Evolution, 2023, **77(7)**, 1667–1681
https://doi.org/10.1093/evolut/qpad075
Advance access publication 27 April 2023
Original Article

OXFORD

Searching for intralocus sexual conflicts in the three-spined stickleback (*Gasterosteus aculeatus*) genome

Florent Sylvestre[1], Claire Mérot[1,2], Eric Normandeau[1], Louis Bernatchez[1]

[1]Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada
[2]UMR 6553 Ecobio, OSUR, CNRS, Université de Rennes, Rennes, France

Corresponding author: Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada. Email: florent.sylvestre.1@ulaval.ca

Abstract

Differences between sexes in trait fitness optima can generate intralocus sexual conflicts that have the potential to maintain genetic diversity through balancing selection. However, these differences are unlikely to be associated with strong selective coefficients and are challenging to detect. Additionally, recent studies have highlighted that duplications on sexual chromosomes can create artifactual signals of intralocus sexual conflicts. Thus, testing the relationship between intralocus sexual conflicts and balancing selection requires stringent filtering of duplicated regions, and dedicated methods to detect loci with low levels of intersex differentiation. In this study, we investigated intralocus sexual conflicts in the three-spined stickleback using whole-genome sequencing (mean coverage = 12×) of 50 females and 49 males from an anadromous population in the St. Lawrence River, Québec, Canada. After stringent filtering of duplications from the sex chromosomes, we compared three methods to detect intralocus sexual conflicts. We found only two genomic regions under potential intralocus sexual conflict that also showed signals of balancing selection. Overall, our results suggest that most intralocus sexual conflicts do not drive long-term balancing selection and are most likely transient.

Keywords: intralocus sexual conflicts, balancing selection, duplications, recombination, three-spined stickleback

Introduction

In species with sexual reproduction, sexes often have different fitness optima for shared traits, resulting in sex-specific selection pressures (Lande, 1980; Rowe et al., 2018). If males and females share the same genetic basis for such a trait, natural selection can favor different alleles in each sex, leading to intralocus sexual conflict (Van Doorn, 2009). Because sexual reproduction randomly shuffles alleles between sexes at each generation, such conflicts may translate into balancing selection, promoting genetic diversity by maintaining male and female-beneficial alleles in the population depending on the selective coefficients in each sex (Connallon & Clark, 2014; Lonn et al., 2017; Zajitschek & Connallon, 2018). Intralocus sexual conflicts have been identified at the phenotype level in a variety of taxa and traits (Cox & Calsbeek, 2009; Foerster et al., 2007; Harano et al., 2010; Merilä et al., 1997). As such, they have the potential to impact multiple genomic regions and to play an important role in genome evolution. In particular, because intralocus sexual conflicts are expected to favor balanced polymorphism, they possibly represent a key process enhancing genetic diversity. However, we still lack knowledge concerning the genomic distribution of intralocus sexual conflict, which impedes testing their predicted impact on genetic diversity.

Direct detection of intralocus sexual conflict implies measuring fitness in males and female samples and identifying genotypes with opposite fitness effects between sexes (Innocenti & Morrow, 2010; Ruzicka et al., 2019). Yet, accurate fitness measurements are at best difficult to obtain in natural populations. Another solution is to search for differences in allele frequency between males and females caused by sex-specific mortality (Rowe et al., 2018). Several recent studies have used this approach to identify intralocus sexual conflicts in whole genome datasets (Cheng & Kirkpatrick, 2016; Dutoit et al., 2018; Lucotte et al., 2016, 2022; Wright et al., 2018, 2019). Recently, Kasimatis et al. (2021) failed to find signal of such conflict despite using dataset with much more statistical power (N = 409,406 human genomes). Confidently identifying signal of intralocus sexual conflict thus remains a challenging task.

Since sexual reproduction erases genetic differences between males and females except on sex chromosomes, differentiation caused by sex-specific mortality is expected to be weak. Moreover, substantial mortality in each sex is required to reach a significant detection level (Kasimatis et al., 2019, 2021). It is unlikely that most intralocus sexual conflicts over survival are under strong selective pressure as this would represent too high a mortality cost for populations to persist. Given the low magnitude and detection power involved in intralocus sexual conflicts, separating signal from noise is a complex task, especially in large and complex whole genome sequencing datasets in which multiple testing allows detection of only the strongest signals. One way to overcome this challenge may be to search for cumulative signals of differentiation over several loci, as opposed to looking for significance at a single locus. This approach improves the detection

Received August 8, 2022; revisions received April 19, 2023; accepted April 26, 2023

power of intralocus sexual conflict in natural populations and makes detecting smaller selective effects possible (Ruzicka et al., 2020).

Another factor to consider is that sequence similarity between autosomes and sex chromosomes can generate artifactual signals of intralocus sexual conflict (Bissegger et al., 2020; Kasimatis et al., 2019; Lin et al., 2022). If a locus is duplicated in a sex-specific genomic region, it can accumulate sex-specific polymorphism (Bissegger et al., 2020; Lin et al., 2022; Mank et al., 2020). If the region remains similar to its original autosomal copies, it is possible that sequence reads from the sex-specific region align to the autosomal copies, creating a spurious signal of differentiation between sexes that can be mistaken for an intralocus sexual conflict. Moreover, duplications of loci on sexual chromosomes are also expected to be part of the process of resolving intralocus sexual conflict (Rice, 1984). This could lead to signals of intralocus sexual conflict that may in fact be caused by resolved sexual conflict (Lin et al., 2022; Mank et al., 2020). The use of a high-quality reference genome with identified sex chromosomes and stringent filtering for potential duplicates is thus essential for the reliable detection of intralocus sexual conflict.

