

Sex matters in Massive Parallel Sequencing: Evidence for biases in genetic parameter estimation and investigation of sex determination systems

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Abstract

Using massively parallel sequencing data from two species with different life history traits -- American lobster (*Homarus americanus*) and Arctic Char (*Salvelinus alpinus*) -- we highlighted how an unbalanced sex ratio in the samples combined with a few sex-linked markers may lead to false interpretations of population structure and thus to potentially erroneous management recommendations. Multivariate analyses revealed two genetic clusters that separated males and females instead of showing the expected pattern of genetic differentiation among ecologically divergent (inshore vs. offshore in lobster) or geographically distant (east vs. west in Arctic Char) sampling locations. We created several subsamples artificially varying the sex ratio in the inshore/offshore and east/west groups, and then demonstrated that significant genetic differentiation could be observed despite panmixia for lobster, and that F_{st} values were overestimated for Arctic Char. This pattern was due to 12 and 94 sex-linked markers driving differentiation for lobster and Arctic Char, respectively. Removing sex-linked markers led to non-significant genetic structure (lobster) and a more accurate estimation of F_{st} (Arctic Char). We further characterized the putative functions of sex-linked markers. Given that only 9.6% of all marine/diadromous population genomic studies to date reported sex information, we urge researchers to collect and consider individual sex information. In summary, we argue that sex information is useful to (i) control sex ratio in sampling, (ii) overcome “sex-ratio bias” that can lead to spurious genetic differentiation signals and (iii) fill knowledge gaps regarding sex determining systems.

Introduction

Recently, the revolution in massively parallel sequencing (MPS) technology has led to the production of many genome-wide datasets, whereby thousands of markers can be easily and inexpensively genotyped in hundreds of individuals for both model and non-model species (Davey *et al.* 2011; Andrews *et al.* 2016). Several MPS studies based on either RAD-sequencing or Genotype-By-Sequencing (GBS) techniques have demonstrated that these markers bring unprecedented insights on the causes and consequences of population structuring (reviewed in Narum *et al.* 2013). The strength of such methods comes from its supposedly random sampling of the entire genome (Davey *et al.* 2013). While the random distribution of markers achieved by these methods is advantageous in many regards, it has one over-looked result that could have consequences for inferences of population structure: some of the markers identified will be located on sex chromosomes, or in regions linked to sex, in species with genetic sex determination. Indeed, Wright (1931) pointed out this bias in genetic parameter estimations, particularly when sampling populations with varying sex ratios or in the presence of sex-biased dispersal. Despite the potential importance of these biases, few MPS studies have focused on the analysis of sex-linked markers (but see Gamble & Zarkower 2014; Kafkas *et al.* 2015; Brelsford *et al.* 2016; Larson *et al.* 2016) and to our knowledge, none have investigated the influence of sex-linked markers on inferences of population structure observed.

In addition to the importance of avoiding potential biases, detecting sex-linked markers in MPS datasets can also provide valuable information on sex determination (Pan *et al.* 2016). Sex is common to almost all living animals and often leads to the evolution of male and female dimorphism, both at the genetic and phenotypic level (Bell 1982). Diverse mechanisms acting at the scale of the genome, chromosomes or cells underlie the morphological, physiological and behavioral differences between males and females. Moreover, sex determination systems vary tremendously among and within taxa (Bachtrog *et al.* 2014), highlighting the challenges in determining the selective forces driving sex determination. In general, the diversity of sex determination systems reported in fish (particularly teleosts) and crustaceans is much more pronounced than that observed in mammals and birds (Bachtrog *et al.* 2014). Yet, the characterization of the

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genetic architecture of sex determination in these taxonomic groups has been limited to a few studies (Legrand *et al.* 1987). The access to new genomic approaches, which are increasingly being used in non-model marine and aquatic organisms (Kelley *et al.* 2016), offers new prospects to investigate the molecular basis of sex determination in this diverse group.

The identification of sex-linked markers can also provide a wealth of other useful information for management, conservation, and aquaculture (Pan *et al.* 2016). First, sex-linked markers can assist in the identification of the sex of an individual, particularly in cases with an absence of clear sexual dimorphism (*e.g.*, at young life history stages). In aquaculture practices, this can help farmers to maintain equal sex ratios of breeding adults and to implement efficient breeding programs (Martínez *et al.* 2014). Second, sex information is often important to include as a covariate in genetic models for finding loci linked to specific traits in order to reduce residual variation (Broman & Sen 2009). Third, knowing the sex of individuals may facilitate the demonstration of sex-biased dispersal, *i.e.*, when individuals of one sex are more prone to disperse (Prugnolle & De Meeûs 2002). Sex-biased dispersal is widely spread among vertebrates and can have important ecological and evolutionary consequences, but there is still little research on this topic in marine organisms, such as fishes and crustaceans, compared to mammals and birds (Mossman & Waser 1999).

Here, we present two empirical examples that illustrate how an unbalanced sex ratio combined with a few sex-linked markers can lead to false interpretations of population structure and to erroneous management recommendations, especially in species with high connectivity as frequently observed in marine and diadromous organisms. Our initial goal was to separately investigate population structure between two groups of American lobsters (*Homarus americanus*) occupying inshore and offshore habitats, and between Arctic Char (*Salvenius alpinus*) collected from two geographically separated regions (east and west) in the Canadian Arctic. In both cases, preliminary multivariate analyses mainly revealed two genetic clusters corresponding to male and female individuals instead of being related to inshore/offshore groups of lobsters or to east/west groups of Arctic Char. To further understand the clustering, we identified sex-linked markers driving the genetic differentiation between male and female in American

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lobster and Arctic Char. To demonstrate the potential impacts of sex-linked markers on the population genetic analysis, we tested for both species how different numbers of sex-linked markers and ratios of samples from each sex can cause biased inferences of population structure. Finally, using the set of sex-linked markers identified, we found potential candidate genes or chromosomal regions linked to sex for American lobster and Arctic Char. We conclude with an exhaustive literature search demonstrating that very few studies performed on marine and diadromous species report sex information, and we argue, in light of our findings, that collecting this information can be critical to avoid biases, especially in high gene flow species.

Methods

Sampling and molecular techniques

American lobster: Commercial fishers collected 203 American lobsters (100 males and 103 females) from 13 sites including eight inshore sites and five offshore sites along the Atlantic coast of North America (Figure 1A; Table S1). The sex of all specimens was determined visually from obvious external morphological differences. Genomic DNA was then extracted using Qiagen Blood and Tissue kits. DNA quality was confirmed using visual inspection on 1% agarose gel followed by quantification with Quantit Picogreen dsDNA assay kits. RAD-sequencing libraries were prepared following the protocol from Benestan *et al.* (2015). Each individual was barcoded with a unique six-nucleotide sequence and 48 individuals were pooled per library. Real-time PCR was used to quantify the libraries. Single-end, 100 bp sequencing was performed on an Illumina HiSeq2000 platform at the Genome Québec Innovation Centre (McGill University, Montréal, Canada).

Arctic Char: Samples of 290 adult anadromous Arctic Char (142 males and 148 females) were collected from six rivers located on southern Victoria Island, Nunavut, Canada (Figure 1B; Table S2). Sex was determined visually by observation of the gonads for a subset ($n = 174$) and based on a genetic assay for another subset ($n = 116$), as described in Moore *et al.* (2016). In brief, the genetic sex was inferred based on the PCR assay described in Yano *et al.* (2013). Six individuals of known sex (three males and three females) were used as controls. Genomic DNA was extracted using a salt-extraction

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protocol modified from Aljanabi and Martinez (1997). DNA quality and quantity were checked on 1% agarose gels and using PicoGreen assays (Fluoroskan Ascent FL, Thermo Labsystems), respectively. Libraries were prepared based on a GBS protocol modified from Mascher *et al.* (2013) using *Pst*I and *Msp*I (details can be found in Perreault-Payette *et al.* in press). Specimens were individually barcoded with unique six-nucleotide sequences and pooled with 48 individuals per library. Libraries were each sequenced on two Ion Torrent Proton P1v2 chips.

