Genomics of freshwater adaptation in three-spine stickleback from eastern Canada

*Alan Garcia-Elfring, [Σ]Mathilde Salamon, [¶]Antoine Paccard, and *Rowan D. H. Barrett

*Redpath Museum and Department of Biology, McGill University, Montreal, QC, Canada

[Σ]Department of Microbiology and Immunology, Emory University, Atlanta, GA, USA

[¶]McGill University Genome Center, McGill University, Montreal, QC, Canada

Abstract

The threespine stickleback is an important species in the study of parallel evolution. However, the evidence for parallel evolution, and the genetic and phenotypic levels, has largely come from the west coast of North America and parts of southern Europe. Here, we use RAD-sequencing of pooled samples to study stickleback marine-freshwater differentiation in Atlantic Canada and test for evidence of parallel evolution. Our results show considerable heterogeneity in differentiation among populations, with some freshwater populations showing high degree of allele frequency divergence relative to marine ones. The strongest candidates were two SNPs near dopamine receptors (*Drd24a* and *Drd2l*). Gene ontology (GO) analysis of candidate genes shows dopamine neurotransmitter receptor activity as one of the most enriched gene functions, along with nervous system development. Our study suggests a more complex evolutionary history of stickleback populations in the Atlantic than previously thought, suggesting multiple colonization events or ongoing gene flow. The genomic regions showing repeated differentiation suggest adaptation to freshwater through changes in behaviour.

Introduction

Knowledge of the genetic basis of adaptation is central to understanding the mechanisms driving the diversification of species. Threespine stickleback fish (*Gasterosteus aculeatus*) are native to the oceans of the northern hemisphere and has been an important species in studies of ecology and evolution for almost a century (Bertin 1925; Heuts 1947a, b; Moodie and Reimchen 1976). Over the last 12000 years, with the melting of glaciers after the last glacial maximum, marine populations of threespine stickleback have colonized freshwater lakes and rivers across the northern hemisphere. Many freshwater populations evolved similar low-plated morph phenotypes, particularly in western North America (Baumgartner and Bell 1984; Colosimo et al. 2005; Hohenlohe et al. 2010). It is thought that alleles that confer higher fitness in freshwater are found at low frequencies in marine populations, with bidirectional gene flow maintaining the genetic variation needed to seed new freshwater

habitats (‘transporter hypothesis’, Schluter and Conte 2009). Today, the threespine stickleback is considered an exemplar of parallel evolution.

However, even early in the development of stickleback as a model species, it was noted that the remarkable parallel evolution among freshwater populations might be a local phenomenon and limited to western North America (i.e., eastern Pacific) and southern Europe (Hagen and Moodie 1982). Taking armour phenotype as an example, in regions of North America, Northern Europe, the Baltic Sea, and eastern Asia the completely plated morphs which is common in marine environments dominates in local lakes (Münzing 1963; Penczak 1965; Hagen and Moodie 1982; Mäkinen et al. 2008; Raeymaerker et al. 2014; Ferchaud and Hansen 2016; Yamasaki et al. 2019). This lack of differentiation between marine and freshwater populations was also noted at the genetic level using allozymes (Rafiński et al. 1989). More recently, a genomic study (Fang et al. 2020a) that looked at stickleback across their distribution showed that high marine-freshwater (M-FW) differentiation is largely confined to the eastern Pacific, with Atlantic populations showing low levels of genomic differentiation. In contrast to populations in British Columbia, many freshwater populations in Eastern Canada are dominated by full-plated ecotype (Hagen and Moodie 1982; Haines et al. 2022), with certain populations showing distinct phenotypes (Scott et al. 2023). However, genomic studies in this region are few (e.g., Fang et al. 2020).

One explanation for why parallel evolution and M-FW differentiation appears to be lower outside of the eastern Pacific is stochastic loss of freshwater-adapted alleles. Threespine stickleback evolved in the Pacific Ocean where they have persisted since for approximately 26 million years ago (Matschiner et al. 2011; Betancur□R et al. 2015). Only much more recently, in the late Pleistocene (36.9–346.5 thousand years ago), did threespine stickleback colonize the western Pacific and Atlantic basin (Orti et al. 1994; Fang et al. 2018; Fang et al 2020). Atlantic populations also show less genetic diversity than those in the Pacific (Fang et al. 2020), a consequence of range expansion. However, most Atlantic populations analyzed thus far are located along the coast of northern Europe.

82 We study marine and freshwater threespine stickleback from eastern Canada, in
83 the provinces of Nova Scotia and Newfoundland where the full-plated phenotypes can
84 be found at high frequencies in freshwater habitats (Hagen and Moodie 1982). We use
85 genome-wide SNPs to characterize M-FW differentiation and look for evidence of
86 parallel natural selection. We seek to gain insight into the evolutionary history and the
87 genetic basis of freshwater adaptation of stickleback populations from eastern North
88 America.

89

90 **Methods**

91 *Field sampling and DNA extraction*

92 We used minnow traps and beach seines to collect 30 adult stickleback (> 30 mm in
93 length) from nine populations in Nova Scotia and Newfoundland, Canada (Figure 1). In
94 Nova Scotia, we sampled three freshwater sites, including two lakes (Pomquet Lake
95 Lake Ainslie) and a stream (Black River), and two marine populations (Antigonish
96 Landing and Porper Pond). In Newfoundland, we sampled two freshwater (Pinchgut
97 Lake and Blue Pond) and two marine populations (Cooks Brook and Humber Arm). We
98 followed a phenol-chloroform procedure for DNA extraction.

99 *DNA sequencing of pooled samples*

100 For DNA quantification, we used a Picogreen® ds DNA assay (Thermo Fisher Scientific,
101 Waltham, USA) and an Infinite® 200 Nanoquant (Tecan Group Ltd. Männedorf,
102 Switzerland). We then normalized the concentration of dsDNA and pooled according to
103 sampling location. Thus, we created nine pools consisting of 30 individuals each. RAD-
104 seq libraries were prepared and sent for sequencing the McGill University and Genome
105 Quebec Innovation Center, Montreal, Canada. Sequencing was carried out on one lane
106 of Illumina HiSeq2500 which produced 125bp paired-end reads.

107 *Bioinformatics*

108 We used the *trim-fastq.pl* script of *Popoolation* (Kofler et al. 2011a) to process raw
109 reads, filtering based on read quality (--quality-threshold 20) and length (--min-length

50). We then mapped the processed reads to the stickleback reference genome (BROADS S1) with the program *Bowtie2* (Langmead and Salzberg 2012), using the --end-to-end mapping option. We then used *SAMtools* (Li et al. 2009) to convert the output SAM files to BAM format and subsequently removed reads with mapping quality below 20 (samtools view -q 20). We generated an mpileup file (samtools mpilep -B) and converted the mpileup file to the synchronized (sync) format using *Popoolation2* (Kofler et al. 2011b), which was used for downstream analyses.

Estimates allele frequency differentiation

We estimated population differentiation using F_{ST}, as formulated by Hartl and Clark (1997), using the *fst-sliding.pl* script of *Popoolation2*. We applied the following parameters: --min-count 2, --min-coverage 5, --max-coverage 500, --min-covered-fraction 0, --window-size 1, --step-size 1, --pool-size 30:30:30:30:30:30:30:30:30:30, --suppress-noninformative. We only analyzed genomic regions assembled at the chromosome level (i.e., scaffolds excluded). We categorized F_{ST} outliers as SNPS in the top 5% of the F_{ST} distribution of a given M-FW comparison. We then looked for overlap of F_{ST} outliers among M-FW population pairs. We quantified F_{ST} outlier overlap among M-FW population pairs using a custom bash script. Loci showing relatively large changes in allele frequency (top 5%) in two or more M-FW comparisons within provinces and across Nova Scotia and Newfoundland were taken as three subsets. We then considered 'F_{ST} parallel outliers' SNPs that overlapped among the three sets.

Identification of candidate genes and analysis of molecular function

Genes potentially under parallel selection are expected to contain outlier loci, or more likely, be near them as we used a reduced representation approach. We applied a custom bash script to map outliers to or near protein-coding genes in the reference genome. We limited our search to a set of 14,252 protein-coding gene annotations with attributes "ID=gene" and "biotype=protein_coding". To gain insights into the traits under selection, we analyzed candidate genes for enrichment of molecular functions using the software *ShinyGO* 0.77 (Ge et al. 2020). This program, which is implemented as a graphical web application, uses a large annotation database derived from Ensembl and STRING-db to link lists of genes to functional categories, like gene ontology (GO). We

140 analyzed whether candidate genes were enriched for GO terms (biological process,
141 cellular component, and molecular function), using the FDR threshold of 10% and
142 keeping the 20 most significant hits. Because our data is based on a reduced
143 representation of the genome, there are likely cases where a causal mutation is not
144 sampled but rather a SNP in linkage disequilibrium to it. We thus analyzed significant
145 enrichment of GO terms for genes with outlier loci or within 5kb of one. We compared
146 our list of candidate genes to the set of 14,252 genes from which our candidate genes
147 were sampled and used the 'best matching species' option.