It is still unclear what proportion of intralocus sexual conflicts play a role in maintaining genetic diversity in natural populations. Their contribution to genetic diversity depends not only on the selective coefficients involved (Zajitschek & Connallon, 2018), but also on the ultimate resolution of such conflicts. Several studies suggest that intralocus sexual conflicts might be easily resolved by the evolution of a sex-specific genetic basis for a given trait or differential gene expression (van der Bijl & Mank, 2021; Wright et al., 2018). In such a scenario, intralocus sexual conflict would not be associated with long-term balancing selection but rather could affect genetic diversity by locally reducing the impact on background selection around the selected loci until the resolution or the end of the sexual conflict (Ruzicka et al., 2020). To better understand the respective roles of these alternative mechanisms, we need to rigorously test the association between long-term balancing selection and intralocus sexual conflict.

Here, we aim to investigate the presence of intralocus sexual conflicts in a natural population of three-spined stickleback (*Gasterosteus aculeatus*). This model species presents several advantages for the study of intralocus sexual conflict: (a) sticklebacks exhibit pronounced sexual dimorphism for a variety of traits (e.g., behavior, coloration, reproductive strategy; (Barker & Milinski, 1993; Chellappa & Huntingford, 1989; Whoriskey et al., 1986); (b) they produce large juvenile cohorts (Craig & FitzGerald, 1982; Poulin & Fitzgerald, 1989), thus having the potential for a high juvenile mortality load; (c) a very high-quality reference genome (Nath et al., 2021) with a recently sequenced Y chromosome (Peichel et al., 2020) is available, which mitigates the issue of duplicated genes on sex chromosomes and potential artifactual mapping of Y linked reads on autosomes.

We performed whole-genome sequencing of 50 male and 50 female three-spined stickleback to search for signatures of intralocus sexual conflicts. After removing one low-quality male from the dataset, we used sex-specific variation in coverage and genetic variability to filter potentially duplicated variants on sex chromosomes. Then, we searched for signals of intralocus sexual conflicts throughout the genome by combining single- and multi-locus approaches, aiming to detect loci with weak polygenic effects. Finally, we tested for the association between potential intralocus sexual conflicts, genetic diversity, and potential balancing selection.

Methods

Ethics statement

This study was approved by the Comité de Protection des Animaux de l'Université Laval (CPAUL, approval number SIRUL 109096). Following the CPAUL permit, we got a permit from the Ministère des Forêts, de la Faune et des Parcs du Québec (permit number 2018 04 11 005 01 S P) for fish sampling.

Sample collection and sequencing

We collected 100 adult anadromous three-spined sticklebacks from the St Lawrence River between May and July 2018, at Baie de l'Isle verte, Québec (48.009961, −69.407070), which belong to a single panmictic population (McCairns & Bernatchez, 2009). To limit the potential impact of environmental heterogeneity and fine scale genetic structure, we sampled all individuals in the same tide pool. Fins were preserved in 95–98% ethanol and we extracted DNA following a salt extraction protocol modified from Aljanabi and Martinez (1997). Samples were then sent to the Centre d'Expertise et de Services Genome Québec (Montréal, QC Canada) for NEB Ultra II Shotgun DNA library preparation and quality checking, followed by whole genome sequencing on nine illumina HiseqX lanes with a targeted coverage of 15×.

Genome alignment and cleaning

Raw reads were trimmed and quality filtered using fastsp version 0.15.0 (Chen et al., 2018) with default settings. We used bwa-mem version 0.7.17-r1188 (Li, 2013) to align reads to the fifth version of the stickleback reference genome (Nath et al., 2021). Males and females were respectively mapped on a reference including or not the Y chromosome reference (Peichel et al., 2020), available on https://stickleback.genetics.ugq.edu. The Y chromosome was trimmed of its pseudo-autosomal region (first 0.34 Mb) as it was already present on the X reference (chrXIX). We used picardstools MarkDuplicates (1.119, http://broadinstitute.github.io/picard/) to identify and trim PCR duplicates, and GATK RealignerTargetCreator and IndelRealiner (DePristo et al., 2011) to locally realign around putative indels. Finally, we clipped overlapping read pairs ends using bamUtils clipOverlap version 1.0.14 (Jun et al., 2015) and removed reads that became unmapped in the process.

