Relative genomic impacts of translocation history, hatchery practices, and farm selection in Pacific oyster *Crassostrea gigas* throughout the Northern Hemisphere

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ABSTRACT

Pacific oyster *Crassostrea gigas*, endemic to coastal Asia, has been translocated globally throughout the past century, resulting in self-sustaining introduced populations (naturalized). The oyster aquaculture industry depends on commercially available seed (hatchery-farmed) or naturalized oysters that can be moved onto a farm (naturalized-farmed), making it critical to understand patterns of genetic variation among populations and farm types. Here we genotype wild or naturalized populations from France, Japan, China, and most extensively in coastal British Columbia, Canada. We also genotype cultured populations from throughout the Northern Hemisphere to compare with naturalized populations. In total, 16,942 markers were identified using double-digest RAD-sequencing in 182 naturalized, 112 hatchery-farmed, and 72 naturalized-farmed oysters (total = 362 individuals). Very low genetic differentiation was observed around Vancouver Island (mean F_{ST} = 0.0019), and low differentiation between countries in Japan, France, and Canada (France-Canada F_{ST} = 0.0024; Japan-Canada F_{ST} = 0.0060), all of which are in the same historical translocation lineage. Chinese populations had much higher F_{ST} (China-Japan F_{ST} = 0.0241). Hatchery-propagated populations had higher inter-individual relatedness suggesting family structure. Within-population inbreeding was not detected on farms, but nucleotide diversity and polymorphism rate were both lower in some farms. Moving oysters from nature onto farms did not result in strong within-generation selection. Private alleles were identified in several hatchery populations grown in BC, suggesting non-local origins. Putative domestication outliers were identified throughout the genome, with some identified consistently on multiple farms. These top outlier candidates were nearby genes involved in calcium signaling and calmodulin activity. Implications of potential introgression from hatchery-farmed oysters depends on whether naturalized populations are considered as a valuable, locally-adapted resource or as an introduced, invasive species, but given the value of the industry in BC and the challenges the industry faces (e.g., climate change, crop losses, abiotic/biotic stressors), this remains an important question.

Keywords: Aquaculture, domestication, farm selection, genetic diversity, hatchery, oyster, oyster farming, marine genomics, Pacific oyster

INTRODUCTION

Wild populations contain standing genetic variation that is critical for adaptation to changing environments (Barrett & Schluter, 2008). When domestic strains of such species exist, genetic differences between wild and domestic lineages can provide an important source of variation for broodstock development (Guo, 2009). Identifying, preserving, and using these genetic resources effectively is essential for sustainable development of aquaculture species (Guo, 2009). Genetic markers that differentiate populations can be useful to assign unknown origin individuals to populations of origin (Beacham et al., 2017), to detect domestic population influence (e.g., introgression of domesticated alleles) (Wringe et al., 2018), and to manage broodstock (Arbelaez et al., 2019). Once markers are identified, they can be used to generate robust, multi-purpose high throughput amplicon sequencing panels (Meek & Larson, 2019) or be added to SNP chips (Gutierrez et al., 2017). Better understanding of the genetics of cultured organisms co-existing with wild populations is needed to provide tools for monitoring the diversity of the species, to direct selective breeding efforts, and to ensure good husbandry practices.

Genetics is particularly useful to understand population connectivity in in aquatic organisms due to the difficulty in directly observing organism movement (Gagnaire et al., 2015). However, marine invertebrates have high fecundity and dispersal, as well as large population sizes, and these factors often result in little to no signal of genetic differentiation (Gagnaire et al., 2015). High fecundity in particular can also result in chaotic genetic patchiness, where signals of genetic differentiation do not correlate with spatial distance and can fluctuate over time (Eldon et al., 2016). This phenomenon occurs due to sweepstakes reproductive success, where a small number of individuals produce many offspring, and the factors leading to this production is largely random, due to random matching of reproductive timing and favourable environment conditions. Sweepstakes reproductive success can therefore result in a low effective population size (Ne) relative to census size (Nc) (Hedgecock & Pudovkin, 2011). Chaotic genetic patchiness has been observed in many marine invertebrates (Villacorta-Rath et al., 2017), including the Pacific oyster (Sun & Hedgecock, 2017). Nonetheless, fine-scale genetic structure has been identified in marine invertebrates such as the American lobster *Homarus americanus* (Benestan et al., 2015) and the sea scallop *Placopecten magellanicus* (Lehnert et al., 2018). The use of high-density markers combined with environmental metadata improves the likelihood of identifying such genetic structure.

High fecundity can also result in high polymorphism rate, and this has been observed in Pacific oyster (Plough, 2016). Producing such numerous gametes requires many mitotic cell divisions, during which mutations accumulate and elevate levels of heterozygosity (Williams, 1975; Launey & Hedgecock, 2001). Sweepstake reproductive success can permit the introduction of deleterious mutations into a population at high frequency (Harrang et al., 2013). In Pacific oyster, high mutational load is expected, with numerous slightly deleterious recessive mutations. This can result in high rates of larval mortality (Launey & Hedgecock, 2001), and severe inbreeding effects (Hubert, 2004; Hedgecock et al., 2005). To counteract such genetic effects, breeders have been advised to cross divergent, high-performing strains to benefit from heterosis in offspring (Hedgecock & Davis, 2007). In a similar context, crossing divergent populations may increase heterozygote advantage, but this requires knowing which populations are genetically distinct.

Bivalve aquaculture is a valuable resource globally. Most of the harvested production originates from aquaculture (89%), and the global, per annum value of farmed bivalves is 20.6 B USD (Wijsman et al., 2019). In BC in 2016, the estimated value of Pacific oyster aquaculture was 14.8 M CAD in revenue (Sun & Hallin, 2018). The oyster aquaculture industry depends on either commercial hatcheries for production, or natural recruitment events where naturally set oysters are transplanted to a farm. When commercial hatcheries are used, the exact country of origin of the broodstock is rarely known, and oysters from multiple sources are combined together and grown on a farm. Whether naturalized (or wild) populations exhibit local adaptation has yet to be demonstrated, but if occurs could allow populations to be more fit to local conditions in terms of food availability, disease, harmful algae, and levels of oxygen, temperature, and pCO_2. Identifying genes involved in either domestication or local adaptation will be informative for guiding selective breeding. Identifying haplotypes specific to broodstock lines will also be useful for tracking introgression of international commercial hatchery populations into naturalized or wild populations. Best practices for broodstock development could both require maintenance of genetic diversity as well as incorporation of alleles conferring higher survivorship under adverse conditions or higher fitness in specific environments. Although relatedness can be determined through pedigrees or low-density panels (Kijas et al., 2019), high-density genetic studies are necessary to pinpoint informative genes and markers.

Farmed Pacific oyster are often grown in areas in which they are not endemic (De Silva, 2012). Pacific oyster originated in East Asia (i.e., coastal Japan, Korea, North China) but has been translocated globally for over a century (Guo, 2009; De Silva, 2012). Pacific oyster has now been introduced to over 66 countries, and grows in self-sustaining (i.e., naturalized) populations in at least 17 countries (Herbert et al., 2016). In Canada, Pacific oyster was introduced repeatedly in the early 1910s until the 1960s to supplement the declining Olympia oyster *Ostrea conchaphila* fishery (Guo, 2009). In France, Pacific oyster was introduced in the 1970s using broodstock (i.e., adult individuals) from British Columbia (BC) and spat (i.e., juveniles attached to substrate) from Japan (Grizel & Heral, 1991). Translocations have therefore resulted in many naturalized populations in coastal BC and Europe (e.g., France, Denmark) (Herbert et al., 2016; Anglès d'Auriac et al., 2017; Reise et al., 2017).

Population genetic analyses to date have not found evidence for bottlenecks during Pacific oyster introductions in BC or France (Sun & Hedgecock, 2017; Vendrami et al., 2018). Very low genetic differentiation between translocated naturalized populations and Japanese populations has been observed (Vendrami et al., 2018). In regions that have been recently colonized with rapid expansion of oyster populations, such as the Wadden Sea of Denmark, lower genetic diversity is observed (Vendrami et al., 2018). In the North East Pacific ocean (e.g. BC and Washington State, USA), low population differentiation was observed using 52 coding SNP markers (Sun & Hedgecock, 2017). This study also identified higher temporal variation than spatial variation, providing evidence for sweepstakes reproductive success (Sun & Hedgecock, 2017). It is not clear whether using a higher density of markers, including non-coding loci, will provide improved resolution of population structure in coastal BC.

Here we address three important aspects of the population genetics of Pacific oyster. First, we examine the genetic diversity and differentiation of oysters within BC by characterizing four naturalized populations, including two collections occurring across multiple brood years. Wild/naturalized oysters from Japan, France, and China

were also genotyped to put the observed genetic differentiation into a broader context. All samples were genotyped using double-digest restriction-associated DNA sequencing (ddRAD-seq), to genotype 16,942 RAD loci and thus represent ~0.255% of the Pacific oyster genome. Second, to better characterize differences between hatchery-farmed and naturalized oysters, several farm populations grown in BC, France, the United Kingdom (UK), and China were genotyped at these same markers. This permitted the investigation of loci under putative domestication selection (i.e., differential selection between hatcheries/farms and nature). Third, to characterize the selective landscape associated with growth on a farm, naturalized oysters moved to a farm were compared to their source populations in three parallel comparisons across the Northern Hemisphere (i.e., Canada, France, and China). Collectively, these analyses provide insight into the relative genomic impacts of hatchery practices, farming, and translocation history on the contemporary genetic diversity that exists in Pacific oysters throughout the Northern Hemisphere.

METHODS

Sample collections

Pacific oyster *Crassostrea gigas* individuals were sampled from a variety of sources, including those growing in nature, either as wild (endemic) populations or as naturalized (introduced) populations (both referred to as NAT), those growing in farms, either from commercial hatcheries (HAT-F) or from NAT populations moved to farms (NAT-F), as well as those from hatcheries destined for farms but not yet transferred onto a farm (HAT). NAT samples were obtained from introduced populations (i.e., BC, France) or wild populations (i.e., China, Japan; Table 1). HAT samples were obtained from Guernsey; the ultimate source of this broodstock is unknown. HAT-F samples were obtained from BC with origins from commercial hatcheries in Canada, US, and/or Chile (exact source unknown), as well as from China, with origins from Chinese populations.

BC NAT collections were conducted during summer 2017 (Figure 1A; Table 1). Each oyster was measured for length and weight for estimating approximate brood year, and a section of the mantle was sampled in 95% ethanol. Naturalized populations were obtained from Pendrell Sound (PEN), Serpentine River (SER), Pipestem Inlet (PIP), and Hisnit Inlet (HIS; Figure 1). Different sizes of oysters for Pendrell Sound and Hisnit Inlet were collected and assumed to be of different brood years. This included small = 3.0-4.9 cm (size class = 2); medium = 5.1-7.0 cm (size class = 3); and large = 11.1-15.0 cm (size class = 6) oysters. Small and medium size classes were obtained for PEN (n = 15 and 20, respectively), and medium and large were collected for HIS (n = 19 and 20, respectively). BC HAT-F populations were sampled from farms located at Deep Bay (DPB) and Rosewall Creek (ROS), BC. BC NAT-F samples were sampled from a population grown on a farm in Baynes Sound with the probable source being Pendrell Sound, BC.