148 **Results**

149 *Data sets and Genomic differentiation among populations*

150 After processing raw RAD-seq data, we identified 18,582 SNPs across all 36 population
151 comparisons. We find that median genome-wide F_{ST} varies widely, ranging from 0.0118
152 to 0.0824 (Table S1; Figure 2). Considerable genomic heterogeneity is found among M-
153 FW comparisons (Figure 3) and between populations from the similar environments
154 (Figure 4). Notably, both the highest and lowest levels of genomic differentiation are
155 between freshwater populations. The least differentiated populations are Pomquet Lake
156 (Nova Scotia) and Pinchgut Lake (Newfoundland), while the most differentiated
157 correspond to Lake Ainslie (Nova Scotia) and Blue Pond (Newfoundland; Figure S1,
158 Table S1).

159 After excluding M-M and FW-FW comparisons, we obtained 25,236 informative
160 SNPs, variable across all 20 M-FW population comparisons. We used this SNP set to
161 look for genomic regions potentially involved in parallel adaptation to freshwater and
162 found 2,100 SNPs as F_{ST} outliers in two or more M-FW comparisons within Nova Scotia
163 (19 SNPs across all six comparisons; Figure S2a). In the four M-FW comparisons within
164 Newfoundland there are 1,306 SNPs in two or more comparisons (Figure S2b). When
165 comparing interprovincial differentiation among 10 M-FW population comparisons, our
166 results show 3,422 SNPs are F_{ST} outliers in at least two comparisons; three SNPs in
167 nine of the 10 comparisons (Figure S2c). In the top 5% of the F_{ST} distribution we find an
168 overlap of 279 SNPs (referred to as 'F_{ST} outliers' hereon) among the three sets M-FW
169 comparisons (NS, NL, and NS-NF), meaning these SNPs are F_{ST} outliers in at least 6/20

170 M-FW comparisons, representing 1.1 % of SNP data set. We found 133 different genes
171 contain or are within 5 kb of any of the 279 F_{ST} outliers (Table S2), with 68 genes
172 containing at least one outlier (Table S3). Notably, two SNPs (chromosome VI position
173 16449327 and chromosome XX position 14009648) fall in the top 5% of the F_{ST}
174 distribution in 19/20 M-FW comparisons: 6/6 M-FW comparisons in Nova Scotia, 4/4
175 comparisons in Newfoundland, and 9/10 interprovincial M-FW comparisons (not an
176 outlier in the Porper Pond and Pomquet Lake comparison). The latter of these two
177 SNPs is 920 bp downstream from a gene coding for a dopamine receptor, *drd2l*
178 (chromosome XX 14002115-14008728).

179 *Gene Ontology*

180 Among genes within 5 kb of an F_{ST} outlier, we found enrichment (FDR = 5%) of
181 biological processes associated with adenylate cyclase-inhibiting dopamine receptor
182 signaling and nervous system development (FDR = 0. 0.043) (Table S4). Molecular
183 functions enriched include neurotrophin binding and dopamine neurotransmitter
184 receptor activity (FDR = 0.019) (Table S5).

185 *Clustering of marine and freshwater populations*

186 Clustering of populations on a phylogram shows a paraphyletic relationship among
187 marine and freshwater populations. A WPGMA tree clusters freshwater populations
188 together, with Lake Ainslie and Black River as one sister clade, Pomquet Lake and
189 Pinchgut Lake as another, and Blue Pond as an out-group (Figure 5). Low bootstrap
190 values (< 50) correspond to branching off marine populations.

191 **Discussion**

192 In this study, we investigated marine and freshwater populations of stickleback along
193 Canada's east coast to gain insights into their evolutionary history and the potential
194 genetic basis of their adaptation to freshwater. Contrary to the expectation of low
195 genome-wide differentiation between marine and freshwater populations in the West
196 Atlantic (Fang et al. 2020a), we found that differentiation among M-FW populations
197 varied widely on the east coast of North America. This suggests a more complex
198 evolutionary history likely involving multiple colonization events or different refugia, and

ongoing gene flow. Evidence of parallel evolution was found as repeated differences in allele frequency at SNP loci across multiple M-FW populations. Notably, we found consistent differentiation of one SNP near a dopamine receptor previously found differentially expressed in freshwater (Di Poi et al. 2016). Likely lacking the alleles for reduced armour found in western North America, our results suggest selection to behaviour or endocrine function played a role in eastern populations persisting in fresh water

Heterogeneous marine-freshwater differentiation in Eastern Canada

Current evidence on the evolutionary history of threespine stickleback indicates this species colonized the Atlantic during the late Pleistocene, as recently as 36.9 thousand years ago (Fang et al. 2018; Fang et al. 2020). During range expansion into the Atlantic, repeated bottlenecks appear to have led to the stochastic loss of some alleles associated with freshwater adaptation, like the *Eda* allele for the low-plated morph (Fang et al. 2020). Loci with alleles conferring higher fitness in freshwater are expected to become rapidly differentiated after freshwater colonization. Therefore, the low M-FW differentiation observed in the Atlantic is thought to be caused by a lack of these alleles in the standing variation present in Atlantic populations relative to Pacific populations (Fang et al. 2020).

Here, we show that although genome-wide M-FW differentiation is indeed low in some population comparisons in eastern North America, other comparisons show a level of differentiation like that observed in the Pacific side (Figure 3). We found the highest differentiation between different freshwater populations. For example, in Newfoundland, stickleback from two lakes isolated from the ocean and only 5 km apart from each other, Pinchgut Lake and Blue Pond, differ considerably in their degree of differentiation relative to marine populations (Figure 4). Blue Pond is a relatively small lake with no input or output streams and is only fed by rainwater, thus immigration from neighbouring sites is unlikely. Barriers to gene-flow likely played a role in differentiating Blue Pond, as genetic drift drives populations apart in the absence of gene flow. An alternative explanation is that there were multiple waves of colonization in Eastern Canada, as occurred in the Japanese Archipelago (Kakioka et al. 2020). The clustering

229 of freshwater populations is consistent with three colonization events, one by the
230 lineage in Blue Pond, one by the lineages in Lake Ainslie and Black River, followed by a
231 more recent expansion to Pomquet and Pinchgut lakes. However, our results are also
232 consistent with freshwater populations that evolved in isolation in different glacial refugia
233 during the last ice age (Mäkinen and Merilä 2008).

234

235 *Evidence of selection on dopamine receptors*

236 Adaptation to similar ecological conditions through re-use of standing genetic variation
237 in a source population is thought to be the dominant mechanism leading to parallel
238 evolution (Schlötterer 2023). The low-plated allele of the *Eda* gene, which is found at
239 low frequencies in marine populations, is known to confer higher fitness in freshwater
240 (Marchinko and Schluter 2007; Barrett et al. 2008; Rouzic et al. 2011). Loss of this allele
241 throughout much of the Atlantic gene pool helps explain why parallel evolution of the
242 low-plated phenotype is not observed in lakes of eastern Canada. However, *Eda* is not
243 the only gene known to be selected in freshwater, as osmoregulatory genes have also
244 been implicated in freshwater adaptation at a global scale, including Na/K ATPase
245 pump and $Na^{+/}K^{+/}2Cl^{-}$ cotransporter (Deagle et al. 2013; Divino et al. 2016; McCairns
246 and Bernatchez 2010).

247 We did not find evidence of molecular evolution near the *Eda* locus, which is
248 consistent with historical observations of stickleback phenotypes in lakes from this
249 region of North America and stochastic loss of allele during range expansion (Hagen
250 and Moddie 1982; Fang et al. 2020). Nonetheless, the west Atlantic stickleback
251 populations sampled here show evidence of parallel evolution, with 1.1% of SNPs
252 showing some evidence of repeated differentiation in M-FW comparisons in Nova
253 Scotia, Newfoundland, and between the two provinces. Notably, we found repeated M-
254 FW differentiation of SNPs near dopamine receptors, *Drd4a* and *Drd2l*, and enrichment
255 of gene functions related dopamine receptor activity and nervous system development.
256 These results suggest that stickleback might have adapted to freshwater through
257 parallel changes in behaviour, like aggression.

258 Individual variation in endocrinology tends to be underutilized in evolutionary
259 biology despite its potential to bridge physiology with ecology and genetics (Williams
260 2008). In zebrafish (*Danio rerio*), the expression profile of dopamine receptors *Drd2c*
261 and *Drd3* have been found to correlate with individual differences in aggression,
262 particularly between dominant and subordinate males (Filby et al. 2010). In stickleback,
263 exposure to a simulated predator lowers the expression of *Drd4a* (Abbey-Lee et al.
264 2018). At the population level, marine and freshwater populations show extensive
265 differences in the behavioural parameters of sociability, aggressiveness, and levels of
266 activity (Di-Poi et al. 2014). A study comparing physiological regulatory networks in
267 freshwater and marine populations of stickleback indicates that evolution of receptors,
268 like *Drd2*, and not ligands (e.g., monoamine levels or metabolites) are associated with
269 population divergence.

