Within Big Reef, only leans differed from siscowets and redfins in terms of both head and body shapes ($P < 0.05$). Within Isle Royale, head and body shapes differed between all four ecotypes ($P < 0.05$) with two exceptions; humpers did not differ from leans in body shape and leans did not differ from redfins in head shape. Within Stannard Rock, leans differed from siscowets and redfins in both head and body shapes ($P < 0.05$). Finally, within Superior Shoals, siscowet body shape differed from all other ecotypes ($P < 0.05$), except for head shape which was

Table 2 MANOVA on body and head shape to investigate the effect of the ecotype, the site of origin, the sex and all interactions

	Body						Head					
Variables	d.f.	Pillai	Approx F	Num d.f.	Den d.f.	Pr (>F)	d.f.	Pillai	Approx F	Num d.f.	Den d.f.	Pr (>F)
Ecotype	3	0.59616	28.7686	12	1392	**<2.2 e−16**	3	0.79729	27.871	18	1386	**<2.2 e−16**
Site	3	0.43694	19.7752	12	1392	**<2.2 e−16**	3	0.90345	33.181	18	1386	**<2.2 e−16**
Sex	1	0.10732	13.8857	4	462	**1.052e−10**	1	0.10040	8.556	6	460	**7.883 e−09**
Ecotype: Site	9	0.47419	6.9486	36	1860	**<2.2 e−16**	9	0.52612	4.966	54	2790	**<2.2 e−16**
Ecotype: Sex	3	0.05388	2.1214	12	1392	**0.01339**	3	0.11061	2.948	18	1386	**3.28 e−05**
Site: Sex	3	0.04189	1.6427	12	1392	0.07412	3	0.04268	1.111	18	1386	0.3344
Ecotype: Site: Sex	9	0.07982	1.0520	36	1860	0.38565	9	0.12499	1.099	54	2790	0.2891

Significant variables are in bold.

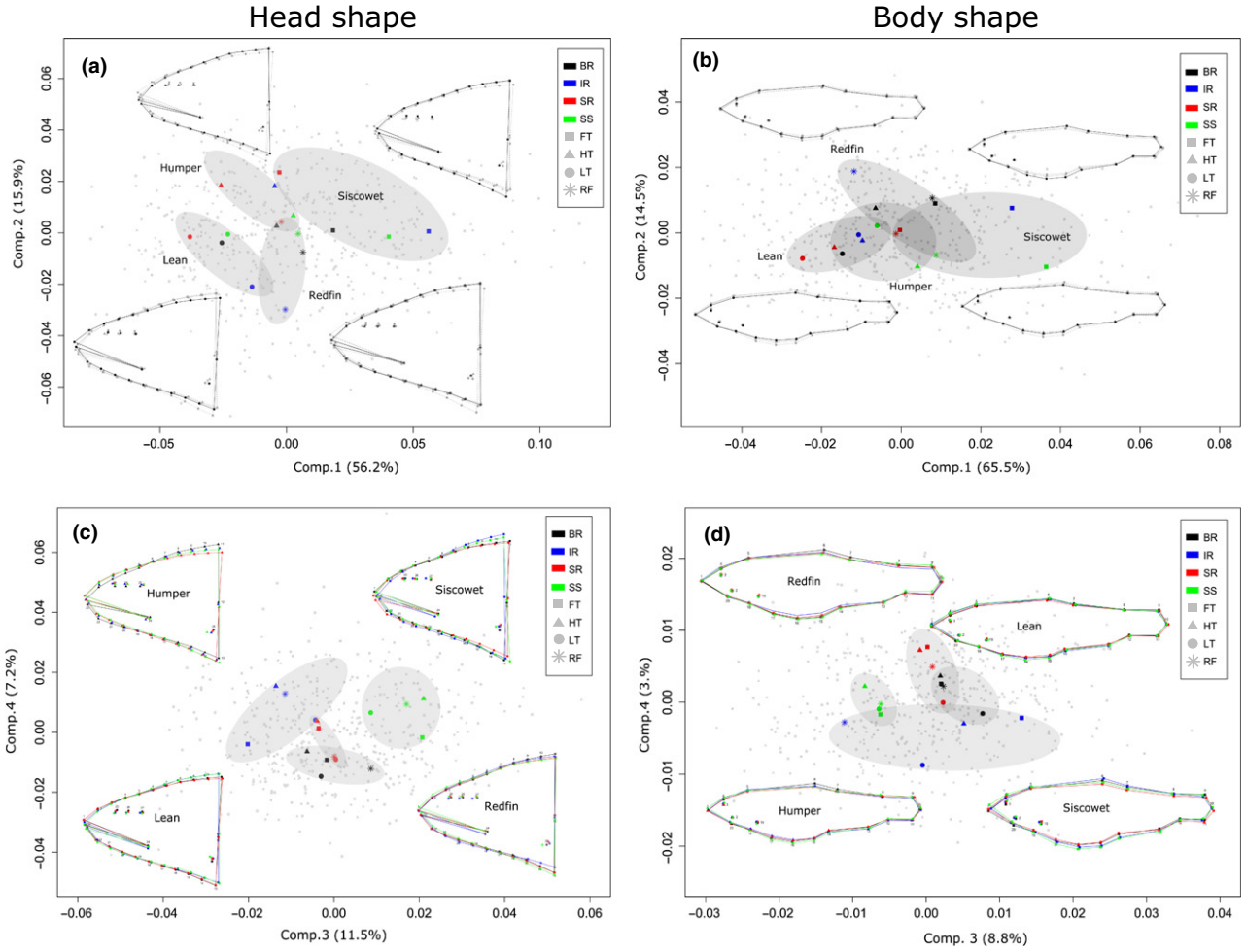

Fig. 2 Between-group PCA on partial warps of 501 lake trout. (a) First and second principal components for head shape representing 56.2% and 15.9% of the variance, respectively, distinguishing the four ecotypes. (b) First and second principal components for body shape representing 65.5% and 14.5% of the variance, respectively, that distinguish leans from siscowets based mainly on belly curvature. (c) Third and fourth principal components for head shape representing 11.5% and 7.2% of the variance, respectively, distinguishing the four sites. (d) Third and fourth principal components for body shape representing 8.8% and 3.0% of the variance, respectively, distinguishing the four sites. The coloured points refer to the mean scores for each ecotype in each site. The sites are as follows: Big Reef (black), Isle Royale (blue), Stannard Rock (red) and Superior Shoals (green). Ecotypes are as follows: Siscowet (FT), Humper (HT), Lean (LT) and Redfin (RF). Under each ecotype are drawn the consensus shapes of all four ecotypes (grey) with the outline of the ecotype in question (black). The shaded ellipses have been drawn for clarity.