Variant calling

We used bcftools v1.12 to call autosomal SNPs using mpileup (-a AD,DP,SP,ADF,ADR, -q 5, -d 20000) coupled with bcftools calls (Li, 2011) (-a GP, GQ, -G -). We kept biallelic SNPs with a good mapping quality (≥30), no mapping quality bias ($p > 10^{-3}$) or read position bias ($p > 10^{-3}$), and a good QUAL score (≥25). We also used custom python3 scripts to calculate Strand Odds Ratio as suggested by GATK (https://gatk.broadinstitute.org/hc/en-us/articles/360036361772-StrandOddsRatio) and discarded all SNPs with a value above 3.5. We considered all genotypes calls with coverage < 4 (uncertain genotype) or > 35 (potential duplicates) as missing data and then removed SNPs with more than 20% missing data. We excluded individuals with more than 10% missing data and ran the process again on the remaining individuals. As rare

variants have very limited statistical power to detect inter-sex F_{ST} differences, we removed all SNPs with minor allele frequency less than 10%. Unless mentioned otherwise, the following analyses were performed using R 4.0.3 (R Core Team, 2014) and python version 3.7.4 (Van Rossum & Drake, 2009).

Detection of large structural variants

Because large inversions are known to play a role in three-spined sticklebacks' evolution (e.g., adaptation to marine/freshwater; Jones et al., 2012; Liu et al., 2018) and can be under strong selection as they form long haplotypes involving several genes, we scanned the genome to identify putative polymorphic inversions in our population and their potential association with intralocus sexual conflicts (see Supplemental method for details).

Population structure

After removing large genomic rearrangements, we searched for potential sex-specific structure that might lead to significant differences between males and females. We imputed our dataset using the most frequent genotype and ran a PCA to search for axes segregating males and females. We also compared the relatedness among males and females using the implementation of the KING equation in vcftools v1.1.16 (Danecek et al., 2011; Manichaikul et al., 2010) to infer pairwise relationship coefficients among individuals.

Duplications on sex chromosomes

Autosomal regions duplicated on sex chromosomes can create sex-specific polymorphism and heterozygosity that can be mistaken for intralocus sexual conflict. Recent work revealed that aligning sequencing data to a reference genome missing the hemizygous chromosome will result in reads from gene copies on the hemizygous chromosome incorrectly mapping on the autosomal copy of the gene (Bissegger et al., 2020; Kasimatis et al., 2019; Lin et al., 2022). Even with a reference genome containing the Y, some duplicated genes can still be mis-assembled in the reference sex chromosomes or be specific to our focus population especially given the fact that its phylogeographic origin, the northwest Atlantic, differs from that from which the reference genome was generated (a freshwater population from British Columbia, Nath et al., 2021; Peichel et al., 2020).

Here we used two methods adapted from Lin et al. (2022) to detect and filter SNPs potentially duplicated on sex chromosomes. Because males and females do not have the same number of copies of the sex chromosomes (males are heterogametic), the absence of a duplicated gene in the reference genome will result in skewed depth of sequencing between sexes (the expected ratio of coverage Females:Males is 4:3 for an X-duplicated gene and 2:3 for a Y-duplicated gene). We used a Wilcoxon rank-sum test to compare median coverage in males and females to detect SNPs with differences in coverage between sexes. To do so we used a 5% significance threshold. If the different copies have accumulated divergence, it can generate sex-specific variation, that we detected using a Fisher exact test to remove any SNPs with a significant difference in the number of surrounding polymorphic SNPs (250 SNPs in each direction) between males and females at a 5% significance threshold.

Detecting "true" signals of intralocus sexual conflict

Intralocus sexual conflicts over survival are expected to lead to differences in allele frequencies between males and females. Because we expect weak levels of inter-sex differentiation, finding the right balance between discarding false positives and maintaining stringency is a complex task. To identify a wide range of potential intralocus sexual conflicts across the three-spined stickleback genome, we combined three approaches with a priori different sensitivities: SNP-by-SNP, cumulative F_{ST}, and multivariate redundancy analysis (RDA). The first method (SNP-by-SNP) aims at detecting SNPs showing pronounced differentiation between sexes with high confidence. For each SNP, we performed a Fisher test on a contingency table (SNP genotype by sex). An SNP was considered as a candidate for intralocus sexual conflict if it had an associated q-value ≤ 5%. Here the q-value is used to control the overall false discovery rate (hereafter FDR): out of N (40 in our case) SNPs declared as significant hits, we expect Nq (2) of them will be spurious. The Benjamini–Hochberg procedure was used to obtain q-values from the empirical distribution of the *p*-values across all 1,701,083 SNPs, using the p.adjust() function in R (Benjamini & Hochberg, 1995).

Because intralocus sexual conflicts might act on complex traits leading to polygenic selection, we also applied a method designed to search for weak, cumulative signal of differentiation between males and females (cumulative F_{ST}). We adapted a method recently proposed by Ruzicka et al. (2020), which suggested looking for an excess of SNPs with significant intersex F_{ST} compared to the random expectation between permuted groups based on a null-model. For each SNP, we estimated intersex F_{ST} as defined in Cheng and Kirkpatrick (2016), where $F_{ST} = \frac{(p_m - P_f)^2}{4*p*(1-p)}$ in which p_m and p_f represent the estimated allelic frequency at a biallelic locus in males and females while p is their average We assessed its significance based on a null distribution of intersex F_{ST} developed in Ruzicka et al. (2020). We then tested if the number of significant SNPs was significantly higher than expected based on random permutations of our dataset. We applied this approach at three different levels (whole genome, per chromosome, and in 250 kb sliding windows with 50 kb overlap) to limit the effect of regions with lower-than-expected inter-sex divergence, which could reduce and cancel the signal of accumulation of significant SNPs in other regions. For the chromosomal and sliding windows level, we estimated the FDR using the Benjamini–Hochberg procedure. We used 1,000 random permutation at the global and chromosomal level but, as we analyzed 8,000 windows, we increased the number of permutations to 20,000 to precisely estimate low *p*-value. As this method is based on calling individual SNPs significant, we applied this approach for three levels of F_{ST} significance: 5%, 1%, and 0.1% at the whole-genome or chromosomal scale, but only considered the 1% threshold for the window approach.