270 The dopaminergic system is also known to inhibit prolactin which, in fish, plays
271 an important function in osmoregulation (Liu et al. 2006; Yamamoto and Vernier 2011;
272 Mancera and McCormick 2019). Prolactin increases retention of ions like Na^+ and Cl^-
273 and decreases water uptake, giving it the name the 'freshwater-adapting hormone' (Liu
274 et al. 2006). Prolactin mRNA levels correlate with local ion concentrations in natural
275 populations of the black-chinned tilapia (*Sarotherodon melanotheron*) adapted to
276 different salinities (Tine et al. 2007). In the spotted scat (*Scatophagus argus*), a
277 euryhaline fish, the renal dopaminergic system responds via *Drd1* to hypo-osmotic
278 shock by inhibiting Na^+/K^+-ATPase (Su et al. 2016). In stickleback, prolactin is linked to
279 freshwater migration and tolerance (Lam and Hoar 1967; Ishikawa et al. 2016; Pavlova
280 et al. 2020; Taugbøl et al. 2022). Thus, the parallel differentiation of regions near
281 dopamine receptors in M-FW comparisons may reflect adaptation mediated by
282 phenotypic changes to behaviour, osmoregulation, or both.

283

284 **Conclusion**

285 Using genome-wide SNPs, our study showed heterogeneous genomic differentiation
286 between marine and freshwater populations of threespine stickleback in eastern
287 Canada. We found evidence of parallel evolution near dopamine receptors, potentially

meaning that behaviour was under selection upon colonization of freshwater lakes. Alternatively, individuals with alleles associated with specific behaviour profiles may have been predisposed for undergoing range expansion into freshwater. Genetic changes near genes for dopamine regulation may also be connected to osmoregulatory adaptation. Overall, our results suggest that parallel evolution in eastern Canada, and perhaps across much of the Atlantic, may be reflected not as morphological changes but as adaptation of hormone-mediated phenotypes.

Bridging text

Chapter 3 presented an investigation of freshwater adaptation and the evolutionary history of post-glacial populations of stickleback from Eastern Canada. In Chapter 4, we focus on the lower limits of contemporary evolution. We use estuary stickleback as a system for glimpsing the first steps of adaptive evolution.

313

314

315

316

317

318

319

320

321

322

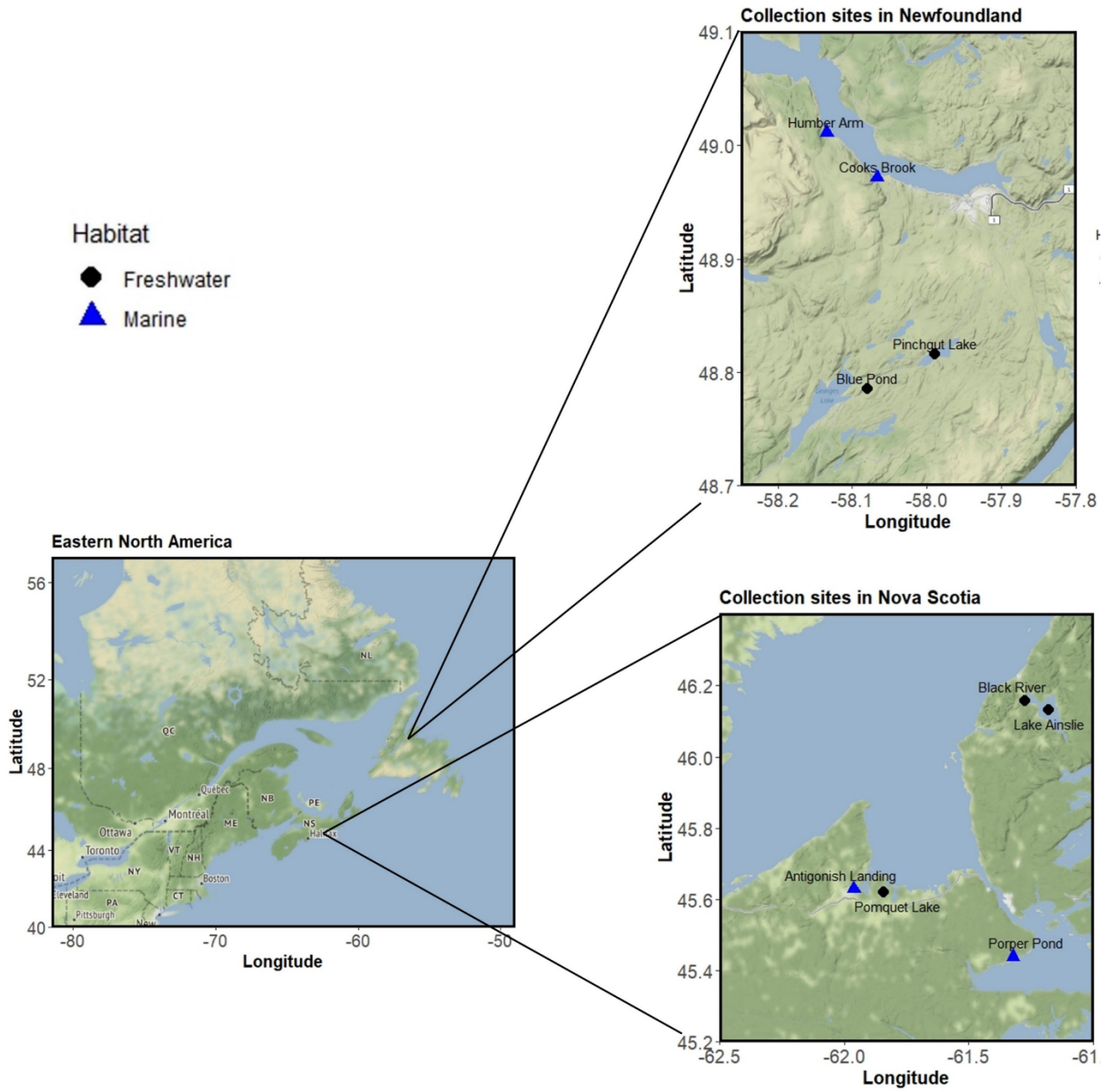


Figure 1. Collection sites in Eastern Canada.