not different from humper's. In addition, leans head shape was different from redfin's ($P < 0.05$). In some cases, similar ecotypes from different sites had significant different head and/or body shapes (Figs 2c,d and 3b, Table S3, Supporting information). Indeed, siscowet head shape differed among sites, whereas body shape was not different between Big Reef and Stannard Rock. Body shape of Superior Shoals leans differed from other leans except Isle Royale's whereas Isle Royale leans differed from Stannard Rock's. On the other hand, Isle Royale lean heads differed from both Stannard Rock and Big Reef leans. Redfins from Isle Royale differed in head and body shapes from all other redfins and lastly humpers were not different from site to site. Despite the fact that not all ecotypes from all sites were morphologically different based on morphometric analyses, we conserved this grouping for the genetic analysis based on the visual inspection of other traits (e.g. size of mature fish, body or fin colours).

Sequencing and SNP calling

Raw reads cleaning and demultiplexing resulted in a total of 1.6 billion reads with an average of 3.2 million reads per individual and a relatively small mean coefficient of variation (CV) of 0.23. The assembly resulted in a catalog containing 1 052 664 loci and a total of 212 804 SNPs (49 399 loci) after the population module. Fifteen individuals

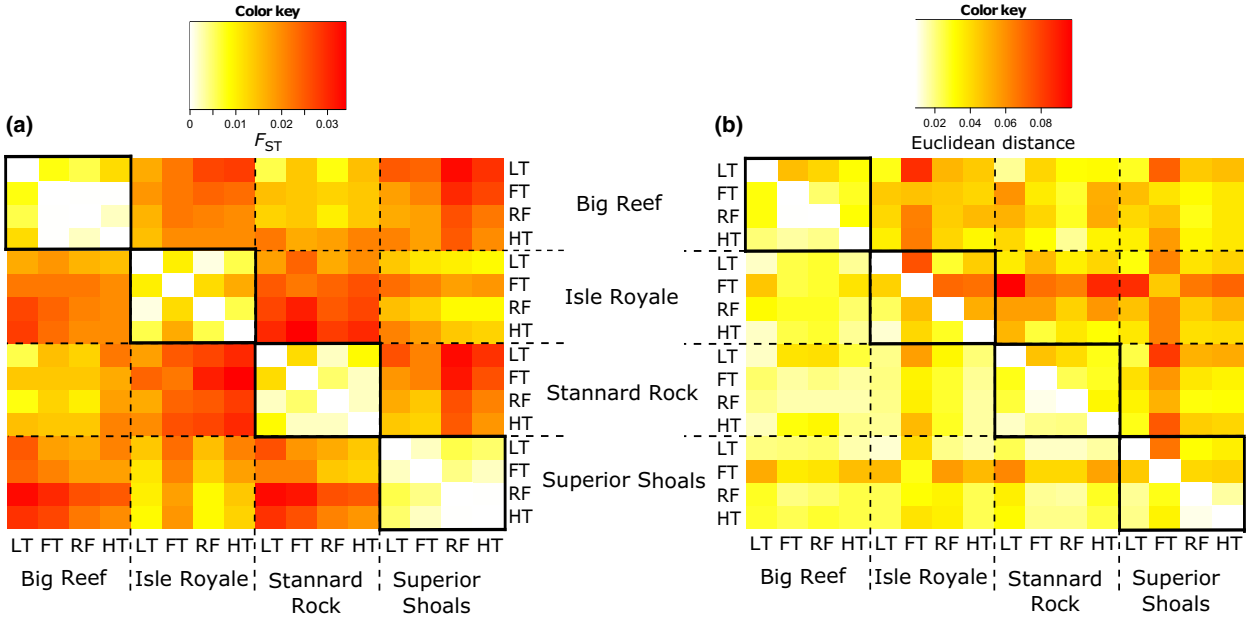

Fig. 3 Heatmaps of (a) calculated F_{ST} values, and (b) calculated Euclidean distances between groups averages for body (below diagonal) and head (above diagonal) shapes for the four ecotypes and four sampling sites. Ecotypes are as follows: Siscowet (FT), Humper (HT), Lean (LT) and Redfin (RF).

Table 3 Number of SNPs remaining after each filtration step

Before filtration	SNP count
Catalog	1 052 664 loci
After population module (presence in ≥70% individuals in ≥2 sites)	212 804 SNP
Filters	
Genotype quality	
Genotype likelihood (≥6)	193 678 SNP
Allelic imbalance (≤5)	
Hardy-Weinberg	
Heterozygosity (≤0.6)	185 445 SNP
Fis [−0.3; 0.3]	
MAF	
Global (≥0.01) and/or	17 812 SNP
Local (site) (≤0.05)	
Population	
Maximum number of SNP per locus (≤8)	13 984 SNP
Position	
1th SNP per locus kept	6822 SNP

Allelic imbalance corresponds to the ratio of the number of sequences for the major allele on the number of sequences for the minor allele.

having more than 40% missing genotype were removed from the analysis. After custom filtration, 6822 high-quality SNPs were retained for subsequent analysis (Table 3).

Genetic diversity and differentiation among sites and ecotypes

Genetic statistics revealed modest but significant F_{ST} among some ecotypes within each sampling site (mean F_{ST} = 0.0055) (Fig. 3a, Table S4, Supporting information). Mean F_{ST} among ecotypes within sites were as follows: Big Reef 0.0047 [0.000; 0.012], Isle Royale 0.0087 [0.001; 0.017], Stannard Rock 0.0053 [0.002; 0.012] and Superior Shoals 0.0032 [0.000; 0.006]. No trend in patterns of genetic diversity was observed between ecotypes within each site (Table 4). That is, there was no evidence that diversity in some ecotypes tended to be higher than in others. On the other hand, F_{ST} among sites were on average three times higher than observed among ecotypes within site (mean F_{ST} = 0.016). For instance, F_{ST} between sites (all four ecotypes pooled) were all significant: Big Reef ↔ Isle Royale 0.017, Big Reef ↔ Stannard Rock 0.009, Big Reef ↔ Superior Shoals 0.022, Stannard Rock ↔ Isle Royale 0.02, Superior Shoals ↔ Isle Royale 0.01 and Stannard Rock ↔ Superior Shoals 0.02. A lower value between Big Reef ↔ Stannard Rock and Isle Royale ↔ Superior Shoals site pairs was consistent with their closer geographic proximity. Also genetic diversity parameters tended to show greater differences between sites, than between ecotypes within sites (Table 4). Namely, genetic diversity, in terms of nucleotide diversity (π) and heterozygosity (H_o, H_e), was notably lower within Stannard Rock in comparison with the three other sites (Table 4).