Finally, we used RDA implemented in the R package *vegan* (Oksanen et al., 2022), a multivariate ordination methods that have been successfully employed in genotype-environment association studies (Capblancq & Forester, 2021; Laporte et al., 2016) to detect weak multilocus signals of selection. This method works by analyzing the covariance of loci in response to environmental variation. In the case of intralocus sexual conflict, it can be applied using the sex of samples as

the "environmental matrix." Again, we ran the RDA on the whole genome, per chromosome, and in 250 kb overlapping sliding windows (50 kb overlap). *p*-Value was obtained by permutational ANOVA for each RDA (1,000 permutations at the global and chromosomal level, 20,000 permutations at the windows level). Windows with a *p*-value ≤ 1% were considered potential targets of intralocus sexual conflicts. We estimated associated FDR level for the chromosomal and window level using the Benjamini–Hochberg procedure.

Comparison of sensitivity of the methods

We used a Wilcoxon rank-sum test to compare the distribution of F_{ST} of significant SNPs in windows potentially associated with intralocus sexual conflict in each of our method and in nonsignificant windows. Significant SNPs were defined as (a) SNPs identified by the SNP-by-SNP method; (b) SNPs with a *p*-value ≤ 0.001 for intersex F_{ST} within windows detected by the cumulative F_{ST} method and (c) SNPs with absolute Z-score above 3 in windows with a significant RDA. For the cumulative F_{ST} approach, we again used the Benjamini–Hochberg procedure to estimate FDR associated with calling a SNP significant at the .001 *p*-value threshold within each window.

GO enrichment and gene analysis

To test if certain biological functions were associated with intralocus sexual conflict, we performed a Gene Ontology (GO) analysis on genes located within in 1 kb of significant SNPs in our dataset. The 1 kb distance was defined according to Kratochwil & Meyer (2015) as being in regions likely to contain cis-regulatory elements. To do so, we annotated a three-spined stickleback transcriptome from Jones et al. (2012) using the SWISS-PROT database and downloaded gene information from the UniProt database. We then used goatools (Klopfenstein et al., 2018) to perform Fisher's exact tests and assess enrichment for biological processes using a 5% threshold for the q-value using the Benjamini–Hochberg procedure to control the false discovery rate. All scripts are available at https://github.com/enormandeau/go_enrichment. As shared SNPs between methods overlapped with only 12 genes, we also report individual gene information from the UniProt database and additional literature if available.

Relationship between intralocus sexual conflicts and genetic diversity

To test whether intralocus sexual conflicts were associated with increased genetic diversity across the genome, we used ANGSD (v 0.931-6-g18de33d; Korneliussen et al., 2014) to estimate Tajima's *D* and π within 250 kb sliding windows across the genome (50 kb overlap). We considered only reads with a mapping quality above 30 and positions covered by at least one read in 50% of individuals.

We compared the distribution of both metrics for windows detected by cumulative F_{ST}, RDA and focusing on windows detected by both methods relatively to the rest of the genome using a Wilcoxon rank sum test. Because patterns of genetic diversity can covary with recombination rate, we performed the same analysis comparing regions of similar recombination rate. We estimated recombination rates using a linkage map from Rastas et al. (2016) from which we used bwa-mem (Li, 2013) to lift over the coordinates of their SNPs to ours by aligning a 500 bp windows around each SNP to our version of the reference genome. Then, we used Mareymap (v 1.3; Rezvoy et al., 2007) to estimate the recombination rate by linear interpolation across the reference genome (loess interpolation span = 0.2, degree = 2). With this, we classified each region of our dataset as low (≤1 cm/Mb), medium (between 1cM/Mb and 5cM/Mb), or high (>5cM/Mb) recombination rates and perform similar Wilcoxon rank sum test between pair of significant and nonsignificant windows of similar recombination rates.

Finally, we wanted to test if some regions of potential intralocus sexual conflicts containing windows identified with cumulative F_{ST} and RDA were associated to elevated Tajima's *D*. We used the 99th quantile (0.0384) of the Tajima's *D* distribution to identify outlier windows associated with potential long term balancing selection.

To refine the scale, we re-estimated Tajima's *D* for all those regions in smaller windows of 25 kb (5 kb sliding windows) following the same procedure. Since recent balancing selection can also lead to increased linkage disequilibrium (LD) (Fijarczyk & Babik, 2015), we used plink (v1.90b5.3, Chang et al., 2015; Gaunt et al., 2007) to estimate LD between pairs of SNPs within these genomic regions and their surroundings (250 kb flanking regions). To simplify the representation of LD patterns, we used a custom python script to estimate the distribution of pairwise LD for windows of 25 kb and represented only the 99th quantile of this distribution. We used the annotation files obtained from the previous GO analysis to extract overlapping gene information for significant SNPs in those and report individual genes information from the SWISS-PROT database and additional literature if available.

Results

Investigating intralocus sexual conflicts requires adequate filtering

Artifactual signatures mimicking intralocus sexual conflicts may be created by both population factors (sampling, structure, inbreeding) and duplications on sex chromosomes. To minimize the impact of such potential artifacts, we studied a single population and applied stringent filtering. We genotyped 1,755,558 autosomal biallelic SNPs (see Methods and Supplementary Table S1 for filtering details) in 50 males and females. One male shown elevated missingness compared to others and was discarded. Grouping on an exploratory PCA suggested the potential presence of large segregating haplotype in the population, so we performed a local PCAs analyses in sliding windows. This identified three regions on chrIX (4.75 Mb from 3.85 to 8.60 Mb), chrXVI (0.95 Mb from 17.1 to 18.05 Mb), and chrXXI (0.90 Mb from 1.8 to 2.7 Mb) that displayed characteristics consistent with polymorphic inversions and potential duplication (Supplementary Figures S1–S3, Supplementary Discussion). However, those structural variants showed neither sex bias nor inter-sex differentiation (Supplementary Discussion) and are thus unlikely candidates for intralocus sexual conflicts and were removed from the dataset. After that, no population structure or sex-specific structure was apparent on a PCA, confirming that our samples belong to a single panmictic population (Figure 1A, Supplementary Figure S4). Investigating pairwise kin coefficients between samples, we confirmed that relatedness among individuals was equivalent between males and females (Figure 1B) and should not influence inter-sexual differentiation.

To focus on current intralocus sexual conflicts rather than sex differentiation or resolved sexual conflicts, we targeted autosomal chromosomes only and implemented

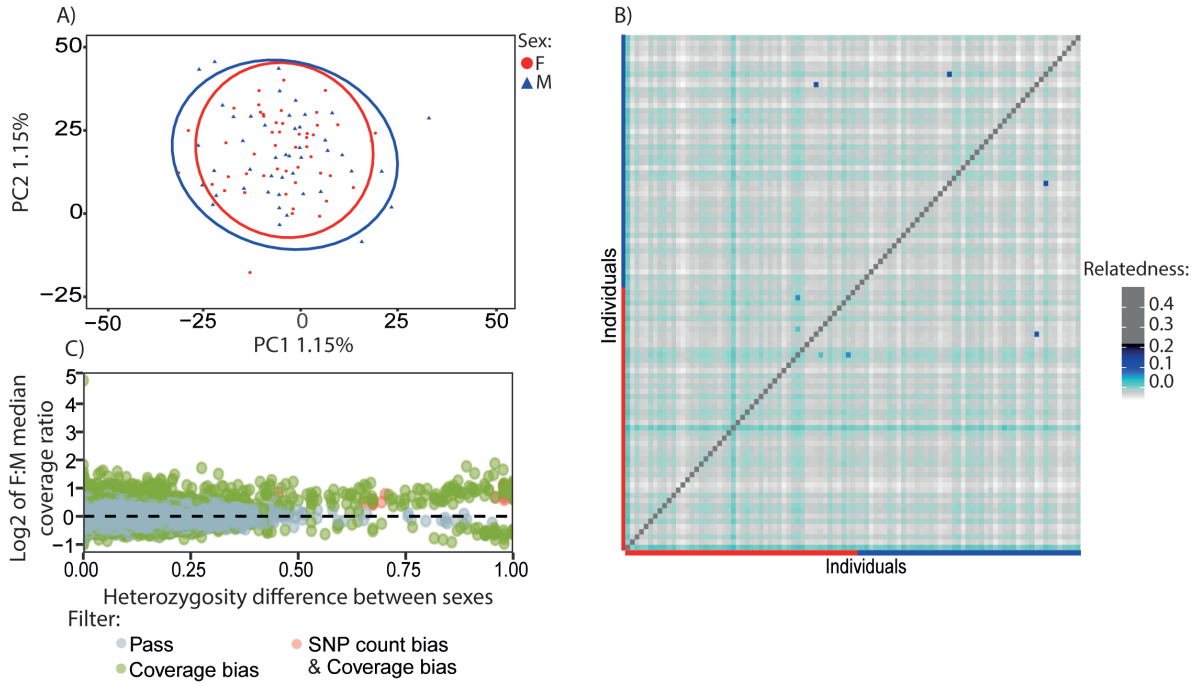

Figure 1. Global patterns of intersex differentiation and duplications on sexual chromosomes. (A) First two axes of a Principal Component Analysis (PCA). Two outlier individuals with high relatedness are not shown. Ellipses represent the 95% confidence interval of point distribution. Circle and the inner ellipse represent females, triangles and outer ellipse represent males. (B) Heatmap of pairwise relatedness as estimated by the KING implementation in vcftools. The first half of both axis represent females, the second half represent males. (C) Variation of female/male coverage ratio as a function of absolute heterozygosity difference between sexes for autosomal SNPs. Green dots represent SNPs with a significantly different coverage between sexes based on a Wilcoxon rank sum test at the 5% significance threshold. Red dots correspond to SNPs with a significant difference in local SNPs density between sexes in a 500 SNPs region around the focal SNP based on a χ^2 test at a 5% significance threshold.

advanced filtering to avoid duplicated autosomal regions on sex chromosomes. We detected 22,825 SNPs with significant coverage bias between sexes and 430 SNPs that showed a significant difference in the number of surrounding SNPs between sexes (in a 500 SNP window surrounding each SNP) (Figure 1C). Some of those SNPs were associated with a sex-specific increase in heterozygosity (Figure 1C), with some alleles being only present in one sex and always in individuals that are heterozygous. Altogether, after filtering out low-quality SNPs and samples, the dataset includes 1,701,083 SNPs genotyped in 49 males and 50 females.

Three complementary methods to identify intralocus sexual conflicts

Single-locus signal of differentiation (SNP-by-SNP)

Because of differential mortality, intralocus sexual conflicts are expected to lead to differences in allele frequencies between males and females. We screened the three-spined stickleback genome for SNPs showing significant differentiation between sexes. For each SNP, we performed a Fisher's exact test. After controlling for FDR, this resulted in 40 SNPs that showed significant differentiation between males and females, representing potential targets of intralocus sexual conflicts.

Cumulative signals of intersexual differentiation (cumulative F_{ST})

We also applied a method designed to search for weak, cumulative signals of differentiation between males and females. We assessed F_{ST} significance using the null distribution for intersex F_{ST} with p-values of 5%, 1%, and 0.1% to call outlier, and compared the number of significant SNPs obtained to random permutation of fish from different sexes. At the genome-wide scale, this method revealed no significant signals of accumulation of intersex differentiation at any level of F_{ST} significance (quantile 95%: mean ratio = 0.995, 95% confidence interval = c [0.991–0.998], quantile 99% = 0.993, [0.986–0.999], and quantile 99.9% = 0.992, [0.979–1.004], Supplementary Figure S5A). At the chromosome scale, however, the pattern was clearly heterogeneous across the genome. We detected suggestive enrichment in putative intralocus sexual conflicts, on Chr XII (p = .05, associated FDR 0.66) for the 5% significance threshold, ChrXIII, ChrXV (p = .018–.019, associated FDR = 0.19) for the 1% significance threshold and chrV (p = .018, associated FDR = 0.36) at the 0.1% significance threshold (Supplementary Figure S5B).

Redundancy analyses

We then performed a RDA to search for multivariate associations between genotype and sex. We applied this method to identify groups of SNPs that covary with sex. Over the entire dataset, we found no evidence for intralocus sexual conflicts (p-value of .447 and R^2 adjusted of 1.101×10^{-5}, Supplementary Table S2). Running our RDA model independently for each chromosome identified two chromosomes in which genetic variation was significantly associated with inter-sex differentiation: chrIX (p-value of .046 and an adjusted R^2 of 3.735×10^{-4}) and chrXII (p-value of 0.019 and an adjusted R^2 of 2.271×10^{-4}) (Supplementary Table S2). However, FDR associated to accepting these p-value were elevated (.48 for accepting both, and .38 if accepting only chr chrXII).

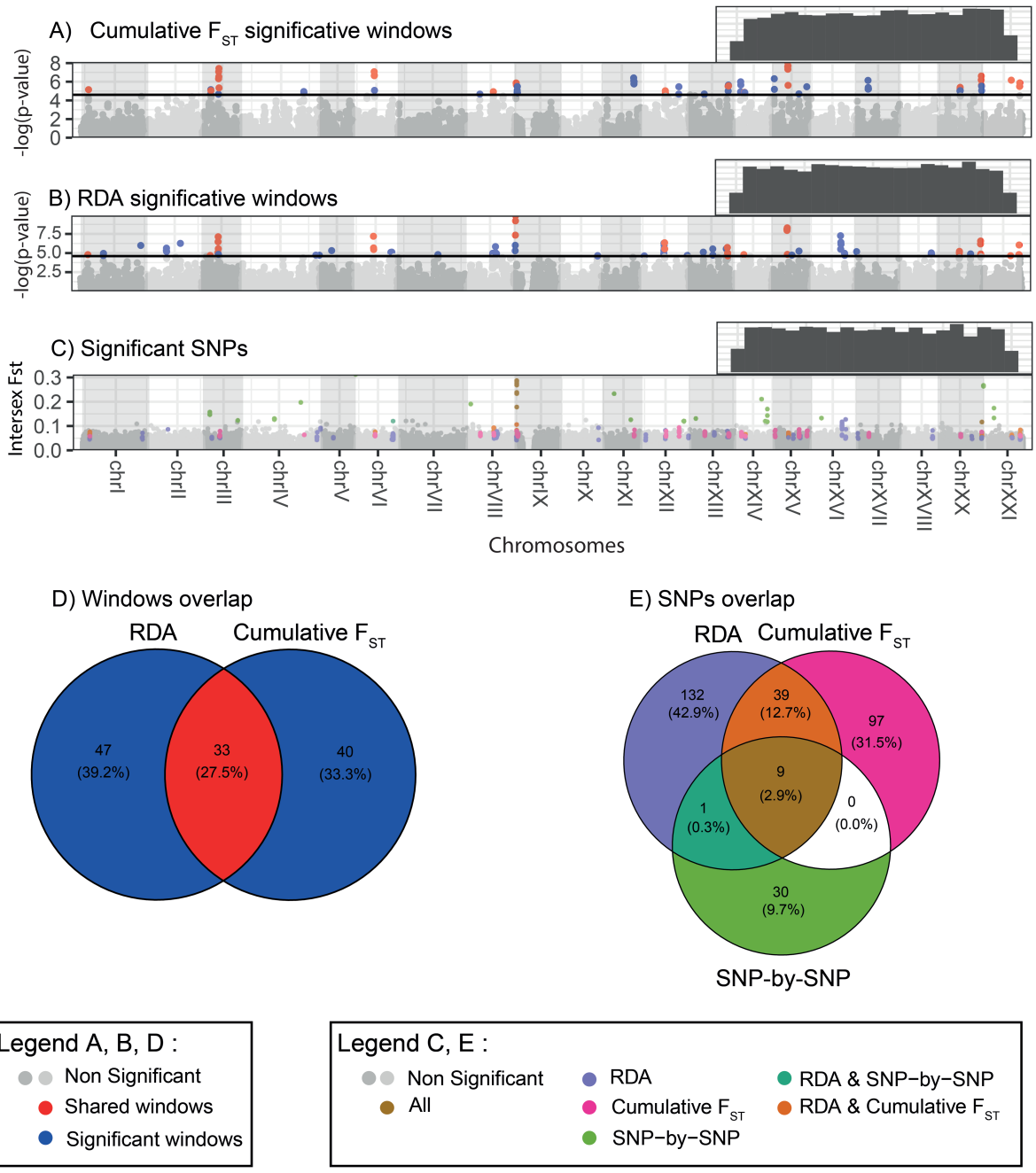

Figure 2. Genomic landscape of putative signatures of intralocus sexual conflicts as detected by three approaches. (A) Manhattan plot of significant 250kb windows using the cumulative F_{ST} approach and (B) the RDA analysis. Blue dots correspond to significant 250 kb windows, and red dots identify windows identified by both methods. The line corresponds to *p*-values of 1% and less. (C) Per-SNP intersex F_{ST}. Colors highlight detection methods (cumulative F_{ST}, RDA, and SNP-by-SNP) and their overlaps. Histograms represent associated *p*-value distribution for (A) cumulative F_{ST}, (B) RDA, and (C) Per-SNP F_{ST}. (D) Venn diagram of the overlap between significant windows detected by cumulative F_{ST} and RDA. (E) Venn diagram of the overlap between significant SNPs detected by all three methods.

Detecting different signal of intersex differentiation

The cumulative F_{ST} and RDA approaches are strongly impacted by the scale at which they are applied, as shown by the differences between the global and chromosomal analyses. To avoid missing local effects that could be masked at the global and chromosomal scales, as well as for identifying more precisely SNPs that might be evolving under intralocus sexual conflicts, we performed the cumulative F_{ST} and RDA analyses in 250 kb overlapping sliding windows along the genome with 50 kb steps, corresponding to a total of 8,000 windows. The cumulative F_{ST} method identified 73 windows with a *p*-value ≤ .01, within which we identified a total of 145 SNPs (associated FDR ranging from 0.008 to 0.32 depending on the windows; Figure 2A). Similarly, focusing on windows with an associated *p*-value ≤ .01 for the RDA approach identified 80 windows and 181 SNPs (Figure 2B). In both cases, the histogram of *p*-value showed no enrichment in the lower tail of the distribution, and the high number of tests led to high FDR (1 for cumulative F_{ST} and 0.94 for RDA), meaning that we lack power to identify precise targets of intralocus sexual conflicts using these approaches.

The two methods showed limited overlap at the chromosomal scale, with only chrXII being detected in both approaches. However, at the window scale, 33 (27.5%) windows are identified by both the cumulative F_{ST} and RDA approaches (Figure 2D). Regarding SNP identification, RDA and cumulative F_{ST} overlapped for 39 SNPs (12.7%) but showed lower overlap with the more stringent SNP-by-SNP approach. Ten SNPS (3.2%) overlapped between SNP-by-SNP and either cumulative F_{ST} or RDA, 9 of which being identified by all three methods (Figure 2C and E). Interestingly, SNPs identified by all three methods were all polymorphic only in males (Figure 3A). However, most SNPs identified by two methods (mostly cumulative F_{ST} and RDA), were polymorphic in both sexes (Figure 3A). More generally, SNPs identified by the SNP-by-SNP method tended to be polymorphic in only one sex, whereas cumulative F_{ST} and RDA were polymorphic in both sexes (Supplementary Figure S6). When measuring male-female differentiation by F_{ST}, we observed that cumulative F_{ST} and RDA detected SNPs with a significantly lower intersex F_{ST} compared to the SNP-by-SNP approach (Figure 3B, p-value $\leq 2 \times 10^{-16}$ for all comparison), possibly providing more sensitivity to detect weak effects of sexual conflicts. All methods detected SNPs with higher intersex F_{ST} than nonsignificant region (p-value $\leq 2 \times 10^{-16}$ for all comparison), and RDA detected SNPs with slightly lower intersex F_{ST} than the cumulative F_{ST} method (p-value = 4.092×10^{-07}).

Intralocus sexual conflicts are not enriched in particular functions

We use SNPs detected as potential targets for intralocus sexual conflict by our three methods to test for GO enrichment in our dataset, first using all detected SNPs (308 SNPs), then focusing on SNPs detected by at least two methods (49 SNPs, which represents higher confidence targets). Using all SNPs, 89 genes were identified, but showed no significant enrichment in particular function. SNPs shared among the three methods overlap with 11 genes, which is not enough to test for GO enrichment. Looking into SWISS-PROT annotation, two of these genes (slc6a4a on chrI, cadherin-12 on chrXXI) are associated to brain-specific functions, with slc6a4a being associated with serotonin transport. Two genes were associated with immune system response (LOC120825389, a predicted immunoglobulin on chrIX, and LOC120831298 on chrXIII, predicted to negatively regulate innate immune response). We identified a second cadherin (cadherin-18) on chrXXI, associated with cell–cell junction. Other functions include calcium channels (LOC120815813, chr III), lipid biosynthesis (agpat4, chrVI), cation transport (slc22a13b, chrXXI), actine-binding and cell mobility (LOC120811547, chrXXI), and map3k4 (chrVI), a mitogen-activated protein kinase that might be involved in various process such as cell proliferation and differentiation.

The relationship between intralocus sexual conflict and genetic diversity depends on the method of detection and is affected by recombination heterogeneity

The relationship between Tajima's D and nucleotide diversity (π) with intralocus sexual conflict varied depending on the detection method (Figure 4). Windows identified by cumulative F_{ST} revealed no difference in nucleotide diversity and Tajima's D between regions associated with intralocus sexual conflict compared to the rest of the genome (Wilcoxon rank sum test, Tajima's D: p = .85 and π: p = .83) while windows identified by RDA show marginally lower Tajima's D compared to the rest of the genome (Wilcoxon rank sum test, $p = 5.9 \times 10^{-2}$) but not significant for π (Wilcoxon rank sum test, p = .11). However, the

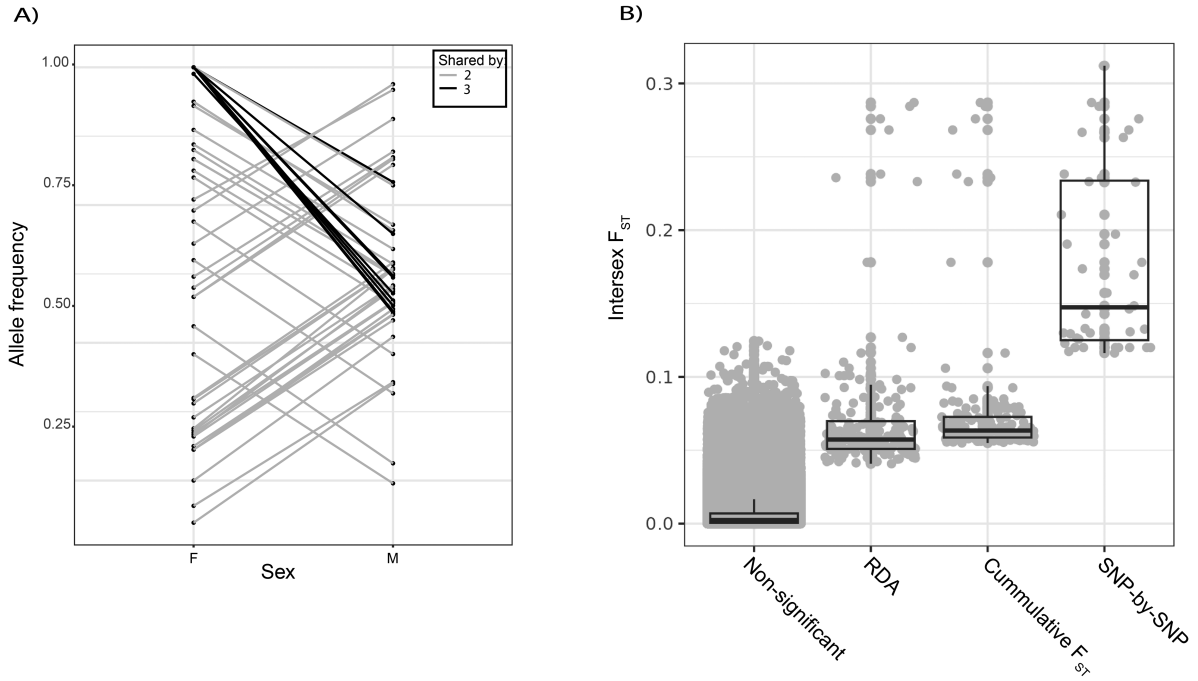

Figure 3. Strength of signatures of intralocus sexual conflicts between methods. (A) Sex-specific allele frequency for SNPs detected by at least two methods. Black line represent SNPs detected in all three methods. (B) Per SNP intersex F_{ST} for significant SNPs detected by each method and nonsignificant SNPs. All pairwise comparisons have a p-value $\leq 4.02 \times 10^{-7}$ based on a Wilcoxon rank-sum test.