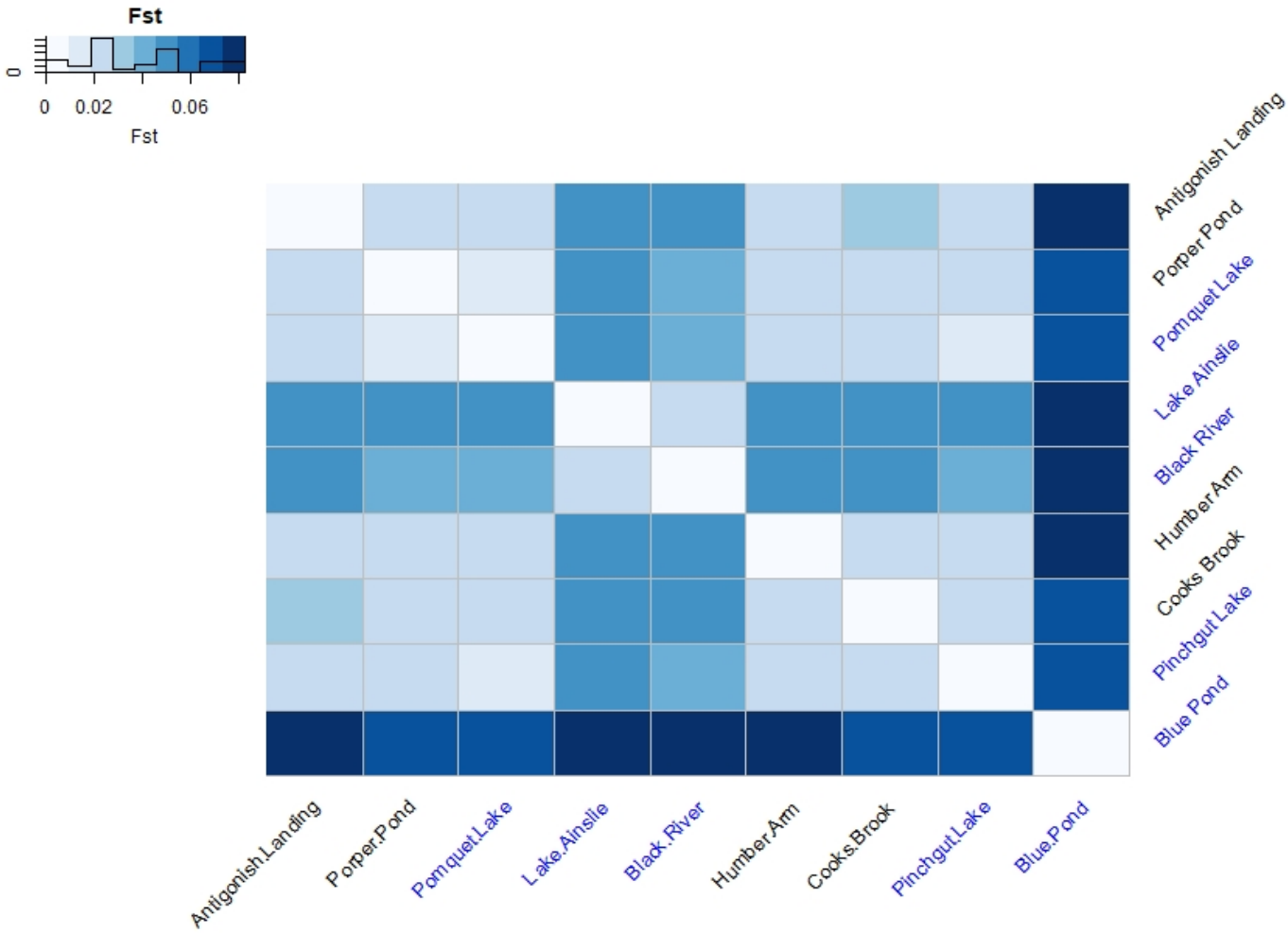
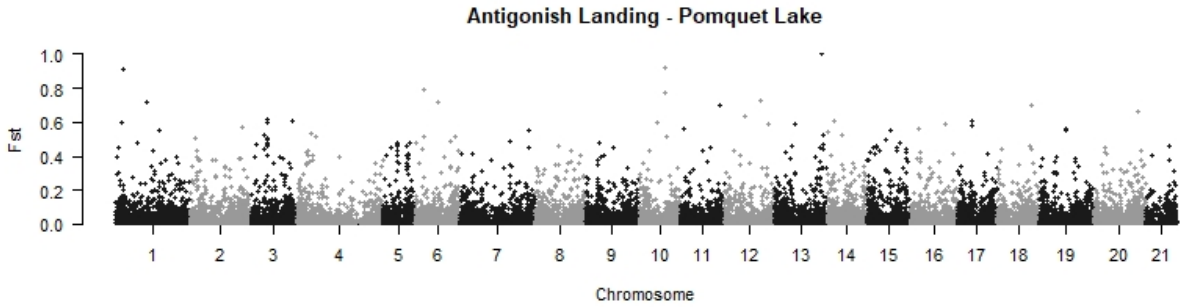
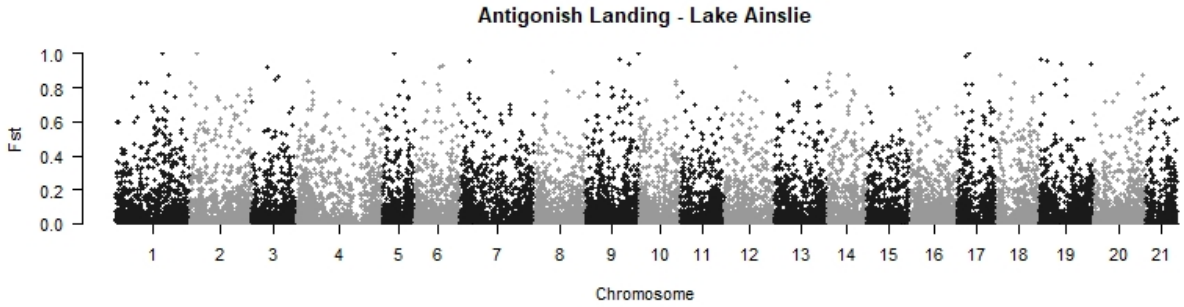
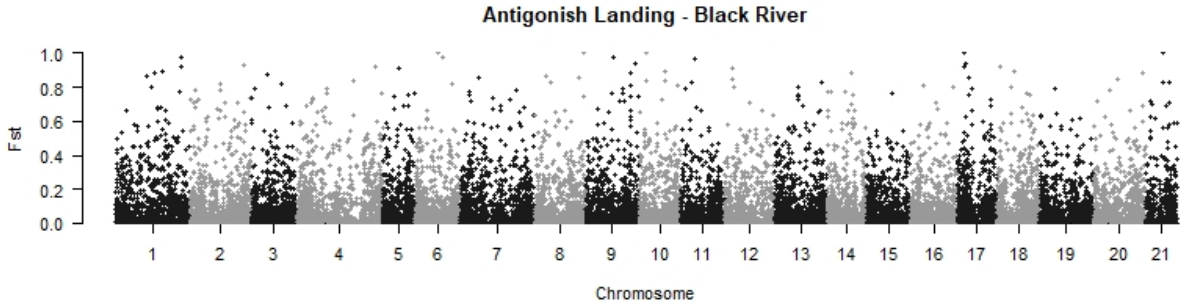
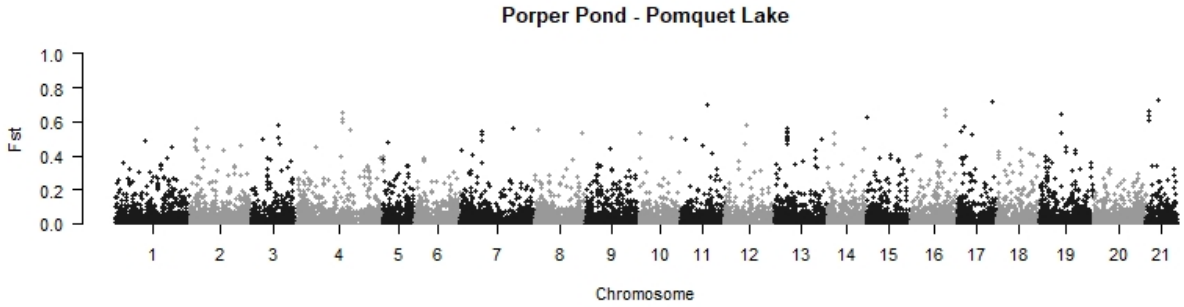


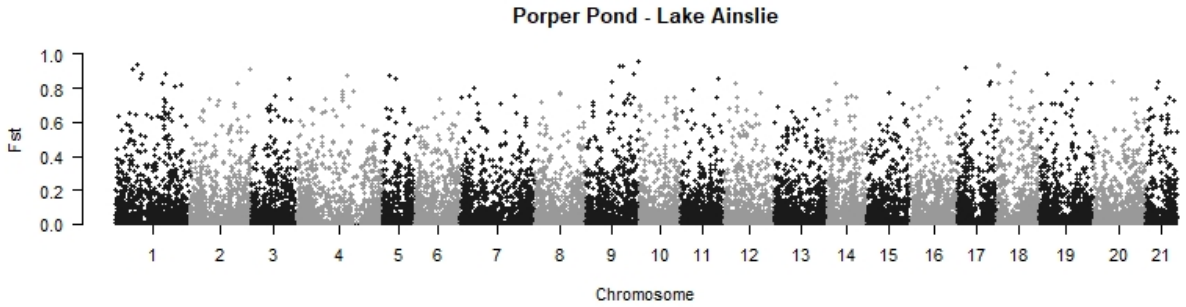
Figure 2. Heatplot depicting average (median) pair-wise F_{ST} with histogram of F_{ST} values. Freshwater sites are labelled blue.

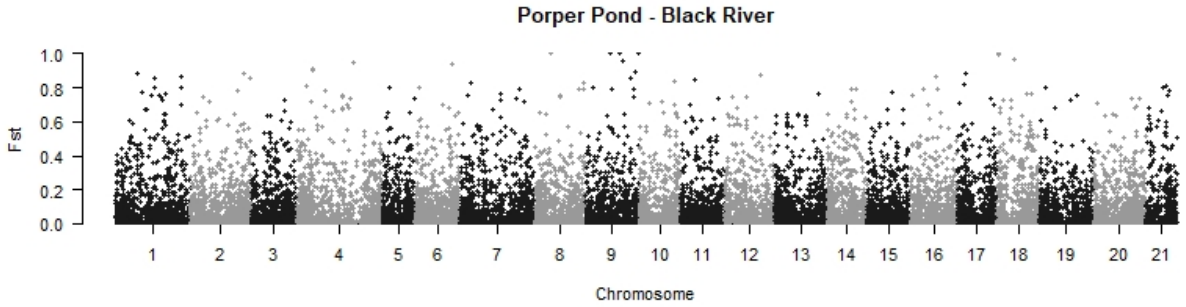

Antigonish Landing - Pomquet Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

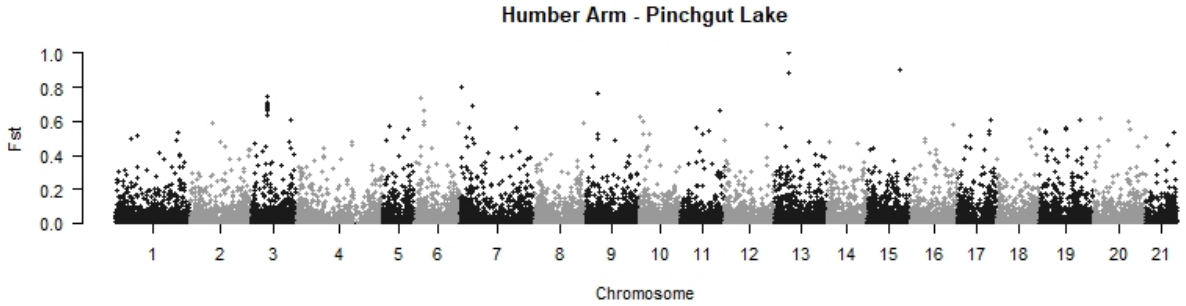

Antigonish Landing - Lake Ainslie
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