Table 4 Population statistics estimated with 6822 SNPs: the observed heterozygosity (H_o), the expected heterozygosity (H_E), the inbreeding coefficient (G_{is}), the effective population size (N_e) and confidence interval in brackets, and the number of polymorphic loci (N)

	H_o	H_e	π	N_e	G_{is}	F_h	N
Big reef							
LT	0.067	0.067	0.000319	415 [356; 498]	−0.007	−1.04E-07	4132
FT	0.074	0.072	0.000353	925 [698; 1365]	−0.027	−1.29E-07	4564
RF	0.073	0.071	0.000337	1341 [646; infinite]	−0.03	−1.44E-07	3238
HT	0.072	0.069	0.000334	NA	−0.034	−1.84E-07	2022
Isle royale							
LT	0.075	0.077	0.000393	181 [171; 194]	0.027	−5.58E-08	4904
FT	0.074	0.075	0.000358	122 [116; 128]	0.012	−5.52E-08	4751
RF	0.079	0.080	0.000387	124 [118; 131]	0.015	−5.41E-08	4663
HT	0.074	0.075	0.000366	192 [178; 209]	0.017	−5.36E-08	4510
Stannard rock							
LT	0.062	0.061	0.000284	487 [408; 603]	−0.011	−9.50E-08	3649
FT	0.062	0.061	0.000285	159 [149; 171]	−0.007	−9.18E-08	3638
RF	0.062	0.061	0.000281	250 [213; 301]	−0.018	−1.03E-07	2923
HT	0.061	0.060	0.000276	199 [174; 230]	−0.016	−1.05E-07	2920
Superior shoals							
LT	0.081	0.082	0.000385	146 [137; 155]	0.01	−6.30E-08	4538
FT	0.078	0.080	0.000378	133 [127; 139]	0.021	−5.14E-08	5022
RF	0.076	0.077	0.000367	94 [91; 97]	0.007	−7.57E-08	5227
HT	0.073	0.074	0.000367	NA	0.012	−7.57E-08	2727

Genome-wide diversity (π) and the increase in individual homozygosity relative to mean Hardy-Weinberg expected homozygosity (F_h) was calculated on the dataset prior to filtration. Effective population size for ecotypes with sample size <15 individuals was not calculated (NA). Ecotypes are as follows: Siscowet (FT), Humper (HT), Lean (LT) and Redfin (RF).

Table 5 Analysis of molecular variance (AMOVA) on 486 individuals and 6822 SNPs. Missing data has been replaced by random picking in the overall pool of allele frequency

Source of variation	% Variance	*F*-stat	*F*-value	SD	c.i.2.5%	c.i.97.5%	*P*-value
Sites as group							
Among sites	0.011	F_{CT}	0.011	0.000	0.01	0.012	<0.001
Among ecotypes within sites	0.004	F_{SC}	0.004	0.000	0.004	0.005	<0.001
Ecotypes as group							
Among ecotypes	−0.002	F_{CT}	−0.002	0.000	−0.002	−0.002	0.95
Among sites within ecotypes	0.015	F_{SC}	0.015	0.000	0.014	0.016	<0.001

Overall, Superior Shoals ecotypes had the lowest effective population size (N_e) estimates while having, with Isle Royale, the highest inbreeding coefficient (G_{is}, F_h) whereas ecotypes from Big Reef had the highest effective population size (N_e) and the lowest inbreeding coefficient (G_{is}, F_h), while Stannard Rock showed intermediate indices. The more pronounced pattern of population differentiation between sites than between ecotypes was also evidenced by the AMOVA which revealed no net genetic variance explained by the ecotype grouping ($F_{CT} = -0.002$) compared with the net and significant genetic variance explained by sites grouping ($F_{CT} = 0.011$) (Table 5). Finally, no significant association was obtained between the F_{ST} matrix and either head ($r = -0.1025$ $P_{value} = 0.862$) or body ($r = 0.1032$ $P_{value} = 0.115$) shape Euclidean distances matrices (Fig. 3).

Clustering analysis

The ADMIXTURE program identified two groups (best K) corresponding to pairs of sites: Big Reef and Stannard Rock vs. Isle Royale and Superior Shoals (Fig. 4a). No migrants from Isle Royale and Superior Shoals were detected in the Big Reef/Stannard Rock cluster, while results suggested the occurrence of migrants and admixed individuals in the Isle Royale/Superior Shoals cluster with a tendency for a greater proportion of migrants in Superior Shoals (Fig. 4a). At K3–K4, Big Reef individuals tended to cluster separately

from those of Stannard Rock although lean trout from Big Reef tended to be more similar to Stannard Rock leans. At K5, Isle Royale could be discriminated from Superior Shoals. Lastly, at K6 all four sites differed and some additional within-site distinctions began to appear. Within Big Reef, leans were distinct from other ecotypes, being more similar to the lean/redfin cluster from Stannard Rock. Within Isle Royale, siscowets were distinct from the other ecotypes, while no obvious difference emerged between ecotypes within Superior Shoals. In addition, some siscowets from Stannard Rock seemed to be similar to Isle Royale siscowets. The NJ population tree mainly grouped ecotypes from different sites together with pair of sites closer geographically also clustering more closely in the tree (Fig. 4b). In addition, as observed