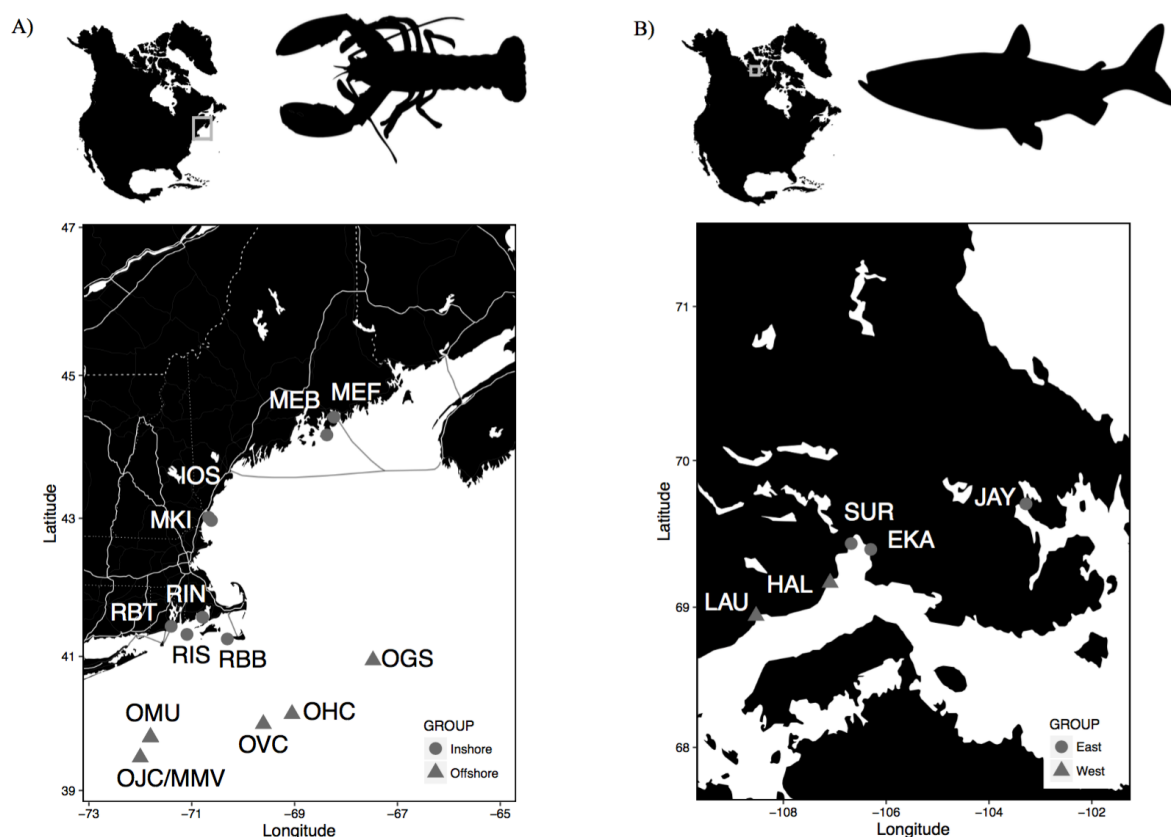


Figure 1. Sampling Locations for American lobster (A) and Arctic Char (B). (A) Inshore sampling locations are shown with a grey circle and offshore locations with a grey triangle. Inshore locations are Isle of Shoals (IOS; n=14), Blue Hill Bay (MEB; n=20), Frenchmans Bay (MEF; n=19), Kittery (MKI; n=20), Brown's bank (RBB; n=17), Beavertail (RBT; n=16), Narragansett Bay (RIN; n=13), Rhode Island Sound Bay (RIS; n=7). Offshore locations are Georges Basin (OGS; n=10), Hydrographers Canyon (OHC; n=16), Jones Canyon (OJC; n=12), MacMaster Canyon (OMU; n=10) and Veatch Canyon (OVC; n=16). (B) Eastern sampling locations are shown with a grey circle and Western locations with a grey triangle. Eastern locations are Ekalluk (EKA; n = 58), Jayko (JAY; n = 58), Surrey (SUR; n = 30). Western locations are Halovik (HAL; n = 87) and Lauchlan (LAU; n = 57).

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Bioinformatics and genotyping

Both the American lobster and Arctic Char libraries were de-multiplexed using *process_radtags* in STACKS (v.1.29 for American lobster and v.1.40 for Arctic Char) (Catchen *et al.* 2013). Raw sequencing data was checked in FASTQC (Andrews 2015). Reads were truncated to 80 bp for lobster and 70 bp for Arctic Char and adapter sequences were removed with CUTADAPT (Martin 2011).

American lobster: Loci were identified allowing a maximum of three nucleotide mismatches ($M = 3$), according to Ilut *et al.* (2014) and a minimum stack depth of three ($m = 3$), among reads with potentially variable sequences (*ustacks* module in stacks, with default parameters). Then, reads were clustered *de novo* to create a catalogue of putative RAD tags (*cstacks* module in STACKS, with default parameters). In the *populations* module of STACKS v.1.29 and following consecutive filtering steps, SNPs were retained when they were genotyped in at least 80% of the individuals and found in at least 9 of the 12 sampling sites. Potential paralogs were excluded by removing markers showing heterozygosity > 0.50 and $0.30 < F_{IS} < -0.30$ within sites. Only SNPs with a global minor allele frequency > 0.02 were retained for the analysis. The resulting filtered VCF files were converted into the file formats necessary for the following analyses using PGDspider v.2.0.5.0 (Lischer & Excoffier 2012).

Arctic Char: SNPs were identified by first mapping the reads to the genome of the closely related Rainbow Trout (*Oncorhynchus mykiss*; Berthelot *et al.* 2014) using GSNAP v2016-06-09 with a minimum of 90% read coverage ($-\text{min-cov } 90$), tolerating 2 mismatches ($-\text{m } 2$) and setting an indel penalty to 2 ($-\text{i } 2$). A subsequent trimming step was conducted with SAMtools v1.2 (Li *et al.*, 2009) to remove unmapped and multi-mapped reads using flags $-\text{F } 1797$ and $-\text{F } 4$, and a minimum mapping quality (MAPQ) of 1, respectively. The binary alignment files (bam) were then used as input for downstream analysis. Genotypes were obtained using STACKS v.1.40 integrated in a workflow developed in our laboratory (Benestan *et al.* 2016a). The catalog of loci was created allowing no mismatches among loci in *cstacks* ($n=0$) and a minimum stack depth of four ($-\text{m } 4$). SNPs were retained if at least 50% of the individuals were genotyped for the marker ($-\text{r } 0.5$) and the locus was present in at least four populations ($-\text{p } 4$). Potential paralogs were excluded by removing markers showing heterozygosity > 0.60 and $F_{IS} < -$

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0.40 $F_{IS} > 0.40$ within samples. Only SNPs with a global minor allele frequency > 0.01 were retained for the analysis.

Discriminant Analysis of Principal Component (DAPC)

For American lobster, Discriminant Analysis of Principal Components (DAPC) was performed in the R package *adeigenet* (Jombart *et al.* 2010). The optimal number of discriminant functions ($n=60$) to retain was evaluated according to the optimal α -score obtained from the data (Jombart *et al.* 2010). For Arctic Char, a Principal Component Analysis was performed in *adeigenet*. As population differentiation was pronounced enough to be observed with the PCA, a DAPC was not conducted.

Sex outlier loci detection

American lobster and Arctic Char: Outlier loci corresponding to the most divergent markers between sexes were identified with a level of differentiation between sexes exceeding random expectations using F_{st} -based outlier analyses. Outlier SNPs were detected with BAYESCAN v. 2.1 (Foll & Gaggiotti 2008). BAYESCAN runs were implemented using permissive prior model (pr_odds) of 10, including a total of 10,000 iterations and a burn-in of 200,000 steps. For both species, these outlier analyses were conducted on the entire data set separated by sex.

Sex ratio and sex-linked marker influence on index of genetic differentiation (F_{st})

To determine the extent to which differing sex ratio influences the detected genetic structure, different proportions of male and female American lobsters or Arctic Char were subsampled from inshore or east and offshore or west, respectively, keeping a total of 50 individuals per group. This generated a gradient of six different sex ratio datasets, representing different sampling bias scenarios, from the most balanced (sex ratio = 25:25/25:25) to the most unbalanced sex ratio (sex ratio = 0:50/50:0).

Considering the three most unbalanced sex-ratio datasets (*i.e.*, 0:50/50:0, 5:45/45:5, 10:40/40:10), we removed sex-linked markers (*i.e.*, here outlier SNPs) according to their F_{st} values (in descending order) and we estimated F_{st} between offshore/inshore for the American lobster and east/west for the Arctic Char. We

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calculated F_{st} values using the function `fst_WC84` in *assigner* R package (Gosselin *et al.* 2016).