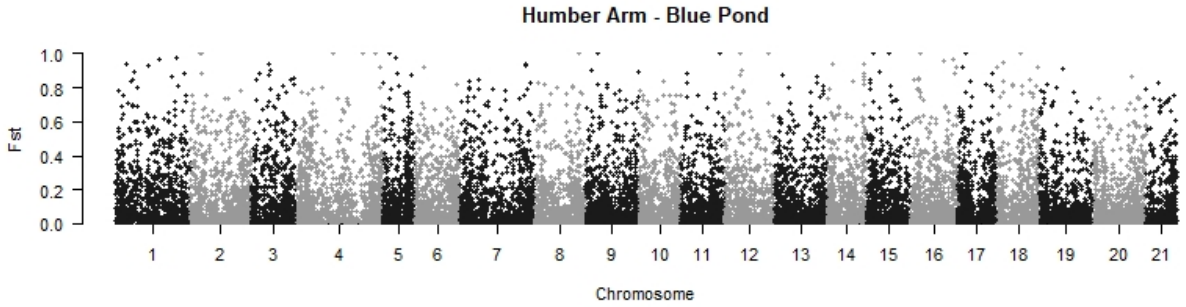

Antigonish Landing - Black River
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

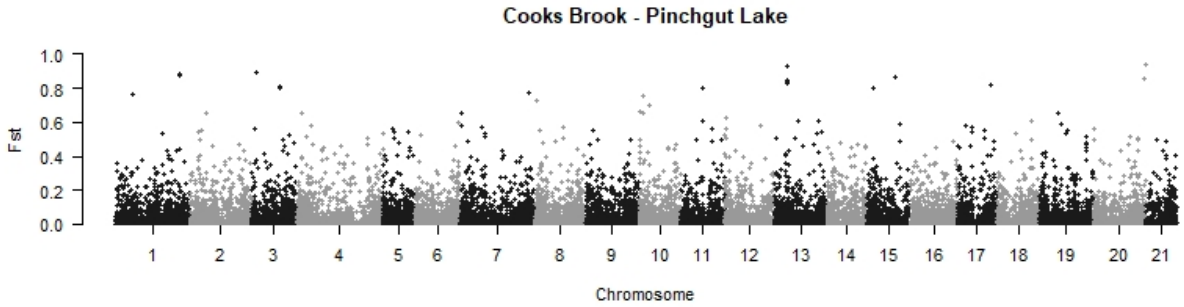

Porper Pond - Pomquet Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

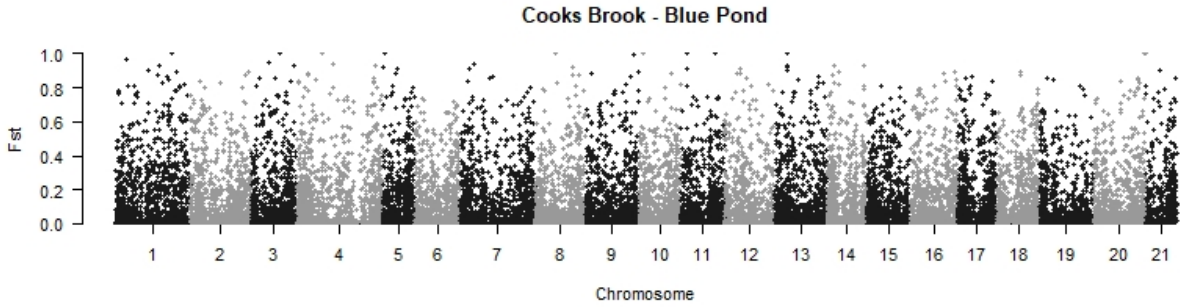

Porper Pond - Lake Ainslie
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

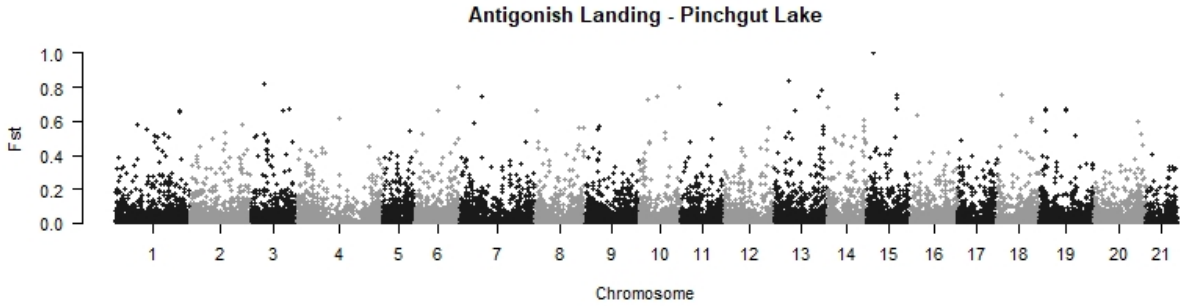

Porper Pond - Black River
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

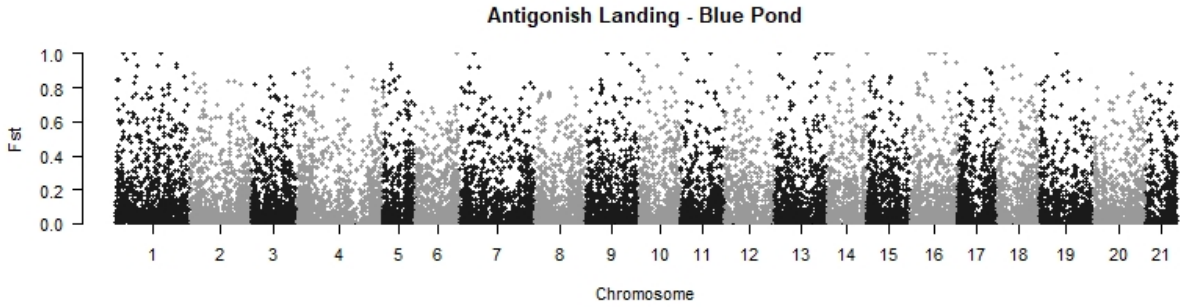

Humber Arm - Pinchgut Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

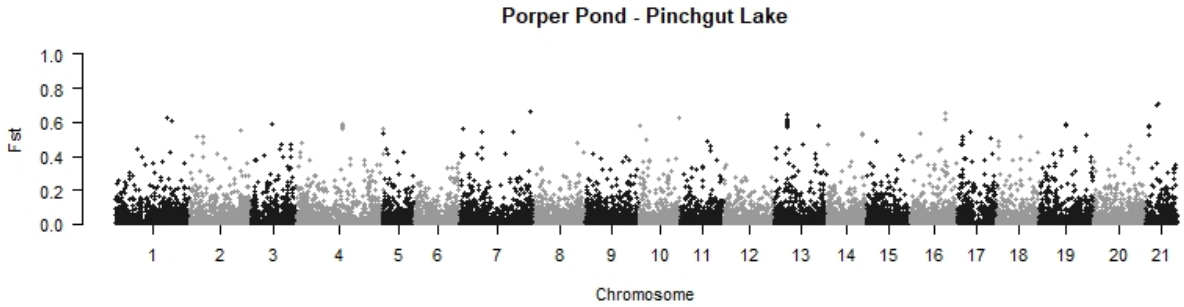

Humber Arm - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

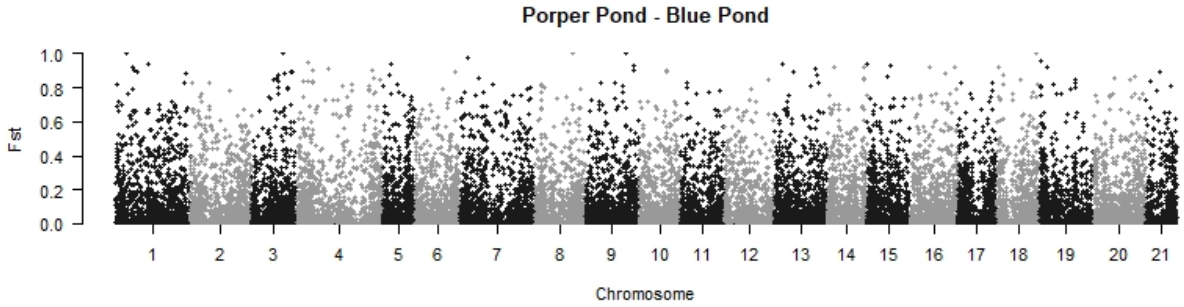

Cooks Brook - Pinchgut Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