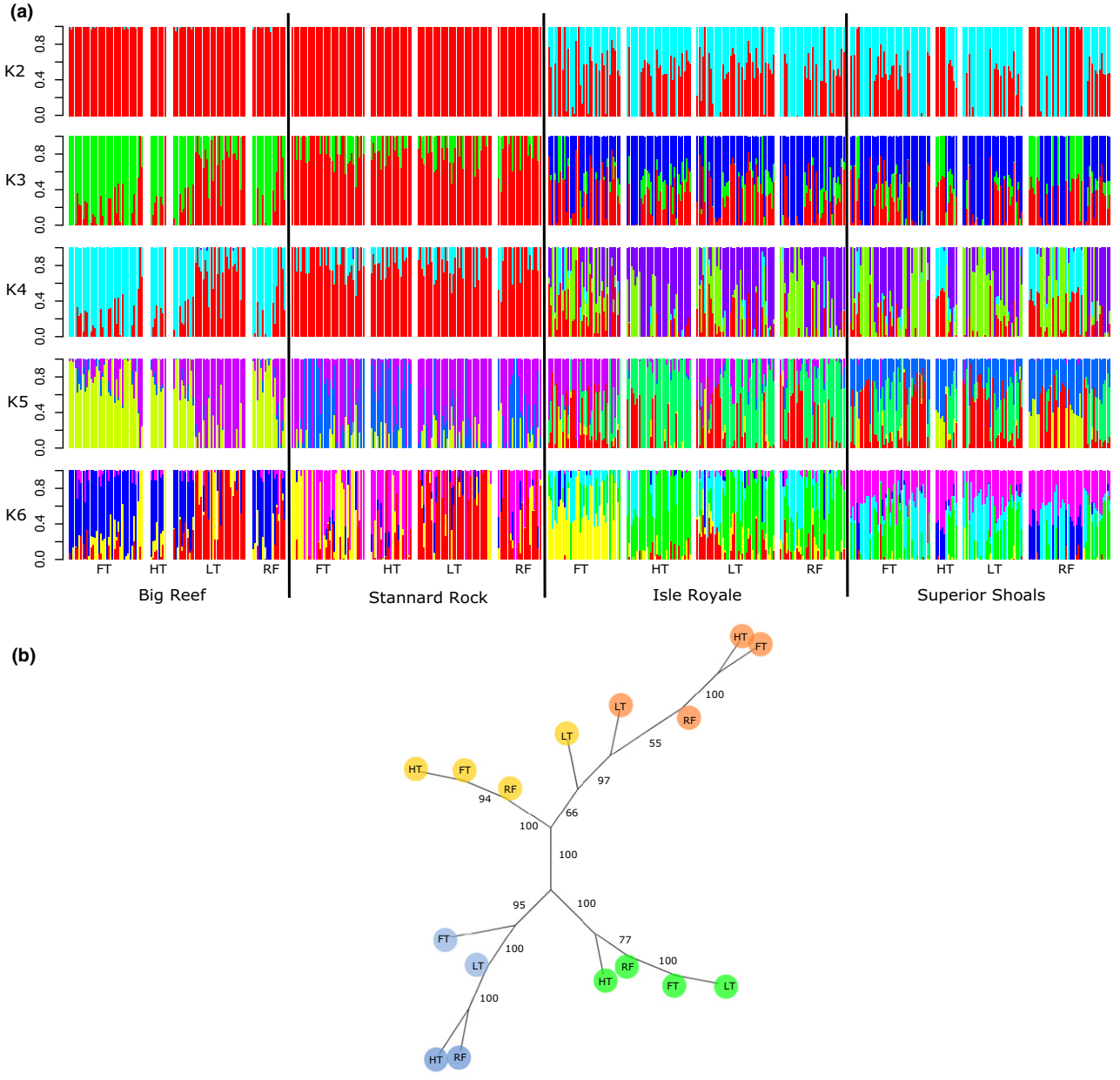

Fig. 4 Population structure analysis of lake trout. (a) Admixture plot based on 486 individuals and 6822 SNPs (including outliers) for different values of K. Individuals are shown by sites and ecotypes. (b) Neighbour-joining tree based on 486 individuals and 6822 SNPs including outliers. Yellow circles 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 represented for each site: Lean (LT), Humper (HT), Redfin (RF) and Siscowet (FT).

in the Admixture analysis, leans from Big Reef were closer to leans from Stannard Rock than from the other ecotypes within Big Reef. ADMIXTURE also showed that siscowets from Isle Royale were most distinct from the other three ecotypes within this site (Fig. 4b).

Population assignment

Assignment success to sampling sites, based on the 6822 SNPs, was high, being 85% on average and up to 95% for Isle Royale and 93% for Stannard Rock (Fig. 5a). Misassigned individuals from Big Reef were only assigned to Stannard Rock and vice versa. Superior Shoals had a lower assignment success (78%) and had misassigned individuals to the three other sites. On the contrary, assignment success to ecotypes was low, being 41% on average, ranging from 12% for humpers up to 61% for siscowets (Fig. 5b). Ecotype assignment success within each sampling site was highly variable, being highest on average within Isle Royale (55%) and lowest within Superior Shoals (21%) and in fact similar to random expectation, while Big Reef (33%) and Stannard Rock (40%) showed intermediate results (data not shown). Assignment success within Isle Royale was 76% for siscowets, 62% for leans, 52% for humpers and 33% for redfins, whereas assignment success within Superior Shoals was 46% for siscowets, 23% for redfins, 18% for leans and 0% for humpers. Within Big Reef, individuals were assigned either to siscowets or leans whatever their current ecotype was. For instance, assignment success for siscowets was 82%, 51% for leans and 0% for humpers or redfins. Stannard Rock showed a similar pattern, where assignment success for siscowets was the highest (76%) followed by leans (74%), while the assignment for humpers (10%) and redfins (0%) was low.

Outlier detection and phenotype–genotype associations

BAYESCAN identified a total of 52 outliers from which 49 occurred between the four sites and three between the four ecotypes (Fig. 6a,b). No outliers were detected between ecotypes within each site. In addition, no outliers were common between sites and ecotype comparisons. For LFMM, the *P*-values were adjusted using a lambda of 0.55 (λ) and population structure was corrected for each analysis using the number of ancestral groups (*K*) identified by ADMIXTURE for the overall data set or within each site separately. According to the ADMIXTURE results, a *K* of five was used for between sites and between pooled ecotype comparisons while for within-site comparisons, a *K* of two was used for Big Reef and Superior Shoals and a *K* of three was used for Isle Royale and Stannard Rock. LFMM identified a total of 670 unique outliers: 554 between sites, and 116 between ecotypes in which 20 were common to both comparisons (Fig. 6a,b). Thus, the number of outliers between ecotypes was lower than that observed between sites. For within-site