International NAT samples included those from France and China. France (FRA) samples were collected as tissue samples of naturally set adult oysters in Northwest France from a beach near Sainte-Barbe (48.765167, -2.994716). China (CHN) NAT samples were collected in the Qingdao region. Both France and China NAT-F collections were made, from a farm in western France and a farm in China. China HAT-F samples were also

collected, one from a short-term culture (QDC, 2nd generation) and one from a long-term culture (RSC, 6th generation).

HAT samples were obtained from a hatchery in Guernsey, UK, but the origin of the broodstock for these samples is unknown (J.P. Hastey, *pers. comm.*). Samples were also obtained from local markets in Japan, but it was not possible to know whether these were farmed or wild origin. All international samples were collected by local researchers (see *Acknowledgements*) or obtained from commercial markets, obtained in accordance with local regulations, and shipped according to international regulations.

DNA extraction, quantification and quality control

DNA was extracted using the BioSprint automated extraction protocol in plate format (QIAGEN), using a proteinase-K digestion and an RNase incubation to reduce RNA presence in genomic DNA, as per manufacturers' instructions. Sections of tissue used for extraction were ~4 x 4 mm. Purified genomic DNA was quantified with a Qubit (High Sens; ThermoFisher) and quality checked for fragmentation using 1% agarose gel electrophoresis. Genomic DNA was normalized to 20 ng/µl for library preparation.

Restriction site associated DNA (RAD) sequencing enzyme selection

To choose restriction enzymes and fragment sizes to produce an optimal number of loci, an *in silico* digest was simulated using the Pacific oyster reference genomes (i.e., GenBank GCA_000297895.1 (Zhang et al., 2012) and the chromosome level assembly (Gagnaire et al., 2018)) in SimRAD (Lepais & Weir, 2014). Initial digests with *Pst*I and *Msp*I indicated low cut frequency due to the low GC content in the oyster genome (33.5%); therefore, *Nsi*I, a 6-base cutter with fewer GC recognition sites was used in place of *Pst*I. Using *Nsi*I and *Msp*I with a size selection of 100-250 bp, in total, 15,770 and 15,319 sites were predicted in the Zhang *et al.* (2012) and Gagnaire *et al.* (2018) genomes assuming that the assembly was 93% and 47% complete, respectively (see code in Data Accessibility). The number of markers was calculated based on the 100% assembly expansion (i.e., a complete genome size of 600 Mb).

Double-digest RAD-sequencing library preparation

In a 96 well plate, 10 µl of 20 ng/ul DNA per individual was fragmented using the high-fidelity enzymes *Nsi*I / *Msp*I (NEB) combined in a double digest, then ligated via sticky ends to a unique forward adapter and a common Y-adapter specific to the Ion Torrent Proton (Thermo Fisher) following previously published procedures (Mascher et al., 2013), using barcodes and size-selection adaptation as described previously (Recknagel et al., 2015). The entire ddRAD-seq protocol is provided on protocols.io (see *Data Availability*). In brief, genomic DNA was digested and ligated to barcodes, then 5 µl of each sample was multiplexed into libraries of between 24 and 48 samples, purified using PCR clean-up columns (QIAGEN), size-selected for a size range of 250-300 bp on a Pippin Prep (Sage Biosciences). After size-selection, four PCR reactions (16 cycles) were conducted and pooled to reduce PCR bias, similar to Mascher *et al.* (2013). Each PCR was pooled and then cleaned up with PCR clean-up

columns (QIAGEN). Pools were quantified by Qubit, and diluted to 200 pM in preparation for sequencing on an Ion Torrent Proton.

Libraries were sequenced on an Ion Torrent Proton using an Ion PI Chip Kit v3 chip (Thermo Fisher), as per manufacturers' instructions. Base calling was done with Torrent Suite 5.10.1 (Thermo Fisher) and multiplexed FASTQ output files with barcodes still present were exported using the Torrent *FileExporter* plugin.

Sequence data pre-processing and alignment against reference genome for genotyping

All sequence processing and analysis occurred within a modified version of *stacks_workflow* (E. Normandeau, *pers. comm.*; see *Data Availability*) with custom analysis scripts and documentation within an additional, separate GitHub repository (see *Data Availability*). Raw FASTQ files were inspected before and after trimming using FastQC v0.11.8 (Andrews, 2010), with output aggregated by MultiQC (Ewels et al., 2016). Universal adapters were removed, and all reads greater than 50 bp were retained using cutadapt v1.18 (Martin, 2011) in parallel (Tange, 2011). All reads were quality trimmed (Phred > 10 in sliding windows of size 15% of the read length), truncated to 80 bp, and demultiplexed with the two enzyme setting (i.e., *Nsi*I and *Msp*I) using *process_radtags* of stacks v2.3e (Catchen et al., 2011; Rochette et al., 2019) in parallel. Custom scripts were then used to combine the same individuals from different sequencing runs, and to prepare the population map for stacks (see *Data Availability*).

Individual read files were aligned to the most complete Pacific oyster genome (Zhang et al., 2012) using *bwa mem* v0.7.12-r1039 (Li et al., 2009) in parallel. Insertion and deletion sequencing errors are known to occur in Ion Proton systems, and therefore non-default flags were used in the alignment, specifically -O 0,0 (no gap open penalties); -E 2,2 (reduced gap extension penalty); -T 0 (no minimum score to produce output) (E. Normandeau, *pers. comm.*). Subsequently, *samtools view* v1.9 (Li et al., 2009) was used to remove unmapped reads (-F 4), non-primary alignments (-F 256 -F 2048), as well as low quality alignments (-q 1), and convert the sam to bam format. Samples with fewer than 1 M reads were removed from the analysis using automated custom scripts (see *Data Availability*), and all remaining samples were used for genotyping with stacks v2.3e (Rochette et al., 2019). A new population map with only retained samples was generated, and the total number of reads per sample, total read alignments, and mapping percentage were calculated using *samtools* and custom scripts (see *Data Availability*).

Genotyping and filtering

Loci were identified with stacks v.2.3e using the reference-based approach with alignments against the most complete Pacific oyster reference genome, estimated at 93% complete (Zhang et al., 2012) with the stacks module *gstacks* with program defaults. The *populations* module and custom scripts (*Data Availability*) were used to identify loci out of Hardy-Weinberg equilibrium in at least three populations ($p < 0.01$) to make a list of markers to exclude (i.e., a blacklist). The *populations* module using the blacklist was then used to output multiple locus genotype files with either a single SNP per RAD-tag, or as microhaplotypes (i.e., all SNPs genotyped and passing filters in a RAD-tag are grouped in a microhaplotype). The single SNP output from *populations* used the following

filtering parameters: -r 0.7 (i.e., minimum proportion of individuals genotyped per population); --min_populations 15 (i.e., genotyped in all populations in the population map); --min-maf 0.01 (i.e., global minor allele frequency filter); --write-single-snp (i.e., restricts data to the first SNP per RAD locus); a blacklist including all loci identified as out of HWE; --fasta-loci (i.e., output one locus per marker in fasta); --vcf (i.e., VCF output) and --plink (i.e., plink format output). For the microhaplotype data output from stacks as a VCF, the parameters were the same as above except allowing multiple SNPs per marker and including the --radpainter flag to output into fineRADstructure input (i.e., SimpleMatrix format) (Malinsky et al., 2016).

Population Differentiation

The single SNP per RAD-tag data was output to plink format (Purcell *et al.* 2007), which was then used to recode the VCF (--recodeA) into the number of alleles per locus, per individual. The recoded VCF was input to *adegenet* (Jombart, 2008) in R (R Core Team 2018) for multivariate analyses (i.e., Principal Components Analysis (PCA) and Discriminant Analysis of Principal Components (dAPC)). The genotypes were input to *hierfstat* (Goudet, 2005) to calculate pairwise F_{ST} (Weir & Cockerham, 1984) using 1,000 bootstraps across loci. When the lower limit of the 95% confidence interval overlapped with 0, there was considered to be no differentiation between the collections, and the interval was therefore transformed to 0.

Genetic Diversity and Relatedness

Observed and expected heterozygosity per population was calculated with the *populations* module. Observed heterozygosity per individual was calculated using vcftools using a single SNP per marker (--het) (Danecek et al., 2011). Per population, per marker estimates of nucleotide diversity (π) was calculated using VCFtools (--site-pi) using the multi-locus genotypes containing a single SNP per marker, and analyzed in R (see *Data Availability*).

Relatedness was calculated using a single SNP per marker based on shared polymorphisms using *related* (Pew et al., 2015) by exporting data from *stacks* into R via *plink* and *adegenet*, then converted to *demerelate* (Kraemer & Gerlach, 2017) to format to *related* input. Shared polymorphisms were calculated using *ritland* (Ritland, 1996), *wang* (Wang, 2002) and *quellergt* (Queller & Goodnight, 1989) statistics for coancestry analysis and inter-individual relatedness (only *wang* retained, as all showed similar trends; *data not shown*). Relatedness was also calculated based on shared microhaplotypes using using fineRADstructure (Malinsky et al., 2016). In brief, the coancestry matrix was calculated using *RADpainter*, then individuals were assigned to populations and a tree built using *finestructure*, then output plotted using scripts provided with *fineRADstructure* (Malinsky et al., 2016).

After inspecting genetic similarity among collections, a single population per general region or broodstock line was selected and private alleles identified using *poppr* (Kamvar et al., 2014). To identify alleles specific to farms, all BC naturalized (including farm transplants) were combined together and compared against the hatchery-farmed populations.