Marker annotation and genomic position

American lobster: There is no reference genome or high-density linkage map available for American lobster and so the approximate locations or associated linkage groups of the sex-linked SNPs could not be determined. Probable proximity between markers was determined by linkage disequilibrium (LD) analysis by calculating LD between pairs of SNPs using the *geno-r2* command available in VCFTOOLS (Danecek *et al.* 2011). The LD data frame obtained with VCFTOOLS was then transformed into an LD matrix to be analyzed using the *heatmap* command in the R environment (Team 2013). In order to determine what genes are associated with these sex-linked markers, the 12 candidate SNPs (outliers identified by BAYESCAN) were queried using BLAST against the transcriptome of the American lobster (F. Clark and S. Greenwood, University of Prince Edward Island, *personal communication*; see details in Benestan *et al.* 2016b). Six of the 12 candidate SNPs were distributed among six different contigs in the transcriptome data. The associated contigs were used as queries in a BLAST search against the SWISS-PROT database (Bairoch & Apweiler 2000). A minimal *E*-value threshold of 1×10^{-6} and percent similarity of at least 70% were used. This yielded a set of two candidate SNPs associated with known genes. Gene ontology (GO) annotation terms were then associated to the candidate SNPs using SWISS-PROT accessions.

Arctic Char: There is no reference genome available yet for Arctic Char, but there is a high-density linkage map available for the closely related Brook Char (Sutherland *et al.* 2016). To obtain approximate positions of the sex-linked SNPs from Arctic Char, the MapComp method (Sutherland *et al.* 2016) was used to pair all of the Arctic Char markers with mapped Brook Char markers using the Atlantic Salmon genome (Lien *et al.* 2016; GenBank: GCA_000233375.4) as the intermediate reference genome. This method connects markers from two different linkage maps by mapping the markers to a reference genome, then pairing markers that map uniquely to the same place or close to each other in the reference genome. This was done as previously described (Sutherland *et al.* 2016), but with ten iterations to permit more than one anonymous marker pairing with a single

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mapped marker, as previously described (Narum *et al. in review*) but with a 1 Mbp maximum distance between the paired markers on a reference genome. This yielded approximate positions for determining the number and identity of linkage groups associated with sex in Arctic Char. To determine which genes are associated with these linked markers, the sex-linked markers were used in a BLAST query against the annotated Atlantic Salmon genome (Lien *et al.* 2016); NCBI Genome ICSASG_v2 reference Annotation Release 100).

Literature search for marine and diadromous species population genomic studies

We performed an exhaustive literature search to document the proportion of population genomics studies that have reported sexing the species analyzed. More specifically, we conducted a literature search of population genomics studies on marine/diadromous species published in peer-reviewed journals from January 2010 to 15 November 2016 using the ISI Web of Knowledge bibliographic database (Thomson Reuters, <http://thomsonreuters.com>) using search keywords (i) “genomics” AND “marine” AND “SNP” yielded 22 hits, (ii) “population structure” AND “marine” AND “SNP” yielded 47 hits, (iii) “RAD-sequencing” AND “marine” yielded 39 hits and (iv) “population genomics” AND “marine” yielded 243 hits, (v) “population genomics” AND “anadromous” OR “catadromous” yielded 11 hits. From these hits, several criteria were used to determine which studies to include in our analyses. First, the paper needed to focus on a marine animal and use a set of more than 1,000 SNP markers. Second, the paper needed to refer to population genomics or related areas such as outlier identification because these are the target areas of research likely to be influenced by the sex ratio bias in sampling. After removing studies on non-marine or non-animal organisms, or those with too low density of markers, a total of 38 and 14 publications were retained for marine and diadromous species, respectively (listed in Table 1 and 2).

Results

Artefactual population structure caused by sex-linked markers

For American lobster, using 1,717 filtered SNPs, Discriminant Analysis of Principal Components (DAPC) was performed on the 203 individuals successfully genotyped to investigate the extent of population structuring between offshore and inshore locations. Instead of finding significant genetic differences between inshore and offshore samples, the first axis of the DAPC highlighted a significant genetic differentiation between sexes ($F_{st} = 0.0057$, P-value = 0.0009), explaining 16.04% of the total genetic variation (Figure 2A).

For Arctic Char, using 6,147 filtered SNPs a principal components analysis (PCA) of genotypes from 290 individuals identified strong clustering that explained 5.74% of the total genetic variation between two groups not corresponding to any particular geographic region (Figure 2C). By using the data on phenotypic and genetic sex, it was clear that samples mainly clustered by sex in this PCA (Figure 2C) and that this genetic differentiation was modest but significant ($F_{st} = 0.0132$, P-value = 0.0002).

Delineating the influence of sex ratio on F_{st} in panmictic or anadromous species

A DAPC and a PCA were run on datasets containing only males for offshore or east region and only females for inshore or west locations for American lobster and Arctic Char, respectively (Figure 2B,D). As expected, the DAPC for American lobster showed a highly significant signal of genetic differentiation between inshore and offshore samples with a F_{st} value in the range typically seen in many marine species ($F_{st} = 0.0056$, 95% $CI_{inf} = 0.0027$ and $CI_{sup} = 0.0088$, P-value < 0.05), which in reality resulted from the extremely skewed sex ratio of this artificial dataset (Figure 2B,D). This outcome contrasts with the panmictic structure observed between inshore and offshore ($F_{st} = 0.0001$, $CI_{inf} = -0.0004$ and $CI_{sup} = 0.0006$, P-value > 0.05) when sex ratio is balanced (sex ratio in the original dataset is equal to 25:25/25:25). As expected, F_{st} between inshore and offshore was highest and most significant when sex ratio was completely unbalanced, *i.e.*, sex ratio equal to 0 ($F_{st} = 0.0055$, $CI_{inf} = 0.0030$ and $CI_{sup} = 0.0092$, P-value < 0.05). F_{st} remained significantly elevated until the sex ratio was 15:35/35:15 ($F_{st} < 0.001$, $CI_{inf} < 0$; Figure 3A).

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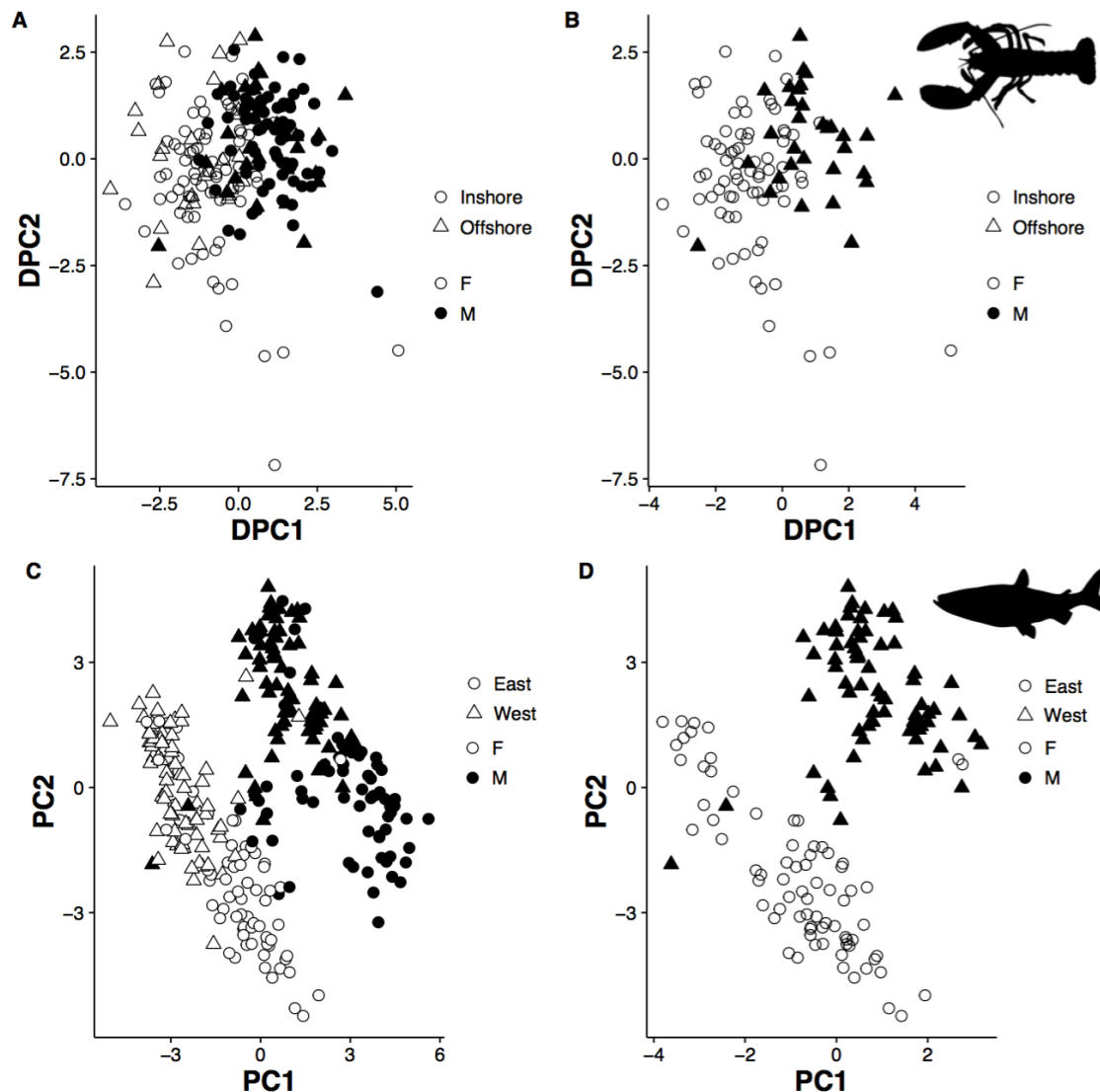