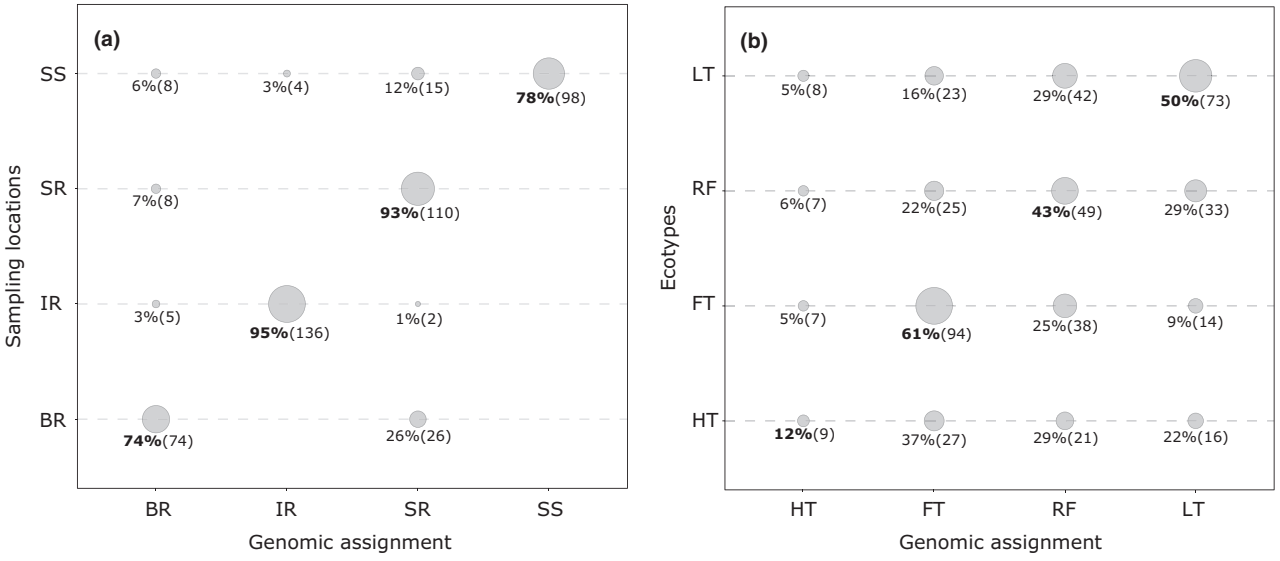

Fig. 5 Assignment success of individuals to their sampling sites (a) or ecotypes (b). Percentage assignment is written below circles with the exact number of individuals assigned within brackets. Percentage of correct assignment to either sampling sites or ecotypes is in bold. Sites are as follows: Big Reef (BR), Isle Royale (IR), Stannard Rock (SR), Superior Shoals (SS). Ecotypes are as follows: Humper (HT), Siscowet (FT), Lean (LT) and Redfin (RF).

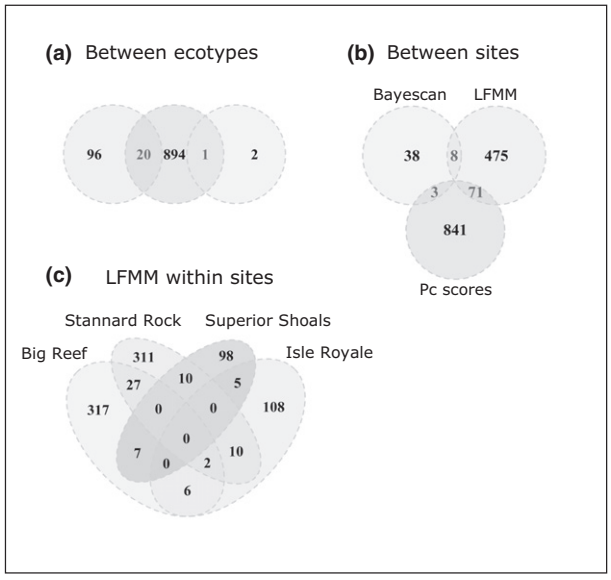

Fig. 6 Venn diagrams of outliers detected by LFMM and BAYESCAN among sites, ecotypes or among ecotypes within sites. (a) Outliers detected among the four sites by BAYESCAN and LFMM including outliers detected by LFMM using morphological PC scores. (b) Outliers detected among the four pooled ecotypes by BAYESCAN and LFMM including outliers detected by LFMM using morphological PC scores. (c) Outliers among ecotypes within each site detected by LFMM.

comparisons between ecotypes, 359 outliers were detected within Big Reef, 131 within Isle Royale, 360 within Stannard Rock and 120 within Superior Shoals. Overall, up to 27 outliers were common between some sites, but none were common to all sites (Fig. 6c). No outliers detected among ecotypes were common between LFMM and BAYESCAN, but eight were common among sites.

Based on the broken stick method, the first four principal components were selected to represent head shape and the first six principal components were selected to represent body shape, for a total of 10 shape variables. Briefly, the *P*-values were adjusted using a lambda of 0.55 (λ) and population structure was corrected using a *K* of five. A total of 915 unique associations were detected with an FDR of 0.05 in which several were common between variables (Table S5, Supporting information). Four of these associated SNPs were common with BAYESCAN outliers (one with the between ecotype comparison and three with the between site comparison, Fig. 6a,b). In addition, 349 of these associated SNPs were common with the previous LFMM analysis. Briefly, 71 were in common with between site comparison, 20 with between ecotypes comparison, 85 with within Big Reef comparison, 48 with within Isle Royale comparison, 91 with within Stannard Rock and 34 with within Superior Shoals comparisons.

Annotation

A total of 2056 loci detected either by BAYESCAN or LFMM between sites, between ecotypes or in association with phenotypic variation were blasted against the rainbow trout genome. After quality filtering, 258 loci that had an annotation in genes were retained (Table S6, Supporting information). From those with a known biological function, markers linked to lipid transport and metabolism as well as visual development and perception were of particular interest given previously documented phenotypic characteristics differentiating lake trout ecotypes (see Discussion).

Discussion

This is the first study to combine genomic and morphometric analyses from all four lake trout ecotypes from several different locations in Lake Superior. This provided the unique opportunity to investigate among- and within-site variation and the extent of parallelism, both at the phenotypic and genomic levels. Both morphometric and genomic analyses revealed within-site morphological and genetic differences between ecotypes, but in general, genetic differences were more pronounced among sites than among ecotypes, even when comparing populations of the same ecotype. Similarly, we observed that values of demographic and genetic diversity parameters generally varied more by site than by ecotype. Moreover, the extent of both morphological and genetic differences among ecotypes observed within site varied from one location to the other, thus creating a continuum of differentiation. In addition, genome scans and association tests identified several loci potentially implicated in local adaptation and phenotypic divergence among ecotypes, among which loci linked to lipid metabolism and transport as well as visual acuity and development are of particular interest (see Discussion below). The relatively large number of outlier loci identified, which globally showed relatively modest levels of genetic differentiation among sites or ecotypes, suggests a polygenic origin of both local adaptation between sites and ecotypic differentiation. We discuss the implications of these results for the understanding of the biological processes responsible for the emergence of the different ecotypes of lake trout as well as for their management.