Detection of domestication outliers

Domestication outliers were considered as those with evidence of differential selection between HAT-F/HAT with NAT populations. Outliers were identified by comparing HAT-F/HAT populations with a NAT population from the closest location to the farm growing the oysters, as exact broodstock source was unknown (see top of Table 2). Four different approaches were applied to identify outliers. Candidate loci were first identified using *pcadapt* v4.1.0 (Luu et al., 2017). Individual comparisons were made for each HAT-F/HAT and NAT contrast (Table 2). The principal components (PCs) retained in the analysis (*k* in pcadapt) were retained based on the flattening of the slope on scree plots to keep only those to the left of the plateau as suggested, retaining *k* = 3, 5, 6, 6, 3 for DPB, ROS, GUR, QDC, and RSC, respectively (Additional File S1). Further, score plots within *pcadapt* were used to identify which PCs separated the samples based on the HAT-F/HAT vs. NAT comparison (Additional File S2). As such, outliers that were significant, as determined by *get.pc* of *pcadapt*, due to the following PCs were retained: DPB =PC1,3; ROS = PC1,3; 1,2,3,4; GUR = PC1; QDC = 1,2,3,4; and RSC = PC1. Outlier p-values were generated, and false discovery rates controlled using *qvalue* (Storey & Tibshirani, 2003; Storey et al., 2019). Markers with $q < 0.01$ for the PCs described above were considered significant. Second, *BayeScan* v2.1 with a prior odds of 100 (Foll & Gaggiotti, 2008) was also used to identify outliers in these contrasts. Third, a Redundancy Analysis (RDA) was conducted using the HAT-F/HAT and NAT collections in Table 2 combined by source type (HAT-F/HAT vs. NAT) as a multi-locus genotype-environment association (GEA) using *vegan* v2.5-5 (Oksanen et al., 2019). RDA, an analog of multivariate linear regression analysis, used the multi-locus genotype data per individual as a dependent variable, and grouping (HAT-F or NAT) as the explanatory variable. The function *rda* was used to compute RDA on the model separating samples based on the grouping factor (e.g., Laporte et al., 2016). An analysis of variance (ANOVA; 1,000 permutations) was performed to determine the significance of the RDAs, and the percentage of variance explained (PVE) was calculated using *RsquareAdj* in the *vegan* package. The identification of outliers was conducted using instructions as per previous work and online tutorials (Forester et al., 2018; Forester, 2019), considering outliers as the markers with loading values greater than 3.5 standard deviations of the mean. The fourth approach taken used per locus F_{ST}, and identified markers in the top 99th percentile of the F_{ST} distribution and considered these as potential outliers.

Outliers significant using both RDA and *pcadapt*, or outliers that show significance for any two statistical tests and are observed in multiple comparisons were considered as top outliers and investigated further. These top outliers were used to search against the reference genome to determine whether the they were within or near predicted genes. The annotation from NCBI, NCBI_annot_rel_101_2017-02-02 from the Zhang et al. (2012) genome was principally used, and genes containing the outlier (e.g., in introns or exons), or those within approximately 10 kb of the outlier position were recorded. The 10 kb distance was used given the rapid decay of linkage disequilibrium observed in the Pacific oyster (Kijas et al., 2019).

Detection of outliers under selection from growing on farm

To identify markers that changed significantly in allele frequency following transplantation from naturalized populations onto farms, a pairwise comparison was conducted between NAT and NAT-F oysters for populations in BC, China and France. The comparison was between a nearby, naturalized population and the transplanted farm population (i.e., FRA vs. FRAF; CHN vs. CHNF; PEN vs. PENF; see bottom of Table 2). Outliers were identified using the same approach as above (i.e., *pcadapt*, *BayeScan*, RDA, 99th percentile F_{ST}). However, scree plots did not indicate a plateau (Additional File S1), suggesting that no PCs explained a majority of the variation, and so *pcadapt* was not used for these comparisons. To determine whether similar signatures of selection were found between different NAT-NAT-F contrasts and potentially identify outliers under parallel selection, per locus F_{ST} was directly compared between each pair of countries (i.e., BC, FRA, CHN).

Alignment of outliers to the chromosome-level assembly

All markers identified using the most complete reference genome (Zhang et al., 2012) that were exported from stacks in *fasta* format were collectively aligned to the chromosome-level assembly (Gagnaire et al., 2018) using *bwa mem* and *samtools*. Only those that aligned to the chromosome-level assembly were used for plotting along chromosomes but for all other purposes both aligned and unaligned markers against the chromosome-level assembly were included. Per locus F_{ST} values calculated on the pairs of populations used in the *pcadapt* contrasts (Table 2) were plotted along chromosomes per contrast, with outliers identified by each tool indicated in the plot using custom scripts (see *Data Availability*).

RESULTS

Sample sequencing and genotyping

In total, 182 naturalized/wild individuals (NAT) were genotyped, including adult individuals from coasts of Vancouver Island, British Columbia (BC; n = 122), France (FRA n = 23), China (CHN; n = 24), and Japan (JPN; n = 13; Table 1; Figure 1A). For naturalized oysters transplanted to a farm (NAT-F), 72 oysters were genotyped, with parallel comparisons in Canada, France, and China (n = 24 for each), to compare to expected source populations. Finally, 112 hatchery-origin oysters grown on a farm (HAT-F or HAT) were genotyped (Table 1).

On average, samples had a total of 2.17 M reads (min = 1.01 M; median = 2.06 M; max = 6.17 M). Aligning to the most complete reference genome (NCBI: GCA_000297895.1; oyster_v9; Zhang et al. 2012) resulted in an average alignment rate of 69.7%. Genotyping with *gstacks* resulted in a total of 723,034 unfiltered loci, with an average effective per-sample coverage of 20.2x (sd = 5.7x, min = 10.3x, max = 48.6x). Loci were then filtered for missing data by only retaining markers that were genotyped in at least 70% of the individuals per population in all populations, that had a minor allele frequency (global MAF > 0.01), and that were not out of HWE equilibrium in more than two populations ($p \leq 0.01$). This resulted in a total of 81,443 SNPs found in 17,084 loci when considering microhaplotypes. When filters were applied to only the first SNP per RAD locus (see

above), a total of 16,942 markers were retained. These 16,942 markers comprised 1,532,384 bp of total sequence, and thus represent 0.255% of the expected 600 Mbp Pacific oyster genome.

Filtered loci were subsequently aligned to the chromosome-level reference genome (Gagnaire et al., 2018) for the purpose of plotting outlier statistics (see below), indicating that loci occurred throughout the genome (Table 3). A total of 9,286 of the filtered RAD-loci mapped to the chromosome-level assembly (54.8% of filtered loci), which fits with the expectation of the chromosome-level genome being approximately 50% present in the assembly (Gagnaire et al., 2018). All markers, including those not aligned to the chromosome assembly, were retained for downstream analyses, except plotting along chromosomes, which only used the markers that aligned to the chromosome assembly.

Genetic differentiation among naturalized locations in British Columbia

Naturalized populations from around Vancouver Island, BC (Figure 1A) ranged from very low differentiation to undifferentiated (Table 4A). On the mainland, very low differentiation was observed between Pendrell Sound (PEN) and Serpentine River (SER) (F_{ST} 95% CI = 0.0017-0.0033; Table 4A; Table S1A for average F_{ST}). Moreover, no differentiation was observed (i.e., F_{ST} = 0 could not be rejected) between PEN and Pipestem Inlet (PIP), even though they are on opposite sides of Vancouver Island. The largest difference in these naturalized BC collections was observed between the two most geographically distant sites by water of those sites sampled in BC, PEN and Hisnit Inlet (HIS), a site further north on the west coast of Vancouver Island (WCVI) (F_{ST} 95% CI = 0.0022-0.0035). Discriminant Analysis of Principle Components (DAPC) also demonstrated highly overlapping genotypic distributions of these locations (Figure 1B).

To evaluate the relative impacts of temporal and spatial effects on population signatures in these BC collections, multiple size classes that were assumed to be different brood years were obtained for HIS and PEN, the most divergent populations. Small (size class = 2), medium (size class = 3), and large (size class = 6) oysters were used in the comparisons (see Methods). Small and medium size classes were obtained for PEN (n = 15 and 20, respectively), and medium and large were collected for HIS (n = 19 and 20, respectively). There was no differentiation between PEN_2 and PEN_3 (F_{ST} not significantly different from 0), and very low differentiation between HIS_3 and HIS_6 (F_{ST} 95% CI: 0.0004-0.0027; Table 4B or Table S1B for average F_{ST}). Size-matched spatial differences between PEN_3 and HIS_3 were greater than temporal differences within a site (F_{ST} 95% CI: 0.0024-0.0048). Therefore, for these sites and years, year class had a minor effect on population structure at HIS and PEN, and spatial effects were larger, suggesting recruitment from within each area.

Genetic differentiation in naturalized/wild oysters throughout the Northern Hemisphere

The maximum differentiation among naturalized sites in BC was F_{ST} = 0.0022-0.0035 (95% CI) between PEN and HIS. This level of genome-wide differentiation was similar to that between naturalized oysters in BC and France (F_{ST} = 0.0016-0.0032; Table 4C or Table S1C for average F_{ST}; Figure 2A). Japan and BC had slightly higher, but still low differentiation (F_{ST} = 0.0047-0.0074; DAPC in Figure 2B).

In contrast, wild Pacific oysters from China were more divergent from the JPN-CDN-FRA lineage (F_{ST} 95% CI: PEN-CHN = 0.0179-0.0229; FRA-CHN=0.0168-0.0218; JPN-CHN = 0.0211-0.0272; Table 4C). The CHN population was found to be more similar to the CHN hatchery populations (i.e., RSC, QDC; DAPC in Figure 2B). For comparison, F_{ST} between BC and China is ~8-fold higher than the most divergent of the BC populations.

Divergence between naturalized and hatchery-farmed oysters

The most genetically distinct oyster populations in this study were consistently hatchery propagated (HAT/HAT-F). In general, to understand this divergence, HAT/HAT-F oysters were compared to their closest wild/naturalized (NAT) populations in terms of physical distance (Table 2).

In BC, the least divergent HAT-F population was ROS (PEN-ROS F_{ST} = 0.0075-0.0096), with a level of differentiation slightly higher than that observed between PEN and JPN (Figure 2B). GUR was more distinct (GUR-FRA F_{ST} = 0.0261-0.0299) and formed a single grouping along the first axis of the DAPC (Figure 2B). The most distinct population in the BC farms was DPB (DPB-PEN F_{ST} = 0.0574-0.0636; see DAPC in Figure 2B). The increasing divergence, particularly for DPB, probably reflects an increasing component of the genome originating from other countries (e.g. Chile).

To identify private alleles by group, similar populations were grouped together to be analyzed, producing collections BC, DPB, ROS, GUR, RSC, QDC, CHN, FRA, JPN (Table 5A). FRA and JPN were included, but private alleles were not expected given the lack of differentiation observed between BC and these collections and the much higher sample size in the BC grouping. In the BC meta-population, 38 private alleles were found. Even with lower sample sizes, HAT/HAT-F populations had private alleles, with DPB having the most in the comparison (n = 49), followed by QDC (n = 17), and GUR (n = 11). Other HAT-F populations, such as ROS and RSC did not have significantly more than zero private alleles (n < 2).

Farmed oysters in China were distinct from those in the France/UK and Canada (see DAPC in Figure 2B). However, the farms were not highly different from the presumed wild collection in China (FST 95% CI: CHN-RSC = 0.0074-0.0100; CHN-QDC = 0.0181-0.0212; Table 2). RSC and CHN therefore had a similar level of differentiation as ROS from PEN in Canada, and QDC and CHN was slightly less divergent than the GUR-FRA comparison in Europe (Table 2). The most distinct population altogether from nearby naturalized oysters was therefore the DPB population (Figure 2B; Table 5A).

In agreement with F_{ST} and DAPC analyses, a Principal Components Analysis (PCA) indicates that the majority of the variation in the genotypic data (single SNP per locus, all RAD-loci) is explained by PC1-3, although the percent variation explained (PVE) was low (Figure S1C). Most samples clustered tightly in the center, and DPB and CHN separated on either side of PC1 (Figure S1A) and GUR separated on PC3 (Figure S1B). Many loci contribute to the differentiation observed in the PCA and DAPC, as observed by marker loading values for the DAPC, although a small number of markers contributed most to this difference (specifically, 16 markers exhibited loading values > 0.002; Figure S2).