Figure 2. Discriminant Analysis of Principal Components (DAPC) and Principal Components Analysis (PCA) of genetic differentiation depending on the sampling scenario (A and C). Results of the DAPC (A) and the PCA (C) performed on lobster and Arctic Char respectively with sex information included. Individuals from the inshore/east and offshore/west regions are represented by different shape symbols, and male and female are represented by black and white symbols, respectively. (B and D) Results of the DAPC (B) and the PCA (D) performed on lobster and Arctic Char respectively, but using hypothetical datasets in which only males were sampled in one of the location (offshore and west respectively) and only female in the other location (inshore and east respectively) showing a false signal of population differentiation driven by differences in sex ratios.

Following the same method described above to simulate differing sex ratio datasets, F_{st} between east and west Arctic Char locations was highest and most significant

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when sex ratio was completely unbalanced, *i.e.*, sex ratio equal to 0:50/50:0 ($F_{st} = 0.0215$, $CI_{inf} = 0.0194$ and $CI_{sup} = 0.0242$, $P\text{-value} < 0.05$). F_{st} then gradually decreased with increasingly even sex ratios until it reached $F_{st} = 0.0064$ ($CI_{inf} = 0.0055$ and $CI_{sup} = 0.0072$; $P\text{-value} < 0.05$) with a sex ratio of 25:25/25:25 (Figure 3B).

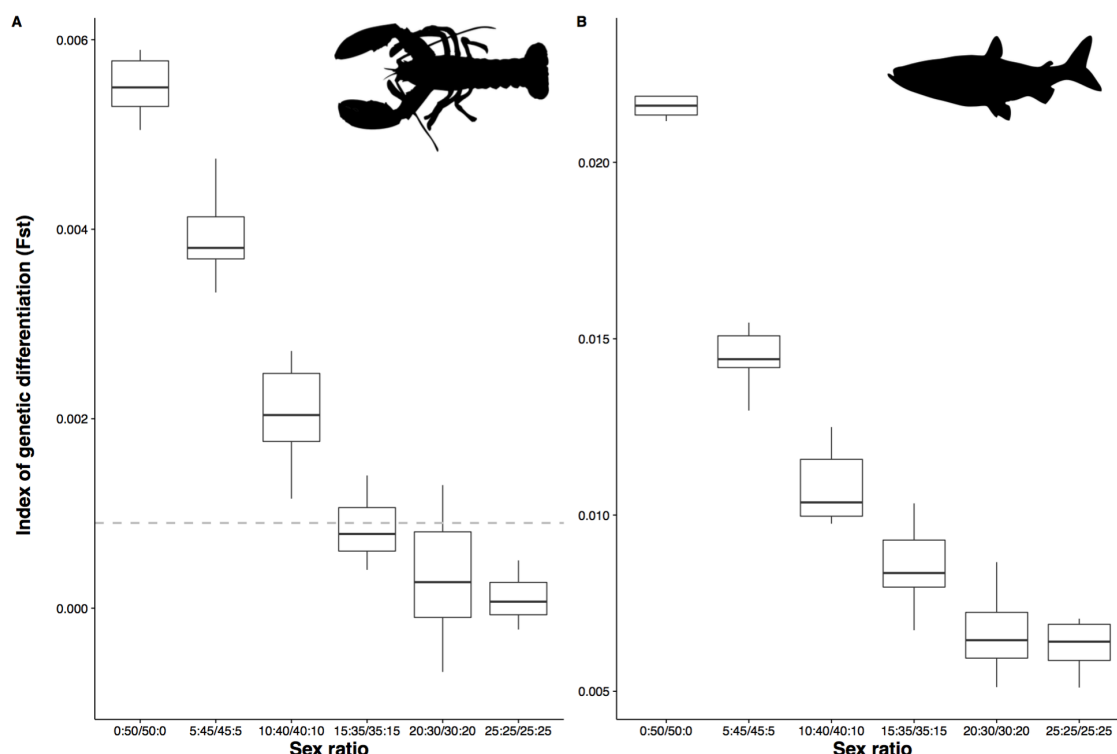


Figure 3. Boxplots showing the influence of sampling sex ratio on F_{st} . (A) American lobster. F_{st} between offshore and inshore according to sex ratio proportion when subsampling 100 individuals with a sex ratio ranging from a complete unbalanced sex ratio (*i.e.*, sex ratio equal to 0:50/50:0) to a perfectly balanced sex ratio (*i.e.*, sex ratio equal to 25:25/25:25). The horizontal black dashed line indicates the threshold below which F_{st} values are no longer significant at $P < 0.05$. (B) Arctic Char. F_{st} between east and west according to the sex ratio proportion when subsampling 100 individuals with a sex ratio ranging from a complete unbalanced sex ratio (*i.e.*, sex ratio equal to 0:50/50:0) to a perfectly balanced sex ratio (*i.e.*, sex ratio equal to 25:25/25:25). F_{st} was still significant for the anadromous, but was overestimated in the skewed sex ratio cases. In both panels, the vertical limits of the box represent one standard deviation around the mean ($n = 10$ individual subsample iterations), the horizontal line within the box is the median, and the whiskers extend from the box to the 25th and 75th percentiles.

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Identifying sex-linked markers in American lobster and Arctic Char

Out of the 1,717 SNPs initially considered for the American lobster, BAYESCAN identified 12 highly differentiated markers between the sexes (Figure S1). These 12 markers have a BAYESCAN F_{st} of 0.0800 on average between the sexes (range = 0.1567-0.1167) whereas the remaining 1,705 SNPs have on average a F_{st} of 0.0030 on average (range = 0.0032-0.0101).

Out of the 6,147 markers initially considered for Arctic Char, BAYESCAN identified 94 markers contributing to the male/female separation (Figure S1). These 94 markers show a BAYESCAN F_{st} of 0.0421 between the sexes (range = 0.0039-0.1140) whereas the remaining 6,053 markers were on average 0.0019 between the sexes (range = 0.0019-0.0036).

Delineating the influence of sex ratio on F_{st} in panmictic or anadromous species

We investigated the influence of the number of these 12 and 94 sex-linked markers on the index of genetic differentiation (F_{st}) calculated between inshore/offshore or east/west for both species, where sex ratio in sampling was unbalanced at different degrees (0:50/50:0, 5:45/45:5, 10:40/40:10). For American lobster, we observed high and significant F_{st} values when no sex-linked marker was removed for the three scenarios. Then, F_{st} progressively decreased with the removal of sex-linked markers (in descending order regarding their F_{st} values) until reaching a small and non-significant value when we removed at least 11 out of 12 sex-linked markers for the most extreme scenario (0:50/50:0; Figure 4A). For Arctic Char, F_{st} progressively decreased from 0.0215 to 0.0064 on average, considering all scenarios, which suggest that F_{st} is more than two-fold smaller when sex-linked markers are removed from the data set (Figure 4B). This decrease reached a plateau when 80 sex-linked markers were removed, which corresponds to almost the totality ($n = 94$) of the sex-linked markers found.