Parallel evolution of lake trout ecotypes?

Parallel evolution, the repeated evolution of similar phenotypic traits, has been documented in many populations within the same species (reviewed in Elmer & Meyer 2011). Shared phenotypic traits that evolved

independently are generally believed to indicate parallel adaptive evolution in the face of shared environmental pressures between locations driving changes to similar optimum (Butlin *et al.* 2013). The evolution of these similar traits can be underlain by similar or different genome architecture (Elmer & Meyer 2011; Ralph & Coop 2014; Bernatchez 2016). Here, similar ecotypes corresponding to previously published descriptions were identified among all sampling sites. That is, a greater proportion of morphological variance, explaining 14.5–65.5%, clustered individual by ecotypes (first and second components of the PCAs, Fig. 2a,b), thus revealing parallelism in morphology between ecotypes from the four sampling sites. It is noteworthy that head shape better discriminated ecotypes than body shape (Fig. 2a and 3b), as reported previously in other lake trout studies both from the Great lakes and elsewhere (Moore & Bronte 2001, 2007; Alfonso 2004; Magalhaes *et al.* 2009; Chavarie *et al.* 2013). Moreover, a greater proportion of markers identified as outliers or associated with phenotypic differentiation was found for head shape compared with whole body shape. More pronounced ecotypic differentiation of head shape could suggest a predominant role for feeding ecology compared with other factors (e.g. locomotion) as the main driver for these morphological differences (Jonsson & Jonsson 2001; Magalhaes *et al.* 2009; Chavarie *et al.* 2013).

The fact that different ecotypes within sites were generally genetically more similar than different populations of the same ecotype among sites suggests that parallel evolution is implicated in the origin and maintenance of ecotypes. Moreover, while both explanations are not exclusive, we cannot refute the possibility that more pronounced genetic similarity within sites might also reflect higher gene flow among ecotypes within sites than among population of a same ecotype among sites. This would also reflect less pronounced reproductive isolation among ecotypes within sites than among populations of a same ecotype among sites. It is also noteworthy that, although to a lesser extent, some morphological components (explaining 3% to 11.5% of variance) could discriminate lake trout by sampling sites (third and fourth components of the PCAs, Fig. 2c,d). In some cases, such as siscowets, leans and redfins, different populations of a same ecotype from particular sites were morphologically divergent, indicating some dissimilarity in morphology. Such intersite differences within ecotype have previously been reported by Bronte & Moore (2007) for siscowet and these were interpreted as either the presence of different reproductive populations or a plastic response to different environmental conditions among sites.

Both outlier detection methods (BAYESCAN and LFMM) differentiated more outlier markers among sampling sites than among ecotypes, again supporting the view that spatial variables (e.g. different environmental conditions or random genetic changes) may be more important than ecotypic differentiation in explaining the observed pattern of population structure in Lake Superior. Moreover, LFMM uncovered markers potentially under selection among ecotypes within all four sampling sites, none being common to all sites. These results also suggest that phenotypic parallelism in lake trout ecotypes is not accompanied by parallelism at the genome level, as reflected by the lack of association between the genetic and phenotypic divergence matrices, whereby the expression of a given ecotype in different sites is controlled by a different genetic architecture. Hypothetically, there may have been random genetic differentiation (drift, founder effects in different parts of the lakes such that subsequent selection driving adaptive changes may have been acting on somewhat different gene pools in different parts of the lakes). This would lead to apparent nonparallelism at the genome level. The absence of parallelism between phenotypic and genotypic differentiation has been reported in many species, including mice (*Peromyscus maniculatus*), cichlids, cavefish (*Astyanax mexicanus*), stickleback (*Gasterosteus* spp.), as well as ciscoes and whitefish (*Coregonus* spp.) (reviewed in Elmer & Meyer 2011; Bernatchez 2016). For instance, ciscoes in Lake Nipigon exhibit four morphological and ecological different species without evidence of corresponding neutral genetic differentiation (Turgeon *et al.* 1999). Similarly, ciscoes from several inland lakes showed variable levels of phenotypic differentiation which was not correlated to genetic divergence (Turgeon *et al.* 2016). Also, Laporte *et al.* (2015) recently documented a clear pattern of phenotypic parallelism in body shape between dwarf and normal sympatric pairs of lake whitefish with similar genomic architecture underlying these traits being observed between some pairs but different genome architecture in others.

Genetic origin of ecotypes

Generally speaking, we found very limited support for a shared genetic origin among populations of the same ecotype. That is, we generally observed fewer genetic differences among ecotypes within sites than among populations (sites) for the same ecotype. The exception to this general pattern was for the lean ecotype for which we observed more genetic similarity between populations from Big Reef and Stannard Rock than between leans and other ecotypes from these locations. Similar results were previously reported by Ihssen *et al.* (1988) and Dehring *et al.* (1981) who showed based on allozymes that lake trout of the lean ecotype from four

different locations differed in allele frequencies. Different markers identified as being under selection among sites provide further support for the independent origin of ecotypes within each site. Alternatively, we cannot rule out that this may also reflect the presence of different genetic architecture underlying phenotypic variation within sites, or that markers under parallel selection were not detected because of insufficient coverage of the genome. Taken together, the combined results obtained for 'neutral' and potentially 'adaptive' markers highlight the contribution of both spatial isolation and local adaptation in shaping ecotypic variation within each sampling site.

Here, relatively large geographic distances between these sites, known for the relatively high occurrence of the four ecotypes separated by ranges of much lower abundance, may have contributed to reduce genetic exchange between spatially isolated populations. Thus, localized movements have been reported for lake trout based on tagging studies where an average movement of approximately 40 km has been reported (Eschmeyer 1955; Kapuscinski *et al.* 2005). Considering that the closest sites in this study are separated by about 69 km (Big Reef/Stannard Rock) to 87 km (Isle Royale/Superior Shoals) and that sites that are the farthest are separated by 98 km (Superior Shoals/Stannard Rock) to 212 km (Isle Royale/Big Reef), the presence of spatially genetically differentiated stocks is consistent with this observation. Spatial isolation could also have been exacerbated by historical water level fluctuations. Lake Superior has a very diverse bathymetric habitat covered by peaks and valleys, thus creating geographical barriers particularly when water levels fluctuated. This situation is thought to have occurred 8000 years ago, which could have triggered the spatial pattern of genetic divergence seen today (Bronte & Moore 2007).