Genetic diversity, heterozygosity and relatedness

Genetic diversity as measured by nucleotide diversity (π) was lowest in the DPB HAT-F population, whereas all other collections had similar median π (Figure S3). JPN was not retained in this analysis because of its lower sample size (n = 13) and the effect of sample size on π. Lower π in DPB was not associated with elevated within population inbreeding (F_{IS}); in fact, DPB had the lowest F_{IS} observed in the study (Figure 3A). The population with the highest inbreeding coefficient was CHN, but this was only significantly higher than the inbreeding coefficient of DPB, ROS, FRAF, GUR populations (Figure 3A). Similarly, several of the HAT/HAT-F collections had the lowest percentage of polymorphic sites, with DPB and GUR having 0.94% and 1.13%, respectively, compared to an average of 1.43% for the other populations (+/- 0.10%) (Table S2). Low read depth can falsely elevate homozygous calls, but this should result in a negative correlation between the number of reads per sample and the inferred inbreeding coefficient, which was not observed (Figure S4).

Comparing different loci, per locus nucleotide diversity was highly correlated across populations, with some loci showing consistently high or low π in multiple populations, even outside of the JPN-CDN-FRA lineage (e.g., within CHN; Figure S5). The least correlated populations with the BC wild population were DPB and GUR (Figure S5).

While some hatcheries attempt to cross distantly related individuals (leading to low F_{IS}), the resulting oyster offspring grown together on a farm tend to be comprised of highly related individuals, due to family structure (Figure 4). The highest relatedness was observed in DPB (Fig 3B). The other HAT-F were not significantly higher than corresponding NAT populations, although the other HAT-F had higher average relatedness in their outlier pairs of individuals (see ROS, GUR, QDC), except RSC. This family structure in the HAT/HAT-F samples was also observed using shared microhaplotypes (Figure 4). The HAT-F samples that were less divergent from the NAT samples (i.e., ROS and RSC) also had elevated inter-individual relatedness relative to the NAT populations, although not as high as the more divergent HAT/HAT-F populations (Figure 4). In contrast, NAT and NAT-F samples were all equally dissimilar from each other.

Signatures of domestication selection

Here we investigate domestication selection, which we define as any markers that are outliers between HAT/HAT-F oysters and NAT oysters. This could reflect either directional selection or relaxed selection in HAT/HAT-F. Using *pcadapt*, between 82 and 367 outliers ($q < 0.01$) were identified per contrast between paired populations of HAT/HAT-F oysters and nearby NAT populations (see top of Table 2). Using RDA, 170 outliers were identified between HAT/HAT-F and NAT populations. RDA indicated that HAT/HAT-F populations could be significantly separated along RDA1 from nearby NAT populations (Figure S6). Using *BayeScan* no outliers were identified. On average, 133 outliers were identified within the 99th percentile of F_{ST} per contrast. The comparisons involving QDC, DPB, and ROS had the most outliers using *pcadapt*, with fewer identified for GUR and RSC. In general, outliers were found throughout the genome (Figure 5).

Top outliers were identified as those that had the strongest evidence per comparison (i.e., significant for both *pcadapt* and RDA), or those that were consistently identified in multiple comparisons with at least two pieces of evidence (i.e., evidence includes significant for F_{ST} 99th percentile, RDA, or *pcadapt*). Top outliers with the strongest evidence and consistent observations included three markers: CLocus_104468, CLocus_625182, and CLocus_704500. These were identified with strong evidence and consistent observations in RSC and GUR, QDC and RSC, and QDC and DPB, respectively. CLocus_104468 was found to be in an intron of *fibrocystin-L*, CLocus_625182 was found to be in an intron of *prion-like-(Q/N-rich) domain bearing protein 25*, and CLocus_704500 was not near any genes. Interestingly, both CLocus_104468 and CLocus_625182, significant for all three outlier approaches for RSC and QDC, respectively, were found on chrD, at position 32,708,807 bp and 9,881,897 bp, respectively. Although the functions of these genes are not particularly known to be related to domestication or to be well characterized at all, these loci were the strongest domestication candidates in the study. An additional 10 outliers were found with the two strong evidence approaches (see Additional File S3), and an additional 17 were found with at least two pieces of any evidence in multiple comparisons. Marker names, evidence type, contrasts, and the genomic location including containing or nearby genes (within 10 kb) are shown in Additional File S3. Putative functions and relation to the Pacific oyster and domestication in general are presented in the Discussion (see below).

Signatures of wild-to-farm movement selection

Signatures of selection were investigated in oysters moved from naturalized populations onto farms (NAT-F). Three parallel comparisons were made from farms and source populations in Canada, France, and China. As expected, oysters that remained in the source population versus moved onto a farm typically exhibited negligible genetic differentiation (see the bottom of Table 2).

Outlier loci were not identifiable for these comparisons with *pcadapt* because scree plots did not indicate any PCs explaining significant proportions of the variation (Additional File S1). RDA found a total of 175 outliers between NAT and NAT-F samples (Figure S7), and these markers were able to differentiate NAT and NAT-F populations (Figure S8). *BayeScan* did not find any outliers. To characterize markers putatively under parallel selection in multiple regions, per locus F_{ST} values were compared among regions (Figure S9). In this way, several markers were found to have F_{ST} values greater than the 99th percentile in pairs of comparisons. However, the amount of parallelism was low, as observed by the lack of markers with high F_{ST} in both comparisons (Figure S9). Two markers were found to be consistent outliers, CLocus_24819 (unknown chromosome) found by RDA and 99th percentile FST in CHN and BC, and CLocus_608966 (chrB) found in FRA and BC.

DISCUSSION

The identification of several thousand genetic markers throughout the genome of the Pacific oyster *C. gigas* allowed us to obtain a detailed overview of the genetic differentiation of oysters in populations across the Northern Hemisphere, including whether there was consistent evidence of reduced diversity in farmed populations.

There were three major findings that emerged from our study. First, the most distinct populations in terms of genetic differentiation were commercial hatchery farmed populations (HAT-F), or populations from China. HAT-F populations often had elevated relatedness, and in one case had reduced genetic diversity. There was no indication of large effects from on-farm, within-generation selection, and therefore the differentiation observed in these HAT-F populations is probably due to demographic effects (e.g., founder effects) as well as domestication selection during breeding. Second, the genetic structure around Vancouver Island was very low, with essentially absent genome-wide differentiation between sites on either side of Vancouver Island (i.e., Pendrell and Pipestem), two regions commonly used for broodstock collections for farming in BC. However, slightly larger differentiation was observed between two distant locations (i.e., Hisnit and Pendrell) than that observed between generations within each location. Third, oysters from China, both farmed and wild, were distinct from the other oysters in the Japan-Canada-France translocation lineage. This work has also identified private alleles specific to distinct farm populations, as well as putative domestication outliers, in some case being identified in more than one HAT-F population.

Genetic differentiation around Vancouver Island, British Columbia

Very weak to negligible population differentiation was observed across BC, as expected for populations of Pacific oyster (Sun & Hedgecock, 2017), and typical of other marine invertebrates (Gagnaire et al., 2015). Low F_{ST} values may be due to weak genetic drift (large effective population sizes) and/or high dispersal, for example as observed in blue mussel (Bierne, 2010). Given the expected transfer of Pacific oysters by human activity around Vancouver Island, such as the common use of both Pipestem Inlet and Pendrell Sound for farmers to collect seed (BC Shellfish Growers Association, 2019), the low differentiation is not surprising. In fact, these two populations were the only pair that had completely undifferentiated populations, with other populations having slightly higher differentiation, potentially reflecting the reduced genetic differentiation due to increased human-mediated transfer.

Stock identification of oysters from the regions around Vancouver Island would not be possible given the results observed here and previously reported (Sun & Hedgecock, 2017). Increasing sample size drastically for populations, or using additional markers putatively under selection could potentially increase power for stock identification (Gagnaire et al., 2015), but given the biology and dispersal ecology of Pacific oyster (i.e., larvae can disperse for up to 20 days at 22°C (Quayle, 1988)), and the anthropogenic movements of stocks, the probability of genetic stock identification of Pacific oysters around coastal BC is very low.

Given the weak differentiation around Vancouver Island, crosses involving oysters sourced from different locations may not confer heterosis (Hedgecock & Davis, 2007). The slight differentiation between Hisnit Inlet and Pendrell Sound could potentially provide this benefit, but without conducting trials of genetically similar or divergent crosses exposed to a variety of environments and perturbations, it is not possible to know if this could have a heterosis effect. By contrast, crossing oysters from Pendrell Sound and Pipestem Inlet would not seem worthwhile given the lack of any differentiation between these two areas.

Spatial effects between the most distant locations by sea outweighed the temporal effects measured for oysters from Hisnit Inlet and Pendrell Sound across two different brood years each. This is in contrast to previous work that found temporal variation outweighed spatial variation around Vancouver Island using a coding gene SNP panel with 52 markers (Sun & Hedgecock, 2017). Sweepstakes reproductive success and chaotic genetic patchiness can cause genetic differences across years, with effects being more pronounced when conditions are less favourable, having a higher impact from matching of rare favourable environmental conditions to the random timing of spawning (Hedgecock & Pudovkin, 2011). For example, in sea scallops, *Placopecten magellanicus*, differentiation between different life stages was observed at one site but not at another (Lehnert et al., 2018). Higher sample sizes for different locations and brood years for a range of locations would better characterize whether time or space has a larger impact on genetic structure in Pacific oyster. However, any differentiation across years is likely to be minor, given the very low differentiation observed even between continents along the translocation lineage.

Genetic differentiation among countries

There was very low differentiation among distant regions, including Japan, France and Canada, implying little if any bottleneck during these introductions (Sun & Hedgecock, 2017; Gagnaire et al., 2018), potentially due to repeated introductions and high propagule pressure (Gagnaire et al., 2018). The introductions of Pacific oyster to France occurred in 1971-1977 either by transplant of adult oysters from BC (~562 tons (t)) or as spat from Japan (e.g., oyster or scallop shell on which spat had been set) (~10,015 t of spat collectors) (Quayle, 1988; Grizel & Heral, 1991). In contrast, transplantations from Japan to Canada probably stopped in the 1950s (Quayle, 1988). The differentiation among Japan, France and Canada reflects the translocation history and time since introduction, where France is similar to both BC and Japan, and BC is more genetically distant from Japan than BC is from France (Figure 2).

Relative to oysters from Canada, France, and Japan, oysters from China were more genetically distinct (Figure 2). Pacific oysters originated in East Asia, so non-farmed oysters from Japan and China could represent wild oysters, with differences between them pre-dating human use. The close similarity of farmed and non-farmed oysters from China, however, suggested a history of repeated movement of wild oysters to farms, or naturalized oysters being released from farms, or both. Further work on the East Asia populations is needed to determine the timing and history of genetic differentiation between oysters from China and the JPN-CDN-FRA lineage to determine whether the differences are reflective of selective breeding and aquaculture effects or more long-term differentiation between the Japanese and Chinese wild populations.

Signatures associated with divergent hatchery populations

Signatures of selection that are different between natural and farmed populations, including markers under domestication selection, can indicate specific genomic regions or markers that have been selected for during the domestication or selective breeding process (Gutierrez et al., 2016; Gutierrez et al., 2018). Here it is not possible

to determine whether the outliers identified were under selection on the farms or hatchery, or whether they have relaxed selection from what they would be exposed to in nature. In any case, outliers identified are under differential selection in nature from in farms, and therefore inform on the different conditions and pressures in these two locations.

In the current study, the most divergent hatchery populations were those with identifiable family structure, and this was a consistent for all countries (e.g., DPB in Canada, GUR in the UK, QDC in China). This may indicate founder effects of the broodstock line, selection within hatcheries or farms, or genetic drift due to low effective population size of the brood line. Interestingly, the most divergent hatchery strain (DPB) also had low nucleotide diversity, which may indicate a lower number of founding individuals, or more ongoing drift in this particular lineage. Hatchery propagation is known to potentially impact both the genetic diversity and the allelic composition of Pacific oyster. For example, hatcheries may cull the slowest growing oysters to keep the crop synchronized, but given that this growth rate is in part genetically determined, this imposes selection on the hatchery population and has been shown to reduce diversity and induce temporally varying genetic structure (Taris et al., 2006). Furthermore, many aspects of reproduction can result in large differences in Ne and N in hatchery or wild populations, including gamete quality, sperm-egg interaction, and differential viability among genotypes (Boudry et al., 2002). This is a strong argument to avoid having too few progenitors in breeding programs and to be aware that the total individuals put into the breeding program may not reflect the genetic diversity that is output. Although best practices would suggest avoiding extreme differences in reproductive success from individual broodstock parental pairs, this requires specific steps to mitigate (e.g. not combining sperm from multiple fathers during fertilization to avoid sperm competition). Given the strong differences between hatchery and wild populations in terms of inter-individual relatedness, and to a lesser extent in nucleotide diversity and polymorphism rate, the impacts of these factors on ecological aspects such as response to environmental perturbation or disease are worthwhile investigating. However, it remains unknown whether farms with lower diversity than others (i.e., lower F_{IT}) are less likely to survive perturbations, or whether they have in fact underwent more selection and may be thus more fit for local conditions. These questions are will be important to address in future work.

The reduced inbreeding coefficient F_{IS} in DPB (and higher heterozygosity) probably reflects the practice of crossing divergent lines, for example selected lines, possibly with the aim to produce heterozygote advantage that may occur due to reducing the impact of negative recessive mutations in the Pacific oyster (Williams, 1975; Launey & Hedgecock, 2001; Ellegren & Galtier, 2016). The lower nucleotide diversity in DPB may reflect either a smaller number of founding individuals, ongoing drift, or selection. The lower F_{IS} observed may result in a single generation of heterozygote advantage (Launey & Hedgecock, 2001), but if these individuals cross again within their cohort, they would have overall reduced nucleotide diversity and thus less chance of carrying beneficial alleles for subsequent adaptation. Further, if they introgress into naturalized populations, they would bring this reduced genetic diversity. The amount of introgression from hatchery populations that are highly divergent from naturalized populations is worth further investigation. However, whether this is of concern is

dependent on whether conservation of naturalized populations is a goal, given that these populations in some cases may be considered invasive, and in other cases may be considered important sources of potentially locally adapted broodstock.

Higher inter-individual genetic variation was observed using multivariate approaches (i.e., PCA, DAPC) within farm populations, particularly those with family structure, than within naturalized populations (Figure S1). A similar result was viewed in selectively bred farmed populations of Atlantic salmon (~12 generation of farming), which showed higher inter-individual variation relative to wild populations (Gutierrez et al., 2016). This was attributed to both the admixture that occurred when the farm population was created and the repeated introgression of other lineages into the broodstock line over time. This increased variation was not observed in all farmed populations, with some populations being more similar to wild populations (Gutierrez et al., 2016). This is similar to the differences in inter-individual variance observed in the present study for DPB (more inter-individual variance than naturalized populations) and ROS (similar to naturalized populations). Further, similar to the present study, many populations of farmed Atlantic salmon had negative inbreeding values (F_{IS}).

Breeding practices can influence the underlying genetics of hatchery strains, which can affect their potential to undergo selective breeding, as well as to have the necessary genetic variation to handle various biotic and abiotic stressors or threats, such as disease, when there is a genotype-environment interaction. An Australian hatchery strain was found to have lower genetic variation, but to have less family structure and no sign of inbreeding; other strains in the same study showed higher family effects than this strain likely through the careful selection of unrelated individuals for crosses (Kijas et al., 2019). Similarly, in the present study, the RSC population from China, even though it has been bred for at least six generations, it shows less family structure than the QDC strain. The QDC strain has a similar level of inter-individual relatedness to the DPB population, but the DPB population has the additional issue of reduced overall nucleotide diversity, indicative of a potential bottleneck or founder effect. This DPB strain would have lower adaptive potential and lower potential to undergo further selective breeding without being introgressed with other divergent strains.

Domestication Outliers

The identification of outliers in multiple populations of oysters has highlighted regions of the genome that may have been subject to domestication selection. Many outlier markers were near or within genes involved in either calcium signalling or resilience to environmental stressors (see Additional File S3). Calcium signalling may have important roles in response to abiotic stress and defense. Genes nearby domestication outliers with calcium signalling related functions include *GTP-binding protein REM 1*, *calcium-binding mitochondrial carrier protein Aralar1-like*, *IQ domain-containing protein K* (may bind calmodulin), *calmodulin-like*, *calcineurin subunit B type 1* (stimulated by calmodulin and conferring calcium sensitivity), and *GTP-binding protein RAD* (involved in calmodulin binding) (Additional File S3). Calmodulin is a multifunctional calcium-binding protein, and given that intracellular Ca^{2+} can function in such diverse responses as response to abiotic stressors such as mechanical stimuli, osmotic stress, and heat and cold shock, as well as being involved in defending against pathogenic infections, this

general category of calcium-binding genes is of particular note. Other genes involved in cellular stability, such as T-complex protein 1 subunit zeta, mitochondrial uncoupling protein 2, tumor suppressor p53-binding protein 1 were also within this list. The immune-related (antiviral) *toll-like receptor 3* was also found to be near an outlier.

An outlier was found in *transient receptor potential cation channel subfamily M member 6*, and interestingly *member 3-like* from the same cation channel subfamily member was found to be a parallel domestication outlier in both Scottish and Canadian domestic Atlantic salmon (López et al., 2019). Several other genes identified here have also been identified as being involved in domestication processes, such as *mitochondrial uncoupling protein 2*, which has undergone loss of function mutations in pig (*Sus scrofa domesticus*) domestication and potentially has led to the reduction of cold tolerance of pigs (Berg et al., 2006) as well as being found to be an outlier in domestic dogs *Canis lupus familiaris* (Udagawa et al., 2014).

The role of these genes in terms of calmodulin function, calcium signalling, and resilience to abiotic stressors may provide more insight into the farm environment and the domestication process thus far in Pacific oyster. Continued efforts to understand the differences in selective landscapes, including the genes involved, between hatchery populations and naturalized populations will inform the pressures that the oysters face in both environments.

Differential selection from one generation on a farm compared to nature

Within-generation outliers putatively under differential selection in naturalized oysters moved onto farms relative to those remaining in nature were minimal, and showed very low parallelism across regions. Aside from the present study, the effect of within-generation selection associated with moving oysters from nature onto a farm is largely uncharacterized. Sources of selection on farms include more crowding and different substrate conditions (e.g. oyster shell substrate held in mesh bags). Alternatively, the outliers may reflect selection in the oysters that remained in the naturalized populations, with relaxed selection in farmed oysters. The lack of genome-wide differentiation between the naturalized and the transplanted populations indicates that sampling of the naturalized population to be moved onto the farm did not impose a bottleneck or founder effect, and that specific families are not greatly enriched on the farm during the transplantation. Given that mesh bags of naturally set oysters are moved onto farms, and that these were sampled in the present study, suggests that oysters naturally settling near one another are not highly related. This is also reflected by the lack of family structure in the NAT-F populations relative to that viewed in the HAT/HAT-F populations by shared microhaplotype approaches. One caveat to our results is that the outliers detected may reflect the non-random sampling performed during transplantation (e.g., reflecting local adaptation to the sampling site), although the low differentiation in general in this study over short distances suggests that this is probably not a major factor.

Farms that grow naturalized oysters nearby farms growing hatchery oysters may represent a good natural experiment to determine what is most beneficial to survival in response to massive mortality events, weighing factors such as heterozygosity, relatedness, and nucleotide diversity. However, environmental conditions would need to be nearly identical for this to be conclusive. An additional approach that would provide insight on this

would be sampling oysters before they are put on farms (or put into an experimental challenge), and then sampling after a mortality event. Given the importance of mortality events for farms and the concerns over changing environmental conditions and the impacts of this on oyster health, these approaches would be both important avenues for future work.

Conclusions

Pacific oysters in Japan, Canada, and France are highly similar and no indications of bottlenecks were observed. Chinese oysters, including wild individuals, were highly distinct from this translocation lineage. The cause of this distinctiveness remains unknown, and may be due to long-term genetic differentiation and/or the effect of selective breeding introgressing from nearby farm populations. The differences between some commercial hatchery populations and naturalized populations were in some cases equal in strength to differences in the most distinct populations across the sampled Northern Hemisphere range. Commercially hatchery populations had more distinct family structure and higher inter-individual relatedness, and in one case had lower genetic diversity. The most differentiated hatchery populations also had higher prevalence of private alleles. Whether the distinctiveness and low genetic diversity of some hatchery-propagated populations is considered to be an issue regarding introgression to locally adapted naturalized populations depends on whether Pacific oyster in Canada is considered a genetic resource for broodstock as well as a harvestable food resource, or whether it is considered an invasive species. The sourcing and movement of oysters from commonly used regions of the coast may have reduced the genetic differentiation between sites such as Pendrell Sound and Pipestem Inlet, which are slightly more similar than expected. Crosses between these two populations therefore do not seem worthwhile in an attempt to gain heterosis. In BC, we did not observe strong temporally-varying population signatures in naturalized populations, and spatial variation had a larger effect. Next steps to understand the specific alleles involved in conferring survival, as well as the role of genetic diversity could use either naturalized populations or hatchery-propagated populations in controlled challenge studies, investigating allele frequencies before and after the selective events to understand the genetic underpinnings of survival to perturbations expected under future climate change scenarios.

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Data Availability

RAD-seq protocol: https://www.protocols.io/view/nsil1-msp1-rad-digest-detailed-protocol-2019-01-18-w95fh86

Complete analysis pipeline: https://github.com/bensutherland/ms_oyster_popgen

Genotyping pipeline (original): https://github.com/enormandeau/stacks_workflow (see fork: https://github.com/bensutherland/stacks_workflow)

Raw data: NCBI Short Read Archive (SRA) BioProject PRJNA550437

Supplemental Materials

Table S1. Mean genetic differentiation (F_{ST}) shown between pairs of populations for **(A)** populations in BC; and **(B)** populations in BC separated by size class; and **(C)** global populations.

Table S2. Population summary statistics with standard error for microhaplotypes (i.e., multiple phased SNPs permitted per RAD locus) from *stacks* using either (A) all variant sites or (B) all variant and fixed RAD loci.

Figure S1. Principal Components Analysis (PCA) of all samples using a single SNP per locus with all loci. Sample IDs are plotted along PC 1 and 2 (A) or PC3 and 2 (B).

Figure S2. Discriminant Analysis of Principal Components (DAPC) marker loading values.

Figure S3. Per population distribution of nucleotide diversity (π), shown as frequencies of specific bins of nucleotide diversity as calculated by vcftools (--site-pi).

Figure S4. Per sample inbreeding coefficient (F) calculated using vcftools plotted **(A)** per sample; and **(B)** as a function of read depth per sample .

Figure S5. Nucleotide diversity (π) for each marker (dot) correlated between each population with the BC wild population.

Figure S6. Comparison between Redundancy Analysis (RDA) and PCA in differentiating populations.

Figure S7. Outliers identified between naturalized/wild oysters moved onto a farm and naturalized populations (NAT-F vs. NAT) using RDA analysis.

Figure S8. Comparison between Redundancy Analysis (RDA) and PCA in differentiating populations.

Figure S9. Per locus F_{ST} values compared between different countries to detect signatures of parallel selection from being moved onto a farm.

Additional File S1. Scree plot showing percent variation explained for all domestication or on-farm selection contrasts.

Additional File S2. Score plots showing sample position within PCA based on each PC for all domestication or on-farm selection contrasts.

Additional File S3. Top outliers, either those identified as being an outlier via both *pcadapt* and RDA, or those with at least two outlier tests (*pcadapt*, RDA, 99th percentile F_{ST}) and present in more than one comparison. Information such as marker name, chromosome-level assembly alignment chromosome where possible, and nearby genes in the reference assembly annotation are provided. Additionally, the general known functions of the outliers from resources such as UniProt are provided for each gene nearby the outlier markers.

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TABLES

Table 1. Sample overview, including collection location, sample type, short form identifier, and sample size for retained, genotyped samples. Collection locations are shown in Figures 1 and 2. Origin of seed was in some cases difficult to determine, and these collections.

Collection Location	Type	Origin of Seed	Identifier	Sample Size
Hisnit Inlet, BC	NAT	BC	HIS	39
Pipestem Inlet, BC	NAT	BC	PIP	25
Serpentine River, BC	NAT	BC	SER	23
Pendrell Sound, BC	NAT	BC	PEN	35
Baynes Sound*, BC	NAT-F	BC	PEN-F	24
Rosewall Creek, BC	HAT-F	US and/or BC	ROS	23
Deep Bay, BC	HAT-F	US, BC, and/or Chile	DPB	20
Guernsey, UK	HAT	Unknown	GUR	22
N. Brittany, France	NAT	France	FRA	23
SW. France	NAT-F		FRA-F	24
Hyogo and Miyagi Prefectures, Japan	*unkn.*	Japan	JPN	13
Qingdao, China	NAT	China	CHN	24
Qingdao, China	NAT-F	China	CHN-F	24
Qingdao, China	HAT-F	China	QDC	24
Qingdao, China	HAT-F	China	RSC	23

NAT = naturalized (or wild); NAT-F = naturalized moved to a farm; HAT-F = hatchery grown on a farm; HAT = hatchery not yet transplanted to farm; * = transplant from Pendrell Sound

Table 2. Outlier overview, including values for both the domestication and on-farm selection comparisons. Outliers were identified per grouping by RDA (single value per grouping comparison; > 3.5x SD of the mean), and per contrast by *pcadapt*. ($q < 0.01$). Outliers in the 99th percentile of the F_{ST} per contrast were also identified. F_{ST} lower limits that overlapped with 0 resulted in the entire interval and average being transformed to 0. The number of outliers per contrast that were detected by at least two methods were enumerated per contrast ('Common outliers'). Note: this includes all outliers, including those that do not align to the chromosome-level assembly.

Type	Comparison	F_{ST} (avg.)	F_{ST} (95% CI)	Outliers (*pcadapt*)	Outliers (F_{ST})	Outliers (RDA)	Common outliers
Domestication						170	
(HAT/HAT-F	DPB-PEN	0.0604	0.0574-0.0636	203	137		53
vs. NAT)	ROS-PIP	0.0080	0.0069-0.0090	309	135		64
	GUR-FRA	0.0281	0.0270-0.0299	82	132		50
	QDC-CHN	0.0196	0.0181-0.0212	367	129		61
	RSC-CHN	0.0087	0.0074-0.0100	83	134		31
On-farm						175	
selection	CHN-CHNF	0	0	NA	134		18
(NAT-F vs.	FRA-FRAF	0	0	NA	140		13
NAT)	PEN-PENF	0.0008	0.00002-0.0015	NA	144		26

Table 3. Markers aligning to the chromosome-level assembly or only to the contig-level assembly are shown as either as numbers or percentages of aligned markers per chromosome. All RAD-loci (n = 16,942) were used for all analysis except for plotting purposes along chromosomes.

Chromosome	**Number of markers**	**Percentage of total aligned**
A	1,058	11.4%
B	732	7.9%
C	882	9.5%
D	1,471	15.8%
E	1,234	13.3%
F	978	10.5%
G	1,027	11.1%
H	581	6.3%
I	762	8.2%
J	561	6.0%
Total aligned	9,286	-
Percent aligned	54.8%	-
Not aligned	7,656	-
Total markers	16,942	-

Table 4. Genetic differentiation (F_{ST}) between **(A)** naturalized oyster populations collected from around Vancouver Island, BC; **(B)** between size classes representing different brood years and locations for naturalized populations around Vancouver Island, BC; **(C)** all pairs of populations in the study, using a single representative for each set of similar populations (e.g. BC naturalized represented by PEN). Statistics are shown as 95% confidence intervals, with lower estimates below the diagonal and upper estimates above the diagonal. Any lower limits that overlapped with 0 resulted in the entire interval being transformed to 0. Full population names and locations are shown in Table 1 and Figures 1 and 2, respectively.

(A)

	HIS	**PEN**	**PIP**	**SER**
HIS	-	0.0035	0.0031	0.0025
PEN	0.0022	-	0	0.0033
PIP	0.0014	0	-	0.0025
SER	0.0009	0.0017	0.0007	-

(B)

	PEN_2	**PEN_3**	**HIS_3**	**HIS_6**
PEN_2	-	0	0.0046	0.0043
PEN_3	0	-	0.0048	0.0043
HIS_3	0.0018	0.0024	-	0.0027
HIS_6	0.0017	0.0019	0.0004	-

(C)

	PEN	**CHN**	**QDC**	**RSC**	**DPB**	**ROS**	**GUR**	**FRA**	**JPN**
PEN	-	0.0229	0.0424	0.0147	0.0636	0.0096	0.0307	0.0032	0.0074
CHN	0.0179	-	0.0212	0.0100	0.0839	0.0308	0.0488	0.0218	0.0272
QDC	0.0371	0.0181	-	0.0285	0.1007	0.0505	0.0671	0.0403	0.0450
RSC	0.0119	0.0074	0.0248	-	0.0756	0.0223	0.0405	0.0135	0.0185
DPB	0.0574	0.0756	0.0920	0.0689	-	0.0722	0.0760	0.0640	0.0668
ROS	0.0075	0.0257	0.0445	0.0188	0.0660	-	0.0372	0.0095	0.0145
GUR	0.0270	0.0431	0.0600	0.0360	0.0697	0.0332	-	0.0299	0.0336
FRA	0.0016	0.0168	0.0346	0.0105	0.0582	0.0071	0.0261	-	0.0063
JPN	0.0047	0.0211	0.0383	0.0148	0.0604	0.0114	0.0291	0.0034	-

Table 5. Private allele counts of the 16,942 markers identified using either **(A)** a single representative from each of the general regions in the dataset, with all other populations in that region included into the same group; and **(B)** a single population from each of the global regions. Population full names, and locations are given in Table 1 and Figures 1 and 2, respectively

(A)

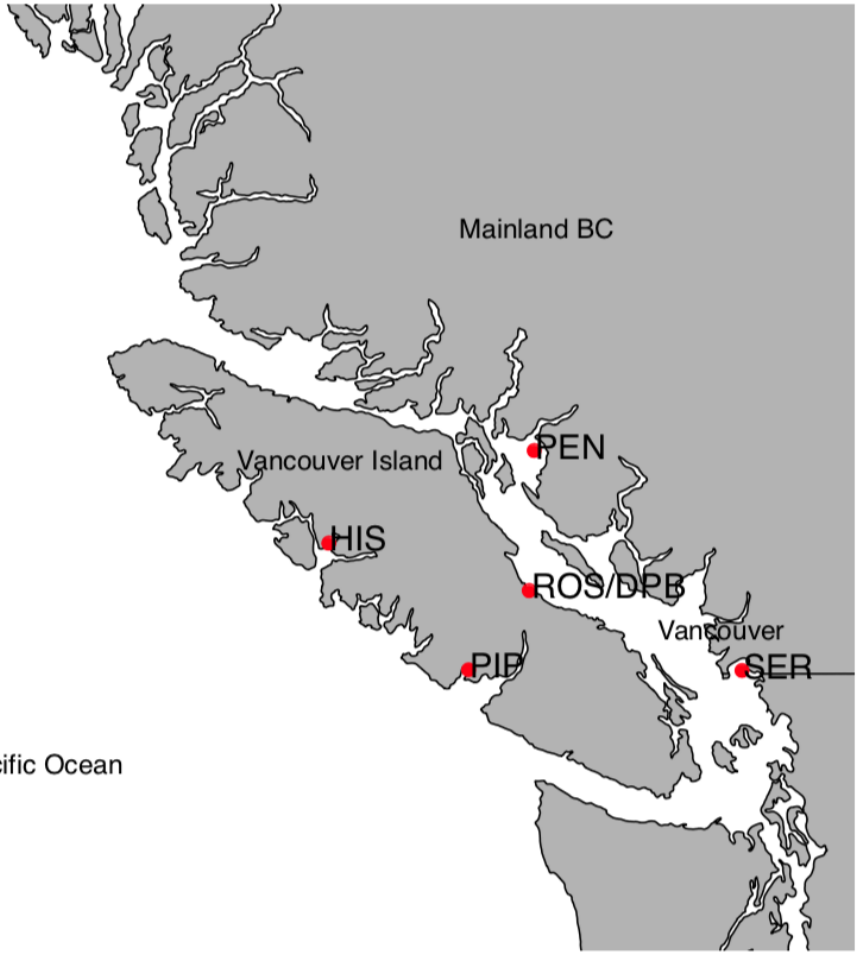
	BC	**DPB**	**ROS**	**GUR**	**RSC**	**QDC**	**CHN**	**FRA**	**JPN**
Private alleles	38	49	0	11	1	17	1	0	0
Sample size	146	20	23	22	23	24	48	47	13

(B)

	PEN	**CHN**	**DPB**	**GUR**
Private Alleles	35	24	20	22
Sample Size	35	24	20	22

FIGURES

(A)



(B)

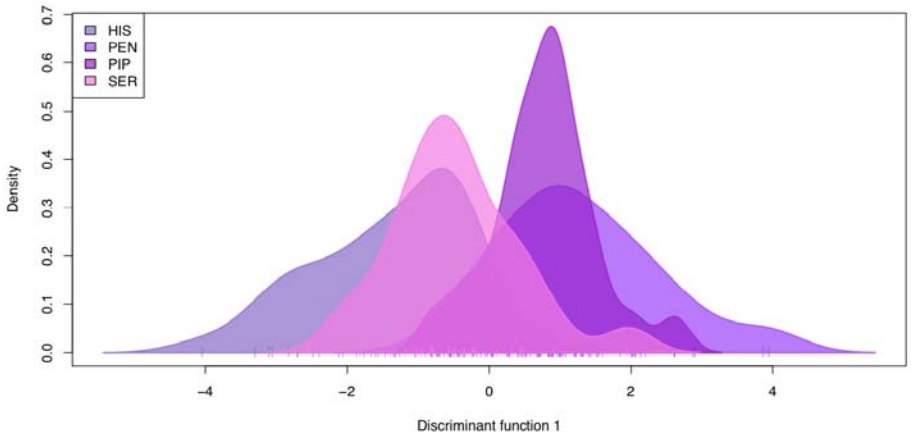
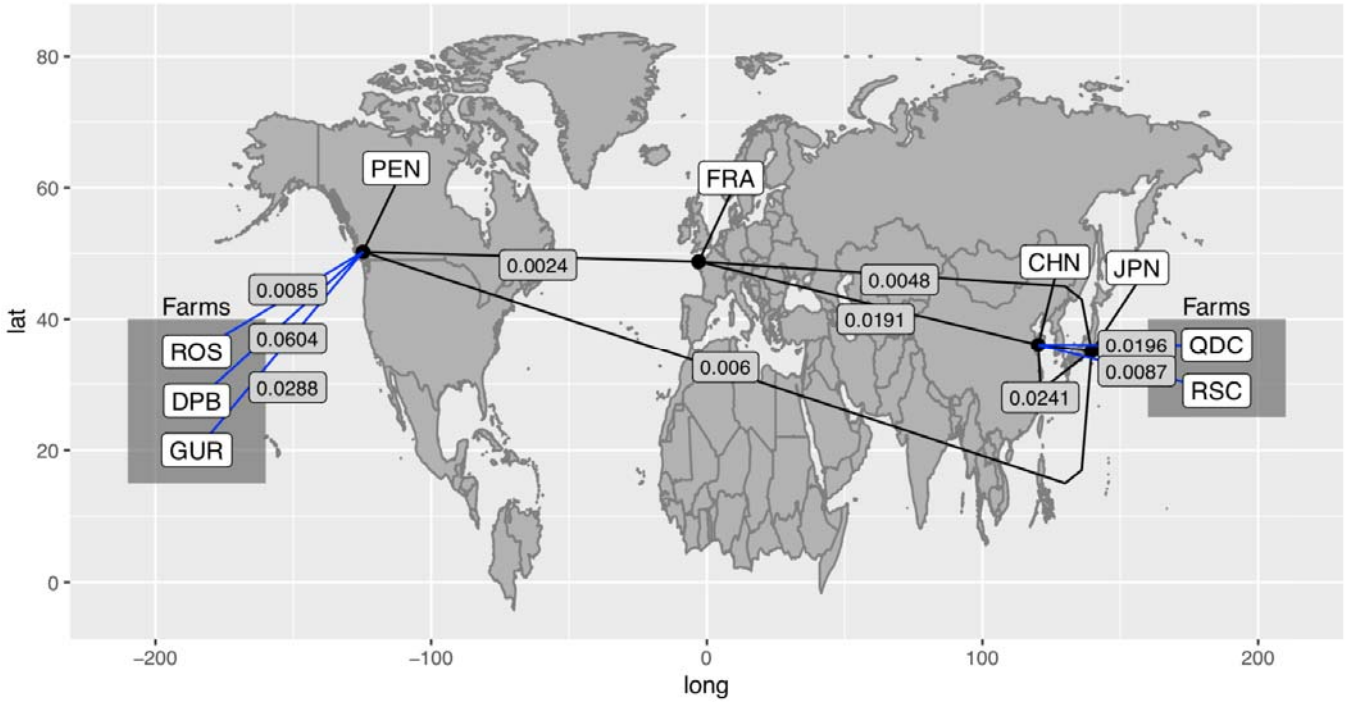


Figure 1. **(A)** Pacific oysters were collected from four locations in southwestern British Columbia. Pendrell Sound (PEN) and Serpentine River (SER) are on the mainland, while Pipestem Inlet (PIP), and Hisnit Inlet (HIS) are on Vancouver Island. **(B)** As shown by discriminant analysis of principal components (DAPC), there was very low to negligible genetic differentiation among these sites. Further, the naturalized populations PEN and PIP are both frequently used by industry for broodstock and are undifferentiated from each other. Populations of commercial-hatchery farms are grown at sites near Rosewall Creek (ROS) and Deep Bay (DPB), shown in (A).

(A)



(B)

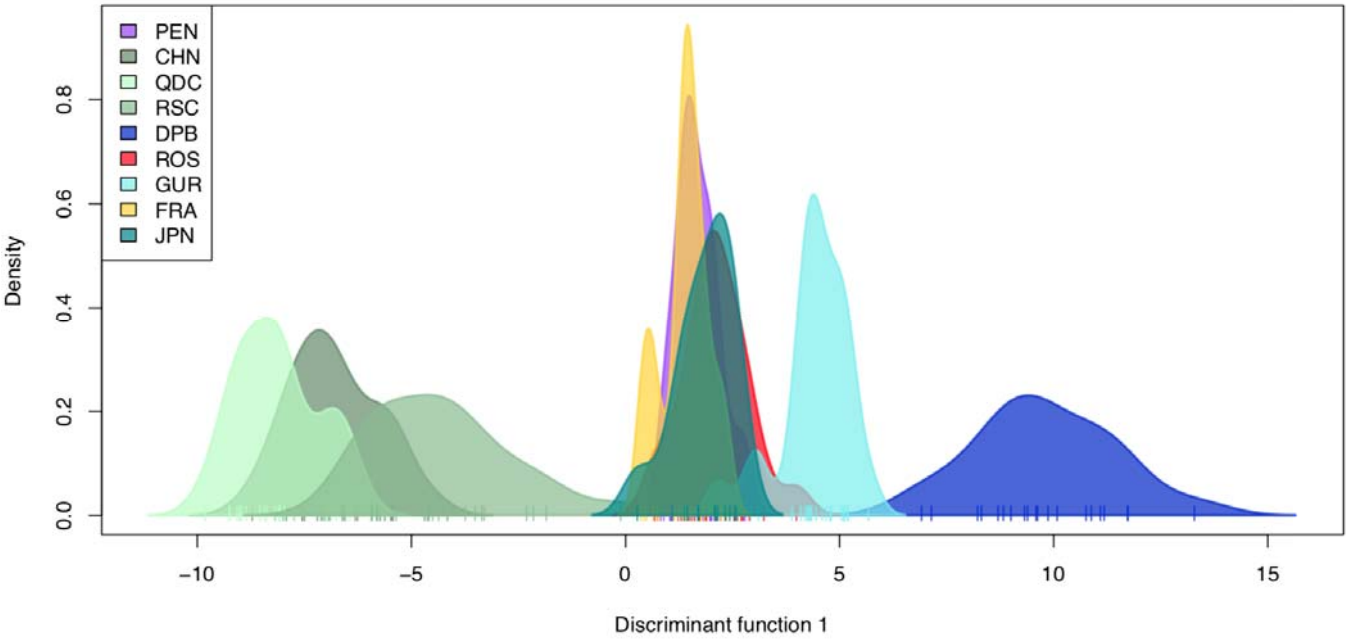
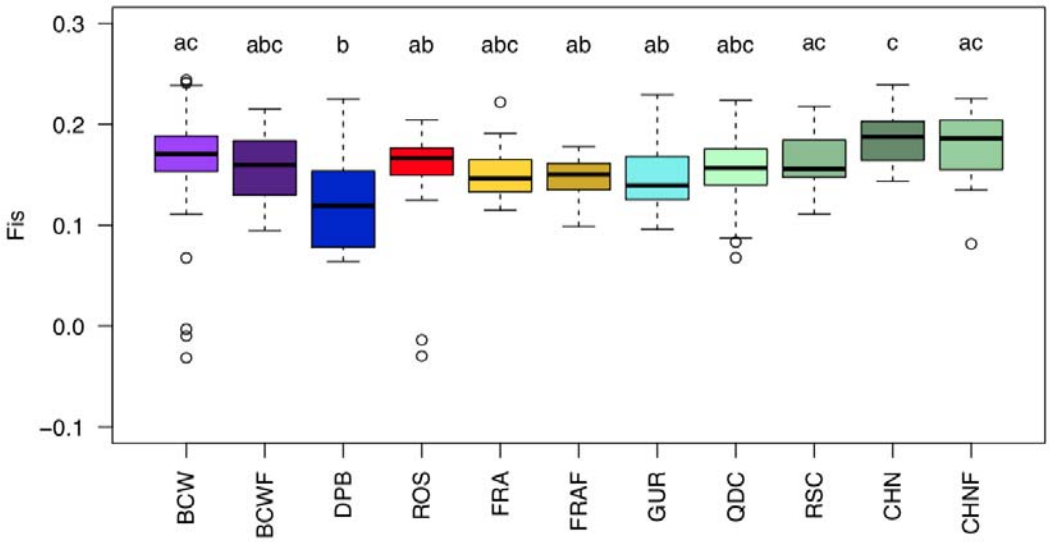


Figure 2. (A) Genetic differentiation among the self-recruiting oysters from Pendrell Sound (PEN), France (FRA), China (CHN), and Japan (JPN) compared to oysters farms growing strains thought to be at least in part descending from the same lineage (ROS, DPB, GUR). China is compared to the farms growing oysters thought to originate from China (QDC, RSC). **(B)** Discriminant analysis of Principal Components (DAPC) using all populations in the analysis indicates the similarity among BC populations, Japan, and France (peaks between 0-4 along discriminant function), among the Chinese populations (peaks between -4 to -10), and the distinct GUR (peak at 5) and DPB populations (peak at 9).

(A)



(B)

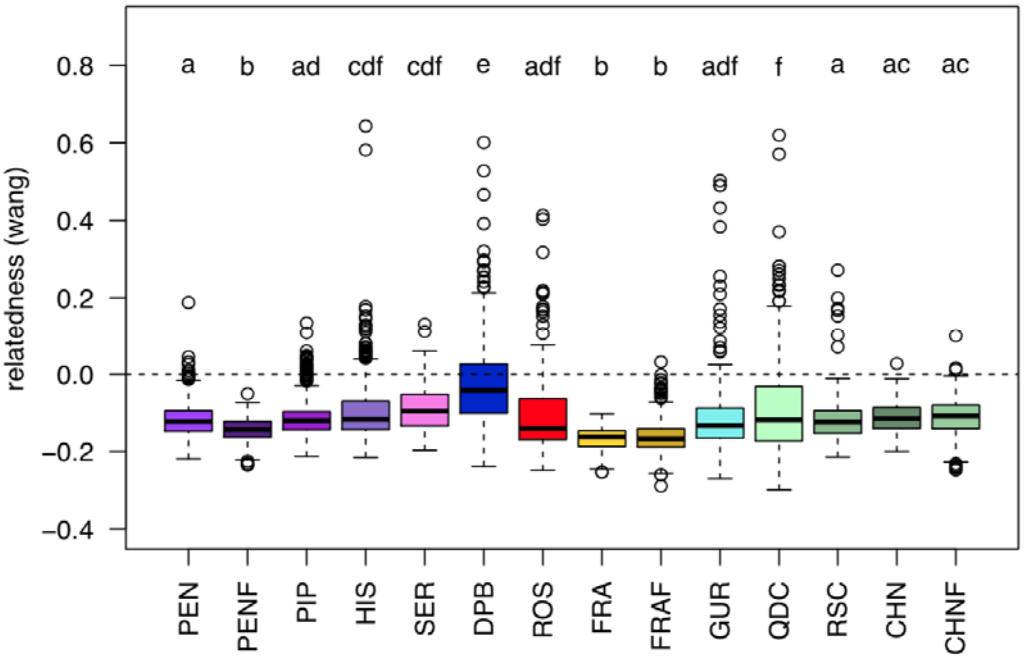


Fig. 3. (A) Per individual within-population inbreeding coefficient (F_{IS}) for all populations. **(B)** Inter-individual relatedness of individuals within each collection as calculated by the *wang* statistic (Wang, 2002). Commercial hatchery-farmed (HAT-F) populations include DPB, QDC, ROS, and RSC, and the hatchery population includes GUR. Significant differences (Tukey HSD $p \leq 0.01$) are indicated when populations do not share any letters above the boxplot. The collection from JPN is not included in this analysis due to lower sample size and impact of sample size on these statistics.

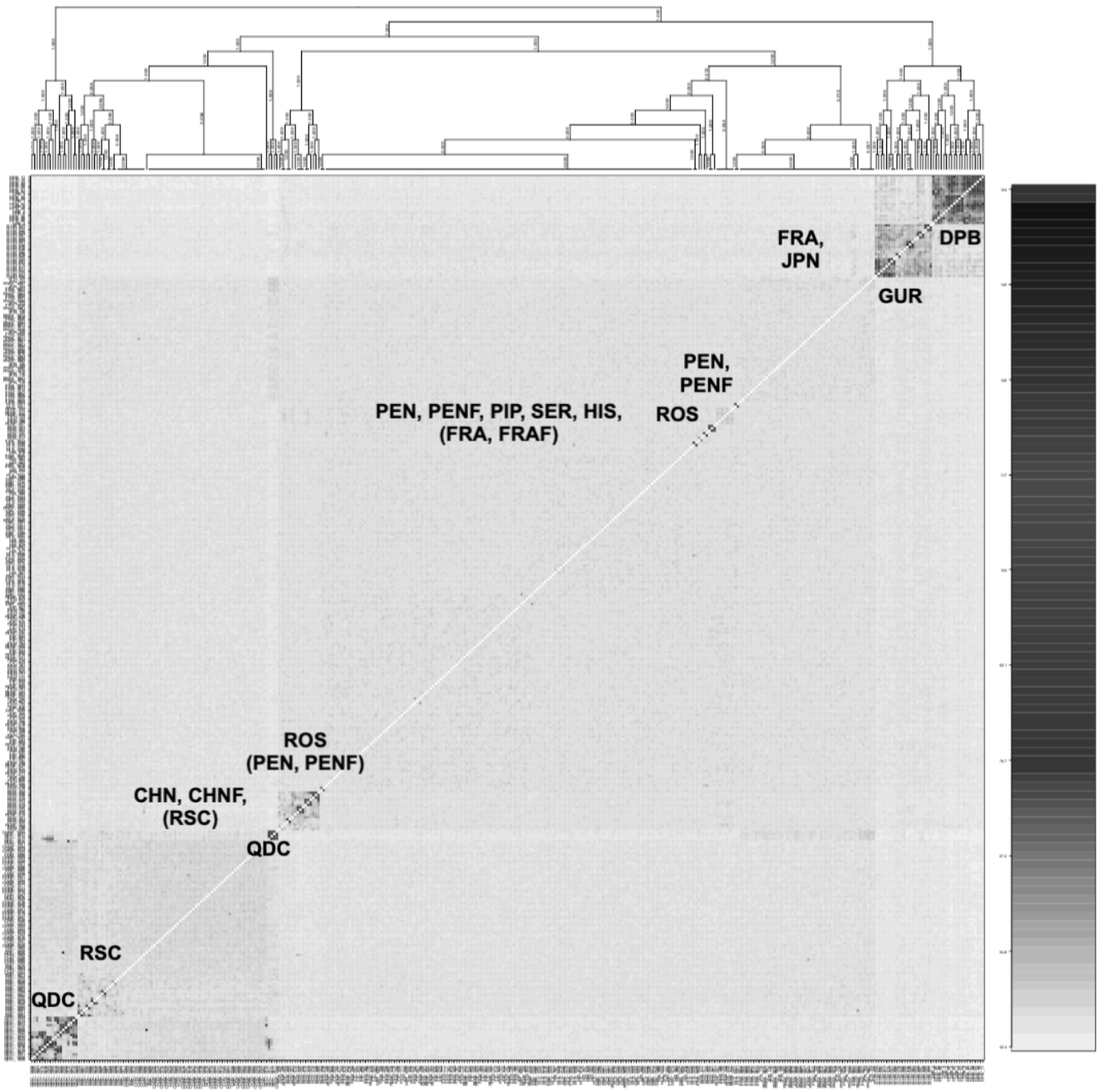


Figure 4. FineRADstructure analysis indicating family structure of hatchery populations and general similarity among groups, as calculated by shared microhaplotypes between samples, including a total of 17,084 loci. Increased shading reflects increased relatedness between individuals.

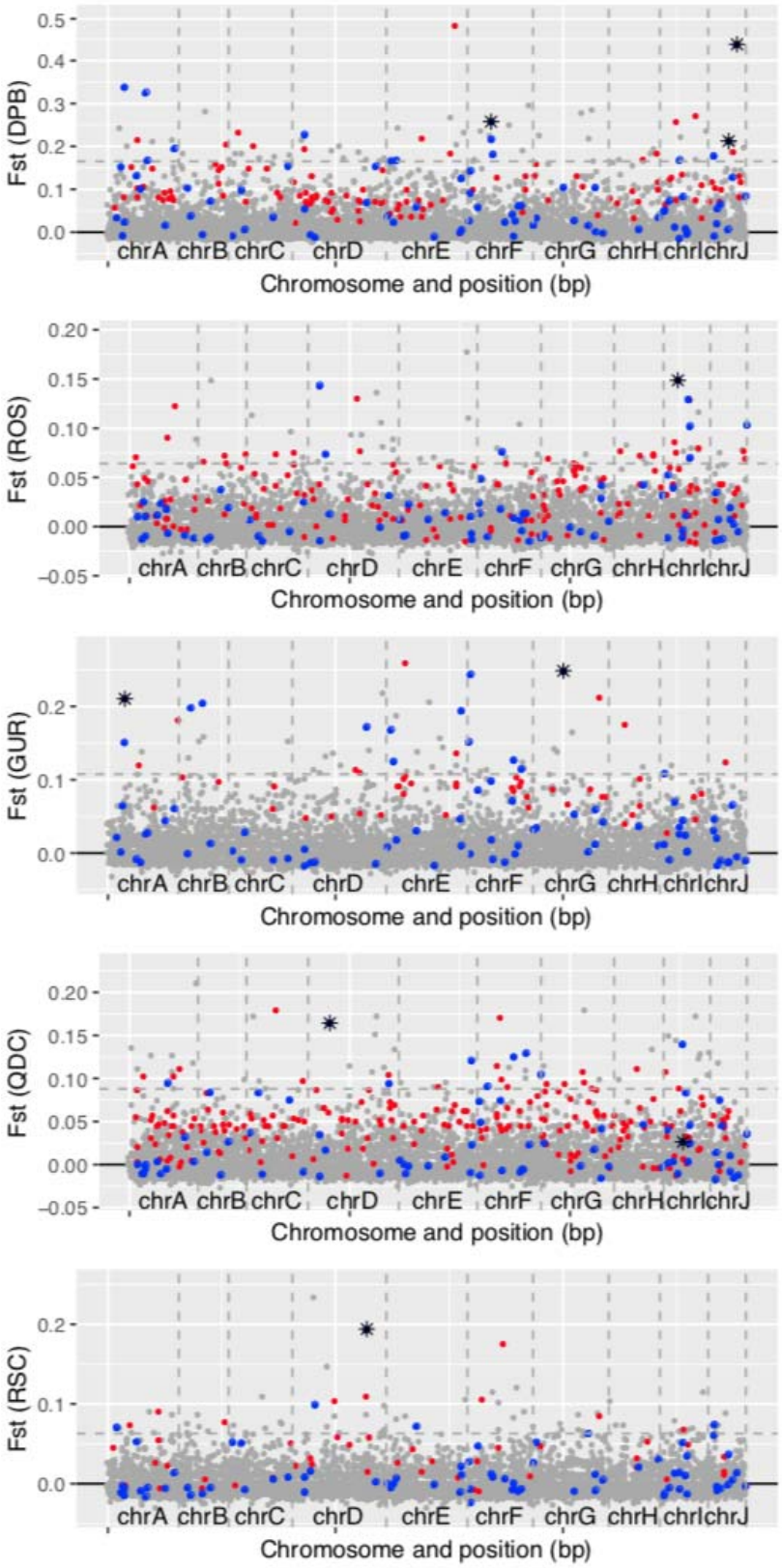


Figure 5. Putative domestication outliers in each comparison shown with per-locus F_{ST} values alongside significant *pcadapt* (red), RDA (blue), and both (black asterisks) indicators. The naturalized/wild population being compared alongside each contrast is the most geographically proximal population (see Table 2). Vertical grey dashed lines separate the chromosomes, and the horizontal dashed line is placed at the 99th percentile of the FST values for the contrast.