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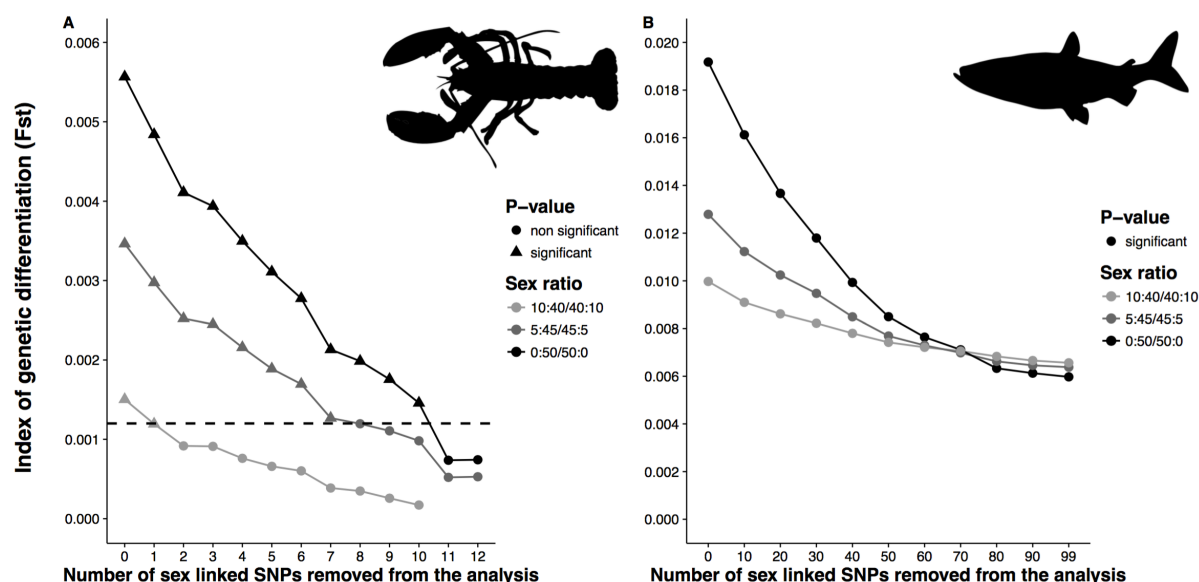


Figure 4. The effect of sex-linked markers on the index of genetic differentiation (F_{st}). (A) American lobster. The line graph displays the influence of sex-linked markers on F_{st} as a function of the number of sex-linked markers removed from the analysis considering three sampling scenario (10:40/40:10, 5:45/45:5, 0:50/50:0). Sex-linked markers are removed in descending order according to their F_{st} values (see Table 1). The dashed line in black indicates the threshold below which F_{st} values are no longer significant at $P < 0.05$. Sex ratio of 0.4 and 0.5 were not included in this analysis because F_{st} values were not significant in these cases (see Figure 4A). (B) Arctic Char. The line graph displays the influence of sex-linked markers on (as a function of the number of sex-linked markers removed from the analysis considering three sampling scenario with different degrees of sex ratio bias (0:50/50:0, 5:45/45:5, 10:40/40:10). Sex-linked markers are removed in descending order according to their F_{st} values.

Characterizing sex-linked markers in American lobster

Linkage disequilibrium (LD) calculations for the 12 sex-linked markers in American lobster revealed two clusters of markers in high LD (Figure S2). One of the clusters includes seven markers with the strongest genetic differentiation between the sexes ($F_{st} > 0.40$; Table 3). Six of these markers displayed heterozygosity excess in males ($H_O = 0.49$, H_O ranging from 0.16 to 0.63) and heterozygosity deficit in females ($H_O < 0.02$; H_O ranging from 0.00 to 0.29), thus providing evidence for a male heterogametic system.

The identities of genes nearby the sex-linked SNPs in lobster were further explored in the six contigs containing the six sex-linked SNP markers, which were located in sequences that had a significant match (more than 90% of nucleotide identity) in the American lobster transcriptome. The polymorphisms associated with two of these

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sequences both occurred in the 3'UTR region of the genes annotated by SWISSPROT database. These genes were *sulfotransferase family cytosolic 1B member 1* (hereafter *SULT1B1*) and *pre-mRNA-splicing factor cwf19* (hereafter *cwf19*), and are involved in steroid metabolism and mRNA splicing, respectively. Both genes that were previously reported to influence sex determination in fishes (Devlin & Nagahama 2002), namely in European Eel (*Anguilla anguilla*; Churcher *et al.* 2015) and Greenland Halibut (*Scophthalmus maximus*; Ribas *et al.* 2015a).

Characterizing sex-linked markers and chromosomes in Arctic Char

From the 6,147 markers, 1,837 could be assigned to the Brook Char linkage map with approximate positions, and this included 45 of the 94 sex-linked markers. Plotting these markers along their approximate locations in the Brook Char linkage map indicates four acrocentric chromosomes with numerous sex-linked markers present, BC13 (8 markers), BC15 (12 markers), BC35 (6 markers), and BC38 (10 markers; Figure 5), which correspond to the ancestral chromosomes 14.1, 19.1, 15.1, 1.2, respectively (Sutherland *et al.* 2016). Three other linkage groups had three or fewer sex-linked markers each (BC07, BC08 and BC25; or 20.1-4.2, 11.2-7.1, and 1.1, respectively).

Using BLAST to align the 94 sex-linked markers against the Atlantic Salmon (*Salmo salar*) reference genome (Lien *et al.* 2016; GenBank GCA_000233375.4) consistently identified the Atlantic Salmon chromosomes homologous to the Brook Char chromosomes that were assigned using iterative MapComp. An additional nine of the 49 non-positioned markers aligned against the Atlantic Salmon chromosomes corresponding to the four highly sex-linked chromosomes, Ssa01, Ssa10 and Ssa09 (Ssa09 corresponds to a fused metacentric chromosome that corresponds to BC35 and BC38; Sutherland *et al.* 2016). Four non-positioned markers were assigned to chromosomes not identified as the four highly sex-linked chromosomes. Often the markers that had not received positions with iterative MapComp either did not have significant alignments or had many equal alignments in the Atlantic Salmon genome.

Sex-linked markers in genome-wide datasets

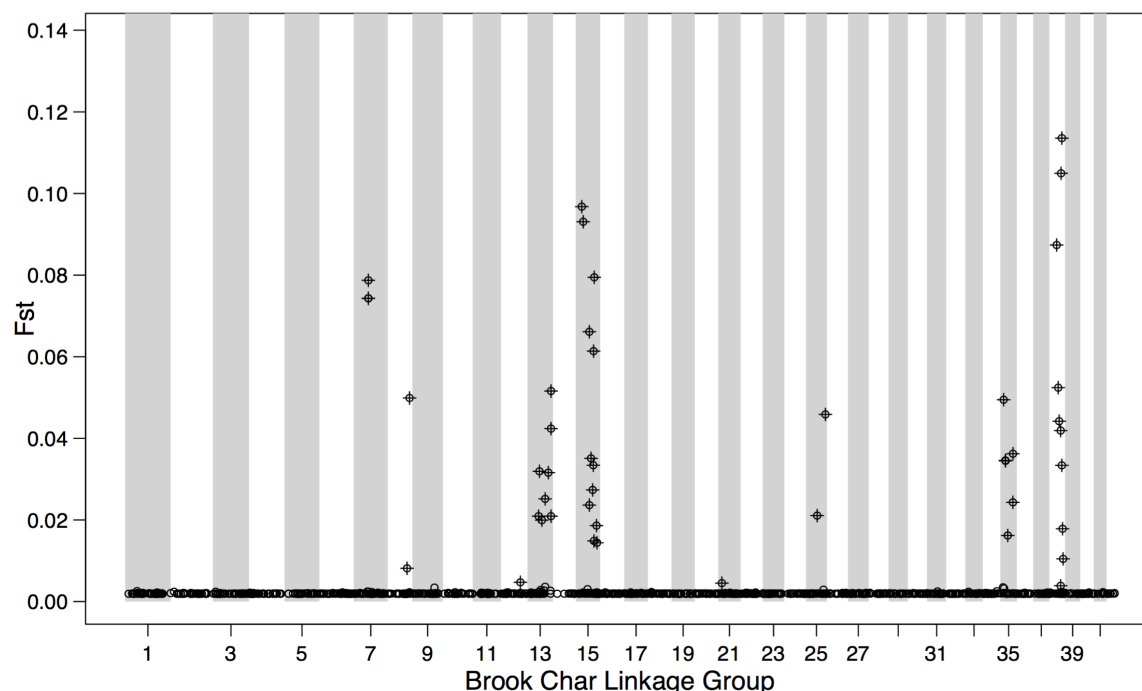


Figure 5. Manhattan plot of BAYESCAN F_{st} between the sexes for Arctic Char markers positioned on the Brook Char genetic map. Arctic Char markers without positions were assigned positions on the Brook Char linkage map using multiple iterations of MapComp to identify linkage groups that were associated with sex in Arctic Char. Plotting the BAYESCAN F_{st} along with marker positions indicates four linkage groups show strong linkage to sex: BC13, 15, 35 and 38. All positioned markers are displayed, and crosses indicate significant BAYESCAN F_{st} markers. Markers that are not associated with sex have very low F_{st} and can be seen along all of the linkage groups at the bottom of the graph.