Our data also revealed a continuum in the extent of both genetic and phenotypic divergence underlying the observed ecotypes ranging from intrapopulation polymorphism to clear genetically distinct populations within a sampling location. The extent of morphological differentiation in both head and body shapes was also variable depending on the site being examined. Although the explanations for this pattern of continuum in morphological divergence are only hypothetical at this time, this could reflect different levels of trophic polymorphism associated with different selective pressures (e.g. competitive interactions), as reported for other species, including lake whitefish (*Coregonus clupeaformis*) (Lu & Bernatchez 1999; Gagnaire *et al.* 2013), arctic charr (*S. alpinus*) (Gislason *et al.* 1999) or threespined stickleback (*Gasterosteus aculeatus*) (Hendry *et al.* 2009). The extent of genetic divergence between ecotypes was also variable depending on the site examined suggesting that different levels of reproductive isolation accompany different levels of phenotypic divergence (see references above, also reviewed by Hendry 2009).

In contrast to our general observation of higher genetic differentiation among sites than among ecotypes within site, a recent study conducted in Great Bear Lake found more pronounced genetic differentiation among lake trout ecotypes than among sampling sites (Harris *et al.* 2014). These authors hypothesized that stronger genetic and morphological differentiation in Great Bear Lake could be due to its more pristine environment and limited human impact compared with Lake Superior where these factors may have altered the original pattern of population structuring. For instance, considerable stocking and fishery harvest has occurred in Lake Superior, which could certainly have had an impact on the extent of population admixture (Guinand *et al.* 2003) compared with Great Bear Lake, which has not been stocked and has only been subject to minor fishery harvest. However, it is noteworthy that stocking has been done essentially for the lean ecotype (Page *et al.* 2004). Consequently, it seems unlikely that this could explain the general pattern of structuring we documented for other ecotypes, although it could possibly explain why leans from different locations were more similar in some cases, as explained above.

In sum, the combined genomic and morphological data support the hypothesis that ecotypic differentiation among lake trout ecotypes from different geographic locations within Lake Superior can be arrayed along a continuum from quasi-panmixia to relatively pronounced reproductive isolation, mimicking the interspecific pattern described by Hendry *et al.* (2009) among lacustrine north temperate freshwater fishes. Consequently, variation along this continuum might profitably be used for studying factors, outlined by Hendry *et al.* (2009), which can promote or constrain progress towards ecological speciation, including plasticity, natural selection, mate choice, geography or historical contingency. However, the present study cannot rule out the possibility that different anthropogenic impacts among sites could have also contributed to the observed pattern of genomic and phenotypic variation. Indeed, a recent study conducted by Baillie *et al.* (2016) highlighted substantive losses of genetic diversity and genetic distances in lean, siscowet and humper trout from postcollapse recovery (1995–1999) compared with contemporary period (2004–2013). This homogenization could be the result of overexploitation, intensive stocking and invasions of non-native species, which could have led to the overlap in breeding or foraging area,

thus increasing hybridization. Biodiversity losses and speciation reversal caused by anthropogenic activities have been recorded in several freshwater species such as Lake Victoria cichlids (Seehausen *et al.* 2008), Great Lakes ciscoes (*Coregonus spp.*; Todd & Stedman 1989) as well as the European whitefish (*Coregonus spp.*; Hudson *et al.* 2013; Bhat *et al.* 2014).

Evidence of local adaptation and functional annotation

In both spatial and ecotypic differentiation however, a much larger proportion of markers potentially under selection were detected by the LFMM method compared with BAYESCAN, the former known to be more sensitive to polygenic effects, suggesting that weak or polygenic selection might be responsible for the observed pattern of 'adaptive differentiation', both spatially and between ecotypes (Rellstab *et al.* 2015). Of the 258 loci for which successful annotation could be retrieved, several were of particular interest and linked to ecotypic differences observed in the present system. Two loci were linked to visual development and acuity of the retina: retinal guanylyl cyclase 2 and retinitis pigmentosa 1-like 1 protein, and one to visual perception: peripherin-2-like. Both markers linked to visual development and acuity were found in significant association with the second component of the head shape analysis from which the highest loading was for the eye position (landmark number 26). Changes in size, location and sensitivity of the eyes have been associated with adaptation to low-light environment (Von der Emde *et al.* 2004). Indeed, larger eyes with a predominance of rods are known to increase visual acuity (Von der Emde *et al.* 2004). Large eyes, close to the snout, have been reported in other deepwater, salmonid morphs similar to the siscowet and humper ecotypes in Lake Superior, potentially reflecting adaptation to low-light condition (Moore & Bronte 2001; Eshenroder 2008; Skoglund *et al.* 2015).

Annotated markers of interest were also linked to lipid binding, transport, regulation and metabolism. A total of three annotated markers were linked to lipid binding: the spectrin beta nonerythrocytic 4-like isoform x1 and 1-like isoform x2 (SPTBN4, SPTBN1) and the calcium-dependent secretion activator 1 (CADPS), and one marker was linked to transport: the lipid phosphate phosphatase-related protein type 4-like (LPPR4) (http://genecards.org). SPTBN4 was found to be in significant association with the first component of the body shape analysis, which was linked to belly curvature, whereas SPTBN1 and CADPS were found to be in significant association with among ecotype analyses and LPPR4 in significant association with head depth. High lipid levels in the muscle of the deepwater siscowet ecotype have long been described and suggested to facilitate vertical migration in the water column by providing hydrostatic lift (Eschmeyer & Phillips 1965; Henderson & Anderson 2002).

Also, Goetz *et al.* (2010) showed that differences in lipid levels between the lean and the siscowet ecotype persist when reared under identical conditions. They also found several differentially expressed genes in controlled conditions between these two ecotypes linked to lipid metabolism. Interestingly, four of the annotated markers in the present study were also found to be differentially expressed by Goetz *et al.* (2010), further suggesting that our study identified some candidate genes involved in the differentiation between these ecotypes. The four markers in common are the alpha-tectorin-like protein, the fk506-binding protein 5-like isoform x3, the galactosamine (n-acetyl)-6-sulphate sulphatase and the peroxisome proliferator-activated receptor. Functional descriptions of most of these genes are still lacking; therefore, mechanistic links between these markers and lake trout ecotypic adaptations remain unknown. In sum, the hypothesis of genetically based adaptation in lake trout is supported by at least a few divergent annotated genes that are linked to biological functions (e.g. vision, lipid metabolism). These same genes are believed to play roles in the local adaptation to different water depths and trophic resource use (Goetz *et al.* 2010).