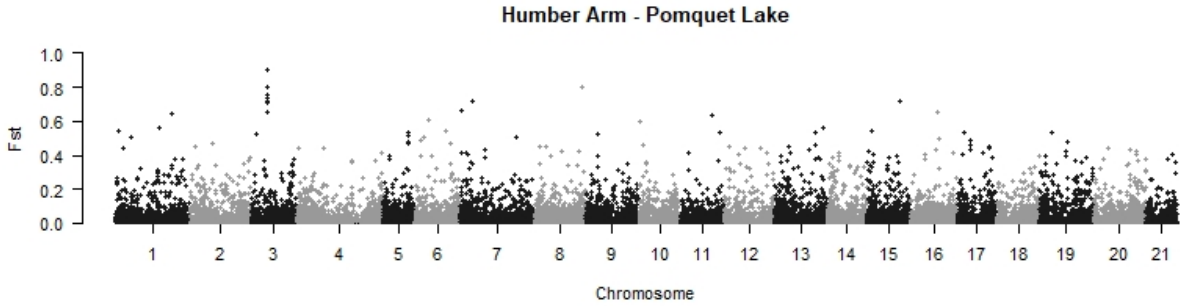

Cooks Brook - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

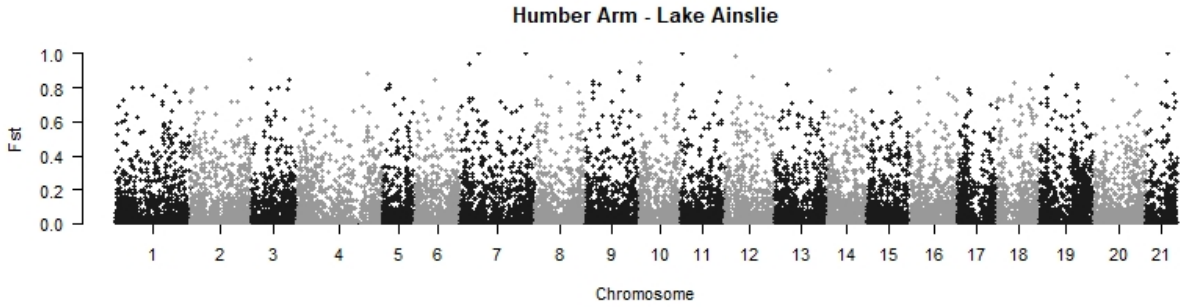

Antigonish Landing - Pinchgut Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Antigonish Landing - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Porper Pond - Pinchgut Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Porper Pond - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Humber Arm - Pomquet Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Humber Arm - Lake Ainslie
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

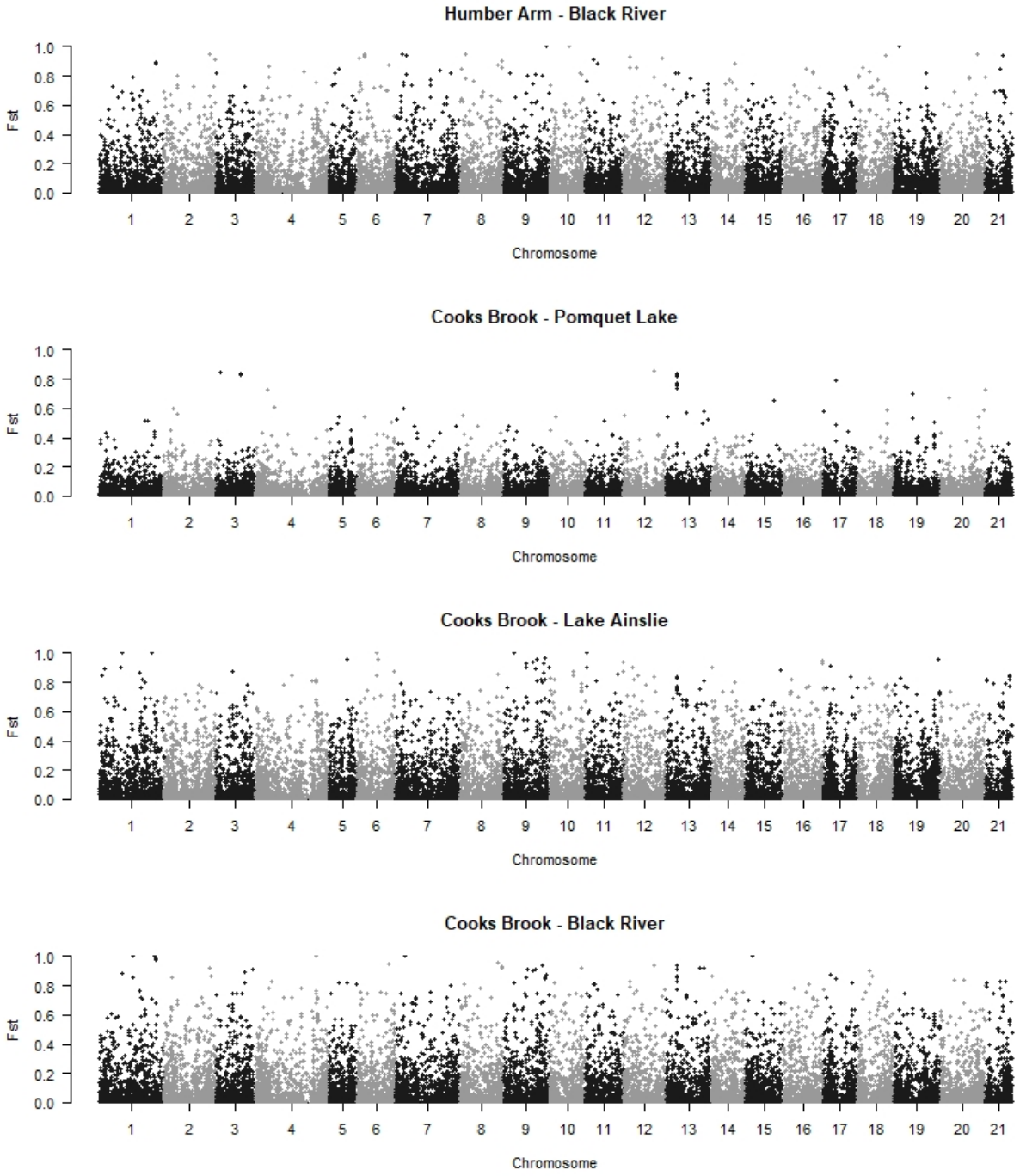
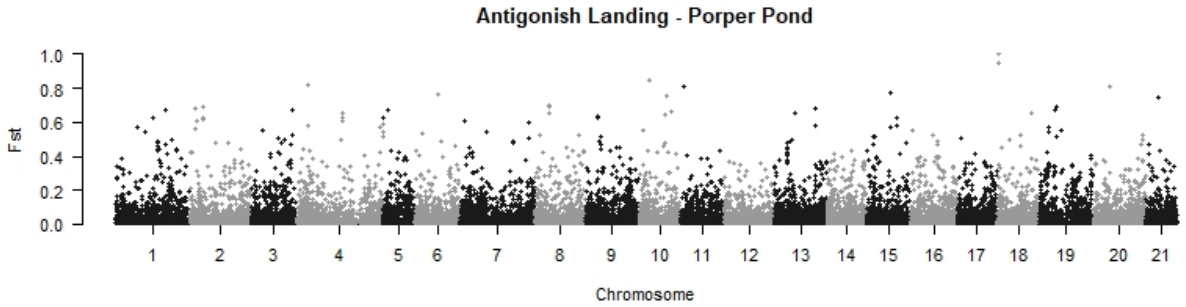
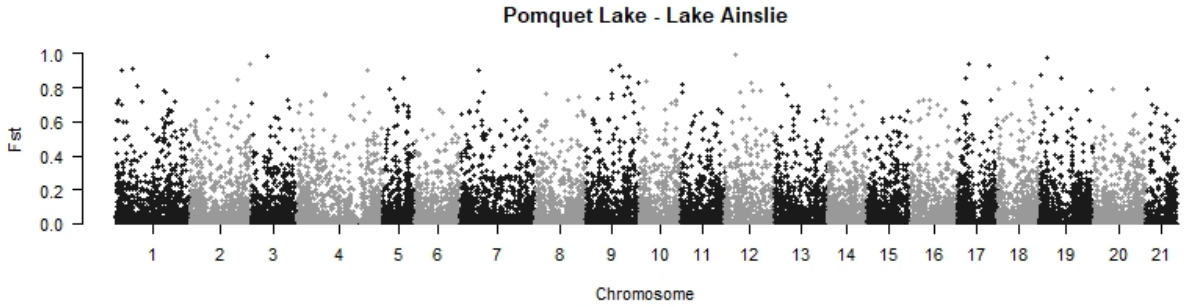
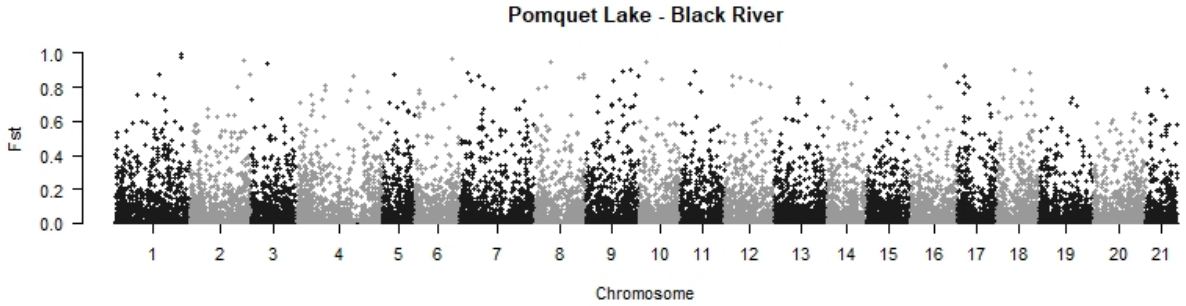
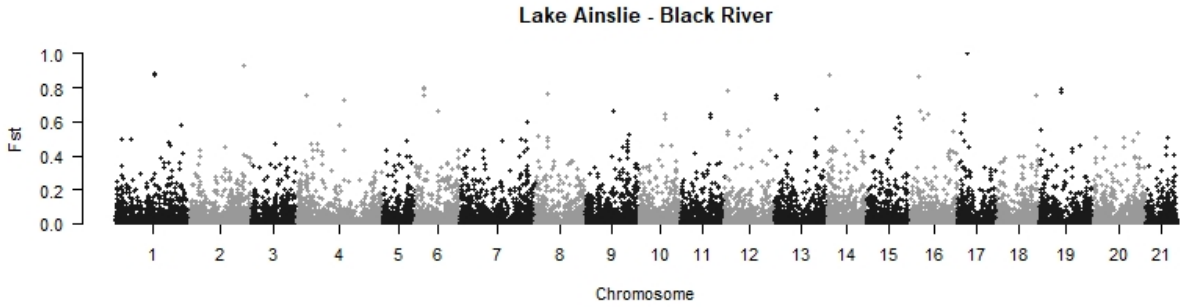


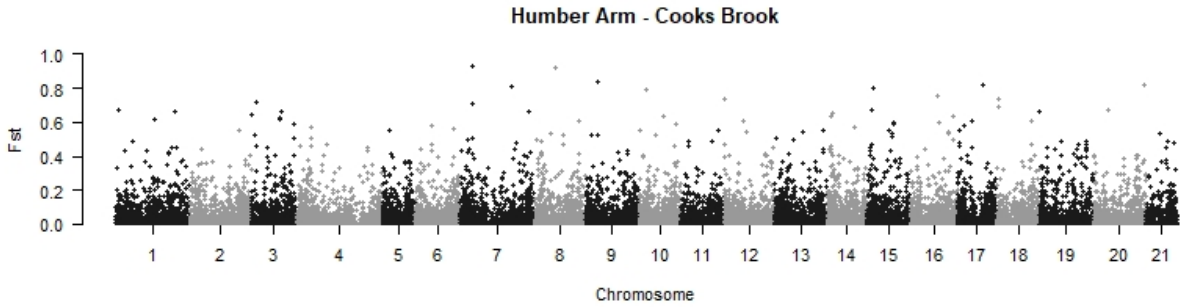
Figure 3. Manhattan plot of FST values showing heterogeneity in 20 marine-freshwater (M-FW) comparisons.

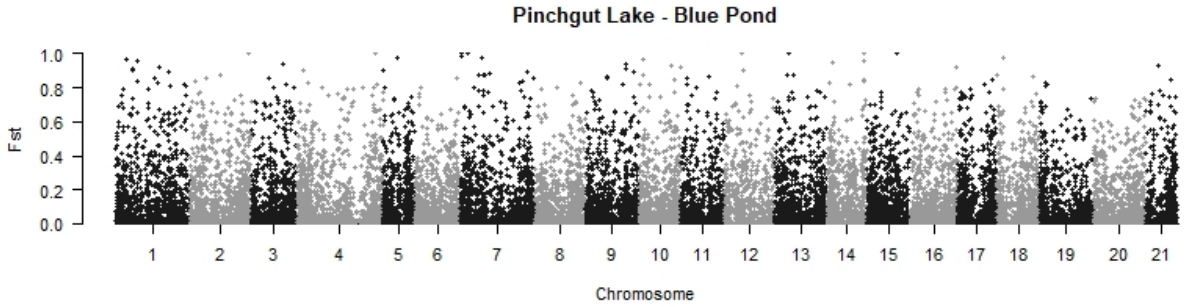

Antigonish Landing - Porper Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

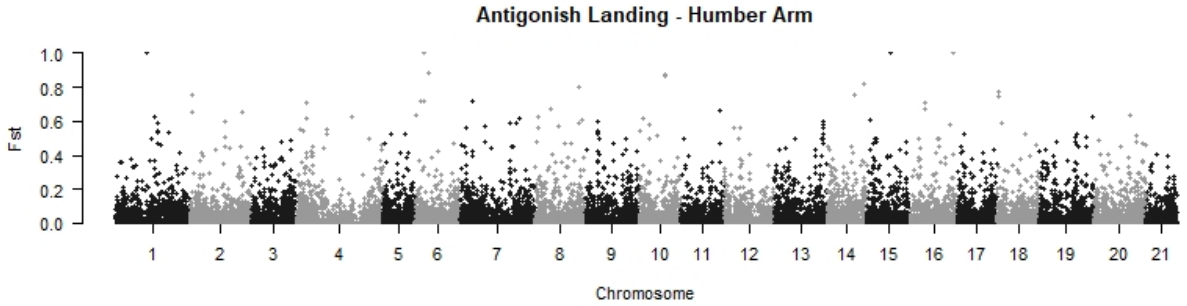

Pomquet Lake - Lake Ainslie
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

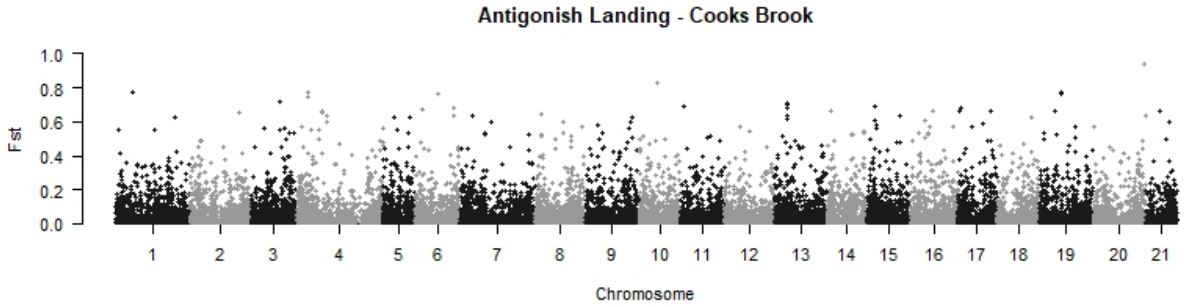

Pomquet Lake - Black River
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

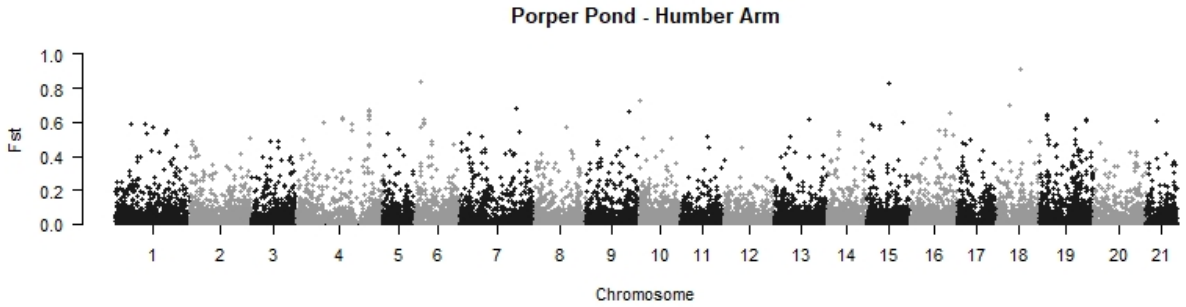

Lake Ainslie - Black River
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

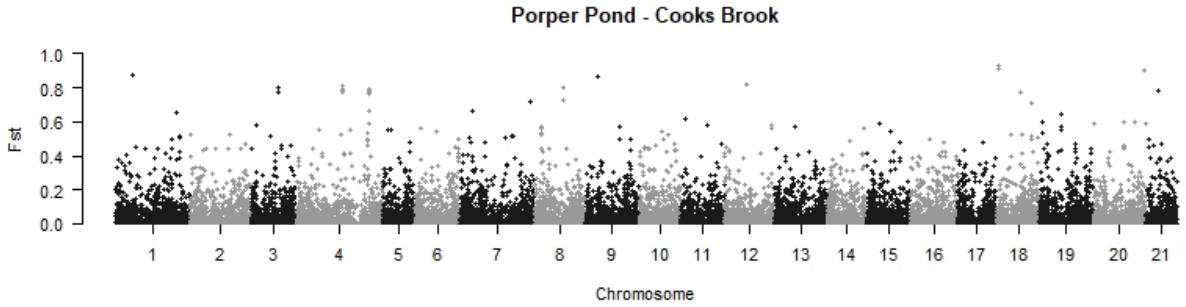

Humber Arm - Cooks Brook
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

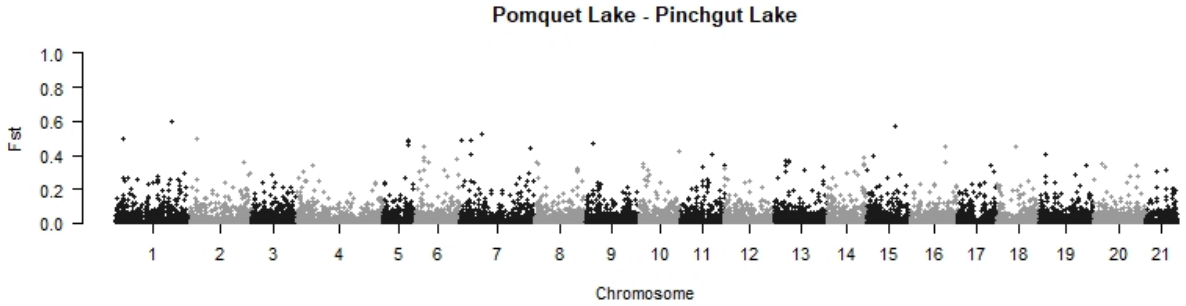

Pinchgut Lake - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

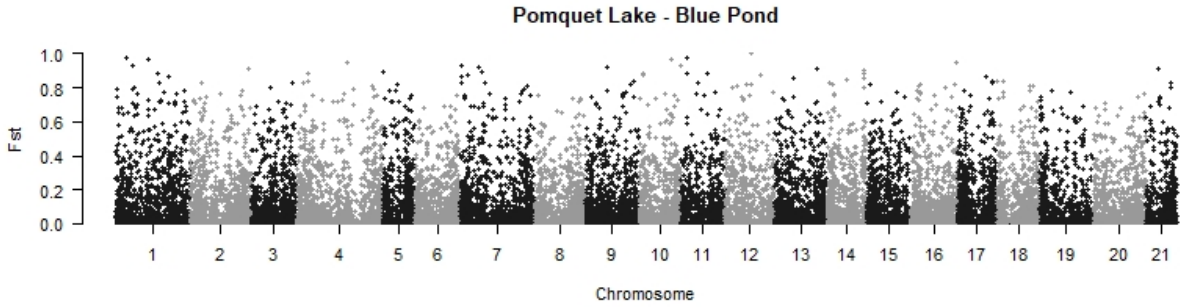

Antigonish Landing - Humber Arm
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Antigonish Landing - Cooks Brook
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Porper Pond - Humber Arm
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Porper Pond - Cooks Brook
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Pomquet Lake - Pinchgut Lake
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome


Pomquet Lake - Blue Pond
Fst
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Chromosome

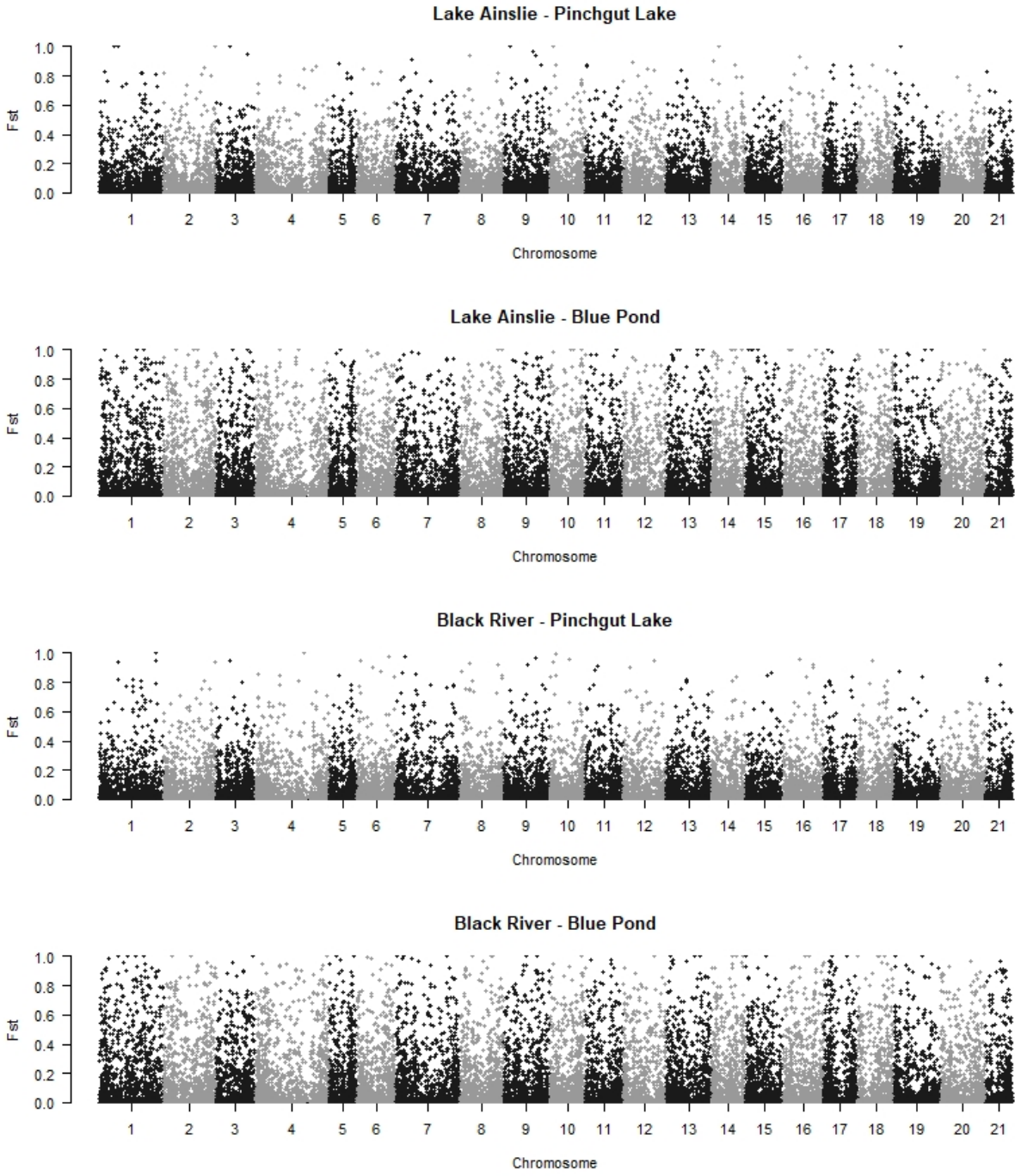


Figure 4. Manhattan plots of populations from similar environments (M-M and FW-FW comparisons).

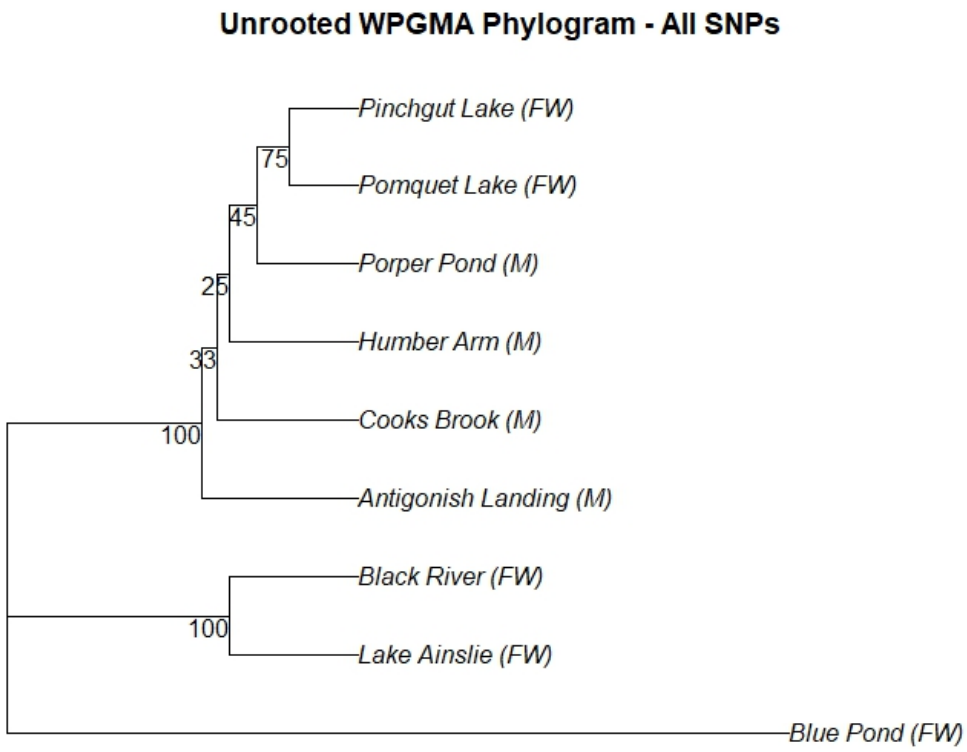


Figure 5. Phylogenetic tree of marine (M) and freshwater (FW) populations.