Using BLAST against the annotated Atlantic Salmon genome, 28 of the 94 markers were found within a gene. For the remaining markers not found in a gene but with significant alignments, the closest upstream and downstream genes were identified along with the distance from the marker to the gene. Two sex-linked markers positioned on the Brook Char sex chromosome (BC35), SNP 86986 and SNP 87087, were on either side of *transcription factor SOX-11-like*, a member of the SRY-related HMG-box gene family associated to sex determination (Graves 1998; Woram *et al.* 2003). This gene was the closest annotated gene to these markers in the downstream or upstream direction, respectively, although the distance was large (~280 kb in each direction). Other identified genes containing sex-linked markers are involved in chromosome segregation and recombination (*e.g.*, *nipped-B-like protein*, *nuclear pore complex protein Nup93*, *bloom*

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syndrome protein and *centrosomal protein of 164 kDa*), putative sex-specific activities (e.g., *talín-2*), or transcription factor activity (e.g., *retinoic acid receptor RXR- α*).

Discussion

Sex-ratio bias in genotyping-by-sequencing studies

Sex-linked markers are expected to be present in all massively parallel sequencing genomic datasets developed on species with a genetic basis for sex determination (Gamble & Zarkower 2014). Despite the ubiquity of these sex-linked markers across taxa, very few population genomic studies on marine or diadromous species have reported information on the sex of samples being analyzed (*see details below*). However, our results clearly demonstrate that the occurrence of such markers jointly with an unbalanced sex ratio in sampling can lead to the observation of a spurious or biased population structure. This, in turn, may result in misinterpreting the biology of the species being investigated and possibly leading to improper management recommendations. For instance, in the case of the lobster study here, with an unbalanced sex ratio this could have led to the conclusion that inshore and offshore lobsters comprise two genetically distinct stocks (and therefore distinct management units) while in reality they comprise a single panmictic unit. This bias is particularly critical for high gene flow species characterized by very weak population structuring, which is typical of many marine and diadromous species alike. In such cases, only a few highly differentiated markers (here 0.7% and 1.5% of the total filtered markers for American lobster and Arctic Char, respectively) can generate a signal of significant genetic differentiation or inflate the signal in the cases of panmictic or low population differentiation, respectively. These outcomes highlight the importance of collecting sex information of individual samples to draw accurate conclusions about population structure of non-model species using genome-wide data sets.

Moreover, sex ratio is obviously an important characteristic of a population and is tightly linked to its dynamics. Therefore, gaining this information is valuable for an efficient and well-designed management plan, especially considering that sex ratio can vary widely in nature. For instance, sex-biased dispersal will strongly affect sex ratio, and this is widespread in birds and mammals (Pusey 1987) but still poorly investigated in

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marine organisms (Burgess *et al.* 2015). Identifying sex-linked markers for identifying the genetic sex of sampled individuals may enable further studies documenting sex-biased dispersal (Yano *et al.* 2013) as well as overcoming the influence of an unbalanced sex ratio on the analyses of genetic structure.

Addressing bias in sex ratio for population genomic studies on marine and diadromous species

Marine and diadromous population genomics studies in animals have become increasingly frequent in recent years, going from a single published article in 2010 to 52 (38 for marine and 14 for diadromous species) articles in 2016 (based on our selective criteria; Table 1). The literature search we performed indicates that in only 9.6% of all studies (5/52; Galindo *et al.* 2010; Bruneaux *et al.* 2013; Johnston *et al.* 2014; Benestan *et al.* 2015; 2016b) information was reported about the sex of the sampled individuals. Most of these studies have a sample size comparable to those of the present study (118 and 359 samples on median for marine and diadromous MPS studies respectively) as well as a comparable number of individuals sampled per location (median N per location range = 20-38). Therefore, all of these studies could potentially have been susceptible to the biases identified here. In the majority of these studies, the number of markers genotyped was higher than ours (7,688 and 9,107 SNPs on median for marine and diadromous MPS studies respectively) but since we demonstrate that only 12 and 94 sex-linked markers (0.7% and 1.5% of our total initial MPS datasets) were sufficient to create a signal suggestive of genetic structure, a greater number of markers will not overcome the influence of a small proportion of sex-linked markers in a high gene flow system such as that observed in the majority of marine species.

Since many MPS studies currently under way may not have access to sex information, one alternative way of overcoming the potential bias resulting from sex ratio differences would be to statistically assess the presence of two genetic clusters not associated with geography or other a priori factors hypothesized to influence genetic structure. Then, one could run a BAYESCAN defining groups based on the two observed clusters and assess the level of heterozygosity shown by the outlier markers found, as we did for the American lobster. However, the heterozygosity method will only work if the

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sex-linked markers are within a sex determining region that is not present on the alternate sex chromosome (i.e. only on Y), or if the sex chromosomes are largely heteromorphic. Nevertheless, this could help MPS studies to ensure that this bias is not present when interpreting patterns of genetic structure.

Sex determination in the American lobster

In crustaceans, as in many other species, sex is determined either by male (XX/XY) or female heterogamety (ZZ/ZW). However, sex chromosomes are difficult to identify in crustaceans because of the large number of chromosomes (e.g., on average 110 chromosomes for American lobster; Hughes 2014) and the small chromosome size (Legrand *et al.* 1987). Although markers associated with sex determination can be identified by approaches such as that used here, or as conducted in salmon lice (*Lepeophtheirus salmonis*, Carmichael *et al.* 2013) but see also (Gamble & Zarkower 2014), most of the crustacean sex determining systems are poorly understood and understudied (Legrand *et al.* 1987). Taking advantage of RAD-sequencing, we provide the first evidence of a male heterogametic system in the American lobster (XX/XY), which is in agreement with one review reporting that male heterogamy is more common in *Subphylum* Crustacea than in the majority of other invertebrate species (Legrand *et al.* 1987). In addition, we demonstrate the potential to efficiently uncover the sex chromosome system of a non-model species using a genome-wide dataset and analysis of heterozygosity excess or deficit.

Candidate genes involved in sexual differentiation in American lobster

We identified two candidate genes linked to sex in American lobster: *SULT1B1*, which is involved in steroid metabolism, and *cwf19*, which acts on pre-RNA splicing. Steroids play important roles in regulating physiological functions related to reproduction and sex differentiation in fishes (James 2011). More broadly, several publications have identified that sulfotransferase genes, such as *SULT1*, are linked to sex determination in house mouse (*Mus musculus*; Dunn *et al.* 1999), mussels (*Mytilus galloprovincialis*; Atasara Şahin *et al.* 2015), European Eel (*Anguilla anguilla*; Churcher *et al.* 2015) and Turbot (*Scophthalmus maximus*; Ribas *et al.* 2015b). For instance, *sulfotransferase 6B1-like*

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gene (SULT6B1) was expressed at higher levels in the livers of sexually mature European Eel males relative to females, which was hypothesised as indicating that this gene may be associated with pheromonal communication during the reproduction of this species (Churcher *et al.* 2015). In addition, one sulfotransferase gene (*hs3st1l2*) was a candidate gene for sex determination in Turbot, being associated with differential expression between sexes at sexual maturity (Ribas *et al.* 2015a). Interestingly, this study also identified *cwfl9* gene as a putative sex determining gene in the Turbot (Ribas *et al.* 2015b).

Both candidate polymorphisms occurred in the 3'UTR region of *SULT1B1* (SNP 2879519) and *cwfl9* gene (SNP 1525332). In particular, the polymorphism located in the 3'UTR region of *SULT1B1* displayed heterozygosity excess in males and heterozygosity deficit in females (see Table 3). The 3'UTR regions have an important role in post-transcriptional control of gene expression, and thus may affect the level of protein being expressed (Hesketh 2004). Several studies have shown that polymorphisms in 3'UTR region modulate the level of transcription of genes (Barrett *et al.* 2012). Here, polymorphisms found in *SULT1B1* and *cwfl9* gene may thus affect transcription, as was documented for European Eel (*SULT6B1* was overexpressed in liver of sexually mature males with a fold change of 7.8; Churcher *et al.* 2015) and Turbot (*cwfl9* was underexpressed in turbot females with a fold change of -1.7 ; Ribas *et al.* 2015b). Although the functional annotation for these two genes in American lobster is unknown, these markers may provide information on the sex determination system of this species, but would require further work.

Chromosomes and genes associated with sex-linked markers in Arctic Char

There is no high-density linkage map available yet for Arctic Char, but low-density linkage maps have indicated that the sex chromosome is homologous between Arctic Char and Brook Char and is homologous to Rainbow Trout RT-25 (Timusk *et al.* 2011). However, Arctic Char may have a metacentric sex chromosome (Timusk *et al.* 2011) whereas in this mapping family, Brook Char has an acrocentric sex chromosome (BC35; Sutherland *et al. in prep*). BC35 corresponds to the ancestral chromosome 15.1 determined from Northern Pike (*Esox lucius*, Rondeau *et al.* 2014), which corresponds to

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RT-25b (Sutherland *et al.* 2016), confirming the previous homology observations (Timusk *et al.* 2011). Six sex-linked markers are grouped on BC35. Considering that the Arctic Char sex chromosome is expected to be metacentric, the Arctic Char chromosome corresponding to BC35 is probably fused with another acrocentric chromosome that is linked to sex here. Other linkage groups, BC13 (14.1), BC15 (19.1) and BC38 (1.2) also show substantial linkage to sex with between 8-12 sex-linked markers being present on each. Interestingly, BC13 (14.1) is the homeologous chromosome to the Rainbow Trout sex chromosome (omySex; 14.2; Palti *et al.* 2015; Sutherland *et al.* 2016), and BC15 (19.1) is homologous to the neo-Y chromosome of Sockeye Salmon (Faber-Hammond *et al.* 2012). The presence of sex-linked markers on these chromosomes related to sex determination in other salmonids indicates the importance of comparative genomics for characterizing the sex chromosomes of the salmonids, which will be possible as more genomes become available.

Sex-linked markers were located on BC35 on both sides (~280 kb up or downstream) of the *SOX-11-like* gene (markers 86986 and 87087), which is interesting given the role of the Sox (SRY-related) family in sex determination (Graves 1998). This is the closest annotated gene down-stream to marker 86986 or up-stream to 87087. Several other sex-linked markers were within genes related to recombination and chromosome segregation, which is interesting given the differences in recombination rate between the sexes (*i.e.*, heterochiasmy) in the salmonids (Sakamoto *et al.* 2000). Genes containing sex-linked markers that were related to recombination included *nipped-B-like protein* (on BC13), involved in holding sister chromatids together during cell division (Losada 2014) *bloom syndrome protein* (on BC15), involved in homologous recombinational repair of double strand breaks during meiosis to suppress crossovers, *centrosomal protein of 164 kDa* (CEP164; on BC38), a centrosomal protein involved in cell cycle and chromosomal segregation (Sivasubramaniam *et al.* 2008), and *nuclear pore complex protein Nup93* (BC15), with a range of activities including transcription regulation and chromosome segregation (Ibarra & Hetzer 2015). A sex-linked marker was also identified near *centrosomal protein kizuna* (BC12) involved in establishing mitotic centrosome architecture. Sex-linked markers were also within genes related to transcription factor activity, including *retinoic acid receptor RXR-alpha* (BC13), a

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member of the steroid and thyroid hormone receptor superfamily involved in sex differentiation in many organisms (Lv *et al.* 2013) and in *SOX*-mediated gene expression regulation (Nikčević *et al.* 2008). Several sex-linked markers were near genes involved in Wnt signaling, which is important for sex determination (Jordan *et al.* 2001), including *Wnt-7b-like* (BC15) and *frizzled-9-like* (BC38). Finally, a sex-linked marker was present in *talin-2* (BC15), which has a truncated version known to be specifically expressed in testes and kidneys and expressed in elongating spermatids (Debrand *et al.* 2009). These genes linked to sex in Arctic Char may provide a better understanding of sex determination and heterochiasmy within the salmonids.

Conclusion

In summary, these results indicate the importance for population genomics studies to collect sex information about individual samples when possible in order to (i) control sex ratio in sampling, (ii) overcome “sex-ratio bias” that can lead to spurious genetic differentiation signals and (iii) fill knowledge gaps regarding sex determining systems. If morphological sex is difficult to determine at some life stages, the identification of sex-linked markers for screening samples may provide a useful alternative solution. Here the exploration of sex-linked markers provided information regarding the sex determination system as well as genes that may be involved in sex dimorphism in American lobster. Furthermore, using comparative genomics within the salmonids allowed us to identify chromosomes that may harbor genes involved in sex determination this ecologically and economically valuable salmonid species, including several chromosomes, which have already been associated with sex in other salmonid species.

Author contributions

For the American lobster, L. Bernatchez, L. Benestan, N. Rycroft, and J. Atema conceived and planned the study. For the Arctic char, L. Bernatchez, J.-S. Moore, L.N. Harris and R.F. Tallman conceived and planned the study. L. Benestan performed all the analyses for the American lobster and Arctic char, analyzed the sex ratio and sex-linked marker effects as well as the BAYESCAN for outlier detection for both species. L. Benestan and J.-S. Moore contributed to the joint analyses between American lobster and

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Arctic Char. B. Sutherland assigned positions to Arctic Char markers and conducted comparative analysis among the salmonid chromosomes. J. Le Luyer performed SNP filtering and with B. Sutherland performed the BLAST search for Arctic Char sex-linked markers. L. Benestan wrote the paper in collaboration with J.-S. Moore, B. Sutherland and J. Le Luyer. H. Maaroufi participated in proteins annotation and SNPs localization. E.N helped for bioinformatics. C.R performed the RAD-sequencing libraries for American lobster. For the Arctic Char, L.N. Harris and R.F. Tallman provided the samples. F. Clark and S. J Greenwood provided the transcriptome dataset. All authors contributed to revisions.

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Data accessibility

All the raw reads will be submitted to NCBI's Short Read Archive and the quality-filtered genotypes will be submitted as vcf files to Dryad upon article acceptance. Code to position Arctic Char anonymous markers on the Brook Char genetic map, combine them with BAYESCAN Fst values, and draw the Manhattan plots can be found on GitHub at the following link: https://github.com/bensutherland/salp_anon_to_sfon.

Tables

Table 1. Marine population genomics studies describing the name of the study, the organism studied, the method used to produce the genetic markers, the number of individuals sampled (N), the number of genetic markers (SNPs), the number of individuals sampled per location (N_{POP}), the index of genetic differentiation observed among the location studied (F_{st}).

Study	Organism	Method	N	SNPs	Sex	N _{POP}	F _{st}
Araneda <i>et al.</i> 2016	<i>Mytilus chilensis</i>	RAD-seq	220	1,240	No	25-39	0.005
Benestan <i>et al.</i> 2015	<i>Homarus americanus</i>	RAD-seq	586	10,156	Yes	30-36	0.0018
Benestan <i>et al.</i> 2016b	<i>Homarus americanus</i>	RAD-seq	562	13,688	Yes	30-36	0.0018
Berg <i>et al.</i> 2015	<i>Gadus morhua</i>	SNP-array	194	8,809	No	8-48	0.0002-0.0709
Berg <i>et al.</i> 2016	<i>Gadus morhua</i>	SNP-array	141	8,168	No	42-51	0.00123-0.0008
Boehm <i>et al.</i> 2015	<i>Hippocampus erectus</i>	RAD-seq	23	11,708	No	5-9	0.0454-0.1012
Bruneaux <i>et al.</i> 2013	<i>Gasterosteus aculeatus</i>	RAD-seq	288	6,834	Yes	48	Unkn.
Cammen <i>et al.</i> 2015	<i>Tursiops truncatus</i>	RAD-seq	156	7,431	No	12-26	Unkn.
Chu <i>et al.</i> 2014	<i>Nucella lapillus</i>	RAD-seq	30	4,000	No	Unkn.	0.0004-0.0474
Corander <i>et al.</i> 2013	<i>Clupea harengus</i>	RAD-seq	2*	4,756	No	6	0.005
Ferchaud <i>et al.</i> 2014	<i>Gasterosteus aculeatus</i>	RAD-seq	60	33,993	No	20	0.056-0.111
Ferchaud & Hansen 2016	<i>Gasterosteus aculeatus</i>	RAD-seq	177	28,888	No	20	0.002-0.458
Galindo <i>et al.</i> 2010	<i>Littorina saxatilis</i>	454 seq	30	2,454	Yes	15	0.03
Gleason & Burton 2016	<i>Chlorostoma funebris</i>	RAD-seq	90	1,861	No	15	0.0042
Guo <i>et al.</i> 2015	<i>Gasterosteus aculeatus</i>	RAD-seq	10*	30,871	No	36	0.0282
Hohenlohe <i>et al.</i> 2010	<i>Gasterosteus aculeatus</i>	RAD-seq	100	45,000	No	20	0.0020-0.1391
Jackson <i>et al.</i> 2014	<i>Epinephelus striatus</i>	RAD-seq	620	4,234	No	14-32	0.002
Lal <i>et al.</i> 2016	<i>Pinctada margaritifera</i>	RAD-seq	156	5,243	No	32-50	0.046
Lamichhaney <i>et al.</i> 2012	<i>Clupea harengus</i>	RNA-seq	400	440,817	No	50	Unkn.
Miller <i>et al.</i> 2016	<i>Haliotis rubra</i>	GBS	80	1,180	No	10	0.003

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Le Moan <i>et al.</i> 2016	<i>Engraulis encrasicolus</i>	RAD-seq	128	5,638	No	24-64	Unkn.
Moura <i>et al.</i> 2014	<i>Orcinus orca</i>	RAD-seq	115	3,281	No	6-21	0.0346-0.334
Nayfa & Zenger 2016	<i>Pinctada maxima</i>	SNP-array	85	1,130	No	25-33	-0.0047-0.004
Pecoraro <i>et al.</i> 2016	<i>Thunnus albacares</i>	RAD-seq	100	6,772	No	10	0.0273
Picq <i>et al.</i> 2016	<i>Hypoplectrus spp</i>	RAD-seq	126	97,962	No	13-43	0.0042
Poćwierz-Kotus <i>et al.</i> 2015	<i>Gadus morhua</i>	SNP-array	95	7,944	No	26-40	0.034
Reitzel <i>et al.</i> 2013	<i>Nematostella vectensis</i>	RAD-seq	30	2,759	No	4-7	0.286-0.622
Rodríguez-Ezpeleta <i>et al.</i> 2016	<i>Scomber scombrus</i>	RAD-seq	122	29,394	No	15-29	0.0157-0.039
Sodeland <i>et al.</i> 2016	<i>Gadus morhua</i>	SNP-array	378	9,187	No	43-48	0.000-0.0189
Stockwell <i>et al.</i> 2015	<i>Scarus niger</i>	RAD-seq	81	4,253	No	24-30	0.007
Xu <i>et al.</i> 2016	<i>Bathymodiulus platifrons</i>	RAD-seq	28	9,307	No	10-18	0.0126
Zhang <i>et al.</i> 2016	<i>Larimichthys polyactis</i>	RAD-seq	24	27,556	No	12	< 0.001
Bradbury <i>et al.</i> 2010	<i>Gadus morhua</i>	EST seq	300	1,641	No	15-26	Unkn.
Jones <i>et al.</i> 2012	<i>Gasterosteus aculeatus</i>	SNP-array	121	1,159	No	4 o 6	0.031-0.383
Therkildsen <i>et al.</i> 2013	<i>Clupea harengus</i>	EST seq	508	1,047	No	14-37	0.000-0.086
Tepolt & Palumbi 2015	<i>Carcinus maenas</i>	EST seq	84	10 809	No	12	0.003-0.134
Bay & Palumbi 2014	<i>Acropora hyacinthus</i>	EST seq	23	15,399	No	10-13	Unkn.
De Wit & Palumbi 2013	<i>Haliotis rufescens</i>	EST seq	26	21,579	No	1-13	0.0003

* = samples are pools

EST: Expressed Sequence Tag

RAD-seq: Restriction-Associated DNA sequencing

GBS: Genotyping-by-sequencing

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Table 2. Anadromous or catadromous (in the case of eels) population genomics studies describing the name of the study, the organism studied, the study goal, the method used to produce the genetic markers (Method), the number of individuals sampled (N), the number of genetic markers (SNPs), the number of individuals sampled per location (N_{POP}), the index of genetic differentiation observed among the location studied (F_{st}).

Study	Organism	Study goal	Method	N	SNPs	Sex	N _{POP}	F _{st}
Bourret <i>et al.</i> 2013	<i>Salmo salar</i>	Pop. structure and outliers	SNP-array	1,431	6,176	No	20-72	0.025-0.758
Candy <i>et al.</i> 2015	<i>Thaleichthys pacificus</i>	Pop. structure and outliers	RAD-seq	494	4,104	No	22-71	0.000-0.0128
Drywa <i>et al.</i> 2013	<i>Salmo trutta</i>	Pop. structure	SNP-array	24	15,225	No	12	0.029
Hess <i>et al.</i> 2013	<i>Entosphenus tridentatus</i>	Pop. structure and outliers	RAD-seq	518	4,439	No	4-35	0.021
Jacobsen <i>et al.</i> 2014	<i>Anguilla spp.</i>	Speciation; Outliers	RAD-seq	60	328,300	No	8-15	0.041
Johnston <i>et al.</i> 2014	<i>Salmo salar</i>	Pop. structure and outliers	SNP-array	503	4353	Yes	49-260	0.0103
Laporte <i>et al.</i> 2016	<i>Anguilla spp.</i>	Parallelism; Outliers	RAD-seq	179	23,659 14,755	No	21-24	0.000-0.001
Larson <i>et al.</i> 2014	<i>Oncorhynchus tshawytscha</i>	Pop. structure and outliers	RAD-seq	270	10,944	No	47-56	0.003-0.098
Moore <i>et al.</i> 2014	<i>Salmo salar</i>	Pop. structure and outliers	SNP-array	9,142	3,192	No	9-100	0.043
Brieuc <i>et al.</i> 2015	<i>Oncorhynchus tshawytscha</i>	Adaptive divergence	RAD-seq	414	9,107	No	21-41	0.000-0.33
Ogden <i>et al.</i> 2013	<i>Acipenser spp.</i>	Pop. structure	RAD-seq	319	140,260	No	8-115	Unknown
Pavey <i>et al.</i> 2015	<i>Anguilla rostrata</i>	Pop. structure and outliers	RAD-seq	379	42,424	No	21-24	<0.001
Pujolar <i>et al.</i> 2014	<i>Anguilla anguilla</i>	Pop. structure and outliers	RAD-seq	259	50,354	No	30-37	<0.001
Rougemo <i>et al.</i> 2016	<i>Lampetra spp.</i>	Pop. structure and outliers	RAD-seq	338	8,962	No	29-53	0.042-0.207

Pop. structure = population structure

RAD-seq: Restriction-Associated DNA sequencing

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Table 3. American lobster. Observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{is}), P-value associated to inbreeding coefficient (P-value) and genetic differentiation index (F_{st}) between sexes (females, $n=100$; males, $n=103$) for 12 highly sex-linked markers identified with BAYESCAN. Markers showing the strongest genetic differentiation between both sexes and belonging to the same LD the cluster are in bold (see Figure S2).

Marker	Females				Males				F_{st}
	H_o	H_e	F_{is}	P-value	H_o	H_e	F_{is}	P-value	
1951841	0.010	0.010	0.000	1.000	0.605	0.496	-0.220	0.027	0.560
3534313	0.000	0.000	--	--	0.634	0.498	-0.273	0.008	0.543
2341697	0.291	0.504	0.423	0.002	0.311	0.383	0.188	0.056	0.514
703660	0.011	0.011	0.000	1.000	0.628	0.501	-0.253	0.013	0.470
1713801	0.000	0.021	1.000	0.006	0.615	0.499	-0.231	0.029	0.440
2033018	0.011	0.032	0.664	0.020	0.563	0.498	-0.130	0.162	0.425
2879520	0.011	0.032	0.664	0.022	0.524	0.493	-0.064	0.371	0.401
434792	0.021	0.041	0.493	0.039	0.484	0.389	-0.244	0.017	0.214
1757708	0.280	0.415	0.326	0.003	0.500	0.485	-0.031	0.462	0.166
1525333	0.261	0.496	0.473	0.001	0.323	0.411	0.215	0.033	0.141
2341745	0.000	0.044	1.000	0.001	0.591	0.496	-0.192	0.052	0.108
794307	0.156	0.373	0.581	0.001	0.156	0.162	0.037	0.525	0.077

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Supporting Information

Table S1. Information on locations and American lobster samples: latitude and longitude, sampling date and number of individuals successfully genotyped (N_{GEN}). Samples were taken by Atema and Gerlach (*unpublished*).

Table S2. Information on locations and Arctic char samples: latitude and longitude, sampling date and number of individuals successfully genotyped (N_{GEN}).

Figure S1. Results of the BAYESCAN analyses. SNPs in grey are significantly more differentiated than expected between male and female American lobster (left panel) and Arctic char (right panel) respectively.

Figure S2. Heatmap of the linkage disequilibrium (LD) for the 12 sex-linked SNPs identified in American lobster. Heatmap illustrating the linkage disequilibrium (LD) for the 12 highly sex-differentiated markers, considering all the males and females sampled at the 13 study sampling sites. Each row and column represents a specific SNP. The shades represent different ranges of LD values, from low (pale grey) to high (in black). The gene tree shown above the heatmap, based on LD values, suggests two different clusters of markers in high LD with each other linkage cluster. The SNPs belonging to the linkage cluster that is the most strongly linked to sex determination are shown in bold and delineated by a black rectangle.