Limitations

Admittedly, we must also consider the possibility that several alternative factors could explain the pattern of continuum in ecotypic divergence observed here. Namely, sample sizes were small in some cases, especially for the humper ecotype, which could have limited our power to detect genetic divergence, namely between humper and redfin ecotypes. Also, our capability of assigning lake trout to different ecotypes based on their morphology varied among sites, which may have created artificially admixed groups of individuals resulting in lower level of differentiation among them. In such a case, however, the clustering analysis performed with ADMIXTURE should have detected such groups of admixed individuals from different populations, which was not the case here. Instead, ADMIXTURE revealed homogeneous groups of individuals, independent of their ecotype in locations where we observed very weak or no genetic differentiation. Arguably, our results do not rule out a role for phenotypic plasticity induced by exposure to different environmental conditions, which will require further common garden studies of other ecotypes (humper and redfins ecotypes) from other locations, as performed by Goetz *et al.* (2010). In fact, phenotypic plasticity may have played

an important role in the diversification of lake trout ecotypes within Lake Superior. Indeed, the presence of environmentally induced (plastic) polymorphism within population has been hypothesized to facilitate the process of divergence (Adams & Huntingford 2004; Pfennig *et al.* 2010). Thus, phenotypic plasticity can promote the emergence of divergent phenotypes on which selection can act (Pfennig *et al.* 2010). In addition, trophic polymorphism may be an effective way to promote speciation by resource use because it may trigger reproductive isolation (Smith & Skúlason 1996; Pfennig *et al.* 2010). Finally, studies on sympatric ecotypes such as cichlids, whitefish and arctic charr have shown, using common garden experiments, that some morphological characters were plastic and others heritable, thus demonstrating the role of phenotypic plasticity in shaping divergence (Adams & Huntingford 2004; Magalhaes *et al.* 2009; Lundsgaard-Hensen *et al.* 2013). Finally, when using methods of reduced genome representation such as RADseq, it is important to keep in mind that only a small subsample of the whole genome variation has been screened. Consequently, some important targets of selection are most likely missed in such studies and results must be interpreted cautiously and accordingly. Here, this means that the interpretations of observed differences with a reduced genome representation are conservative.

Management implications

The maintenance of genetic diversity, and thus the potential of a species to evolve in the face of a changing environment, is central in conservation genomics and fishery management (Toro & Caballero 2005). Improper management may lead to depletion of the resource and/or impaired resilience by decreasing genetic diversity or eroding local adaptations (Laikre *et al.* 2005; Zimmerman *et al.* 2009). Management units are groups of conspecific individuals among which connectivity is sufficiently low so that each group should be managed separately (Palsbøll *et al.* 2007). The delineation of these management units is still debated and has usually been based upon the rejection of panmixia (Waples & Gaggiotti 2006) or the absolute amount of population divergence between populations (Palsbøll *et al.* 2007). Thresholds above which populations should be considered distinct management and demographically independent units do not exist, but a dispersal level <10% has been suggested (Palsbøll *et al.* 2007). Based on our results, the primary basis to define management units in lake trout of Lake Superior should be the sampling sites rather than ecotypes as we observed pronounced levels of net genetic differentiation and high assignment success (varying between 74 and 95%) among sites compared with net genetic differentiation and very low assignment success (varying between 12 and 61%) among ecotypes. Yet, depending on locations, ecotypic differentiation must also be considered as ecotypes were also genetically distinct in some cases, such as Isle Royale in particular. Also, evidence of local adaptation was uncovered, and therefore, caution must be taken within sites to avoid depletion of locally adapted traits by stocking or exploitation. Since the extirpation of lake trout from most of the Great Lakes other than Lake Superior, stocking programmes have been developed in some lakes without success (Page *et al.* 2003). Matching stocking sites with proper ecotype could increase re-introduction success (Zimmerman *et al.* 2009). Based on this study, we would advocate for re-introduction and translocation of lake trout from the least genetically differentiated site, namely Superior Shoals as this would provide the full range of ecotypic differentiation within a quasi-panmictic gene pool, a situation that would be reminiscent of the early stage of ecological speciation (Smith & Skúlason 1996; Hendry 2009). Moreover, such intrapopulation polymorphism may increase survival in a new environment while maintaining genetic diversity and potential for local adaptation (Wennersten & Forsman 2012). In addition, our results provided limited evidence for local adaptation associated with ecotypic differentiation at this location, which could improve survival in a different lake environment given that local adaptation is typically associated with trade-offs wherein locally adapted individuals exhibit higher fitness in their local environment compared with individuals from a different population and environment (Kawecki & Ebert 2004). However, further studies on the extent of population differentiation throughout Lake Superior will be necessary not only to better define boundaries to gene flow but also characterize potentially adaptive traits in other localities.

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A.M.M. and F.G. provided samples and visual identification. L.B. and A.M.M. conceived the study, and A.P.-P. did the laboratory work, analysed the data and wrote the manuscript. C.P. assisted in data analysis and writing the manuscript. E.N. helped for bioinformatic analysis. P.S. and L.B. provided the funding for the present study and helped editing of the manuscript. All authors approved and edited the manuscript.

Data accessibility

Individuals raw sequences are available at the Sequence Read Archive (SRA) (Study Accession no. SRP096183), and necessary data for genomic and morphometric analyses are available at Dryad doi: 10.5061/dryad.k713n.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Landmark and semi-landmark positions digitized on fish body and head.

Table S1 Models and parameters selected to assign an ecotype to each fish from the four sites separately.

Table S2 Between-group PCA analysis of group distance among the four ecotypes pooled (Siscowet (FT), Humper (HT), Redfin (RF), Lean (LT)) with 10 000 permutation for head and body shape.

Table S3 Between-group PCA analysis of group distance among ecotypes within and among sites with 10 000 permutation for body (below diagonal) and head (above diagonal) shape.

Table S4 Paiwise differentiation (F_{ST}) and genetic diversity indices calculated between each pair of ecotypes within and among sites with 6822 SNPs.

Table S5 SNPs associated with variation in head and body shape detected by LFMM.

Table S6 Hits against the Rainbow Trout genome (*Oncorhynchus mykiss*) of loci sequences identified as potentially under selection according to BAYESCAN or LFMM between ecotypes or between sites as well as in association with phenotypic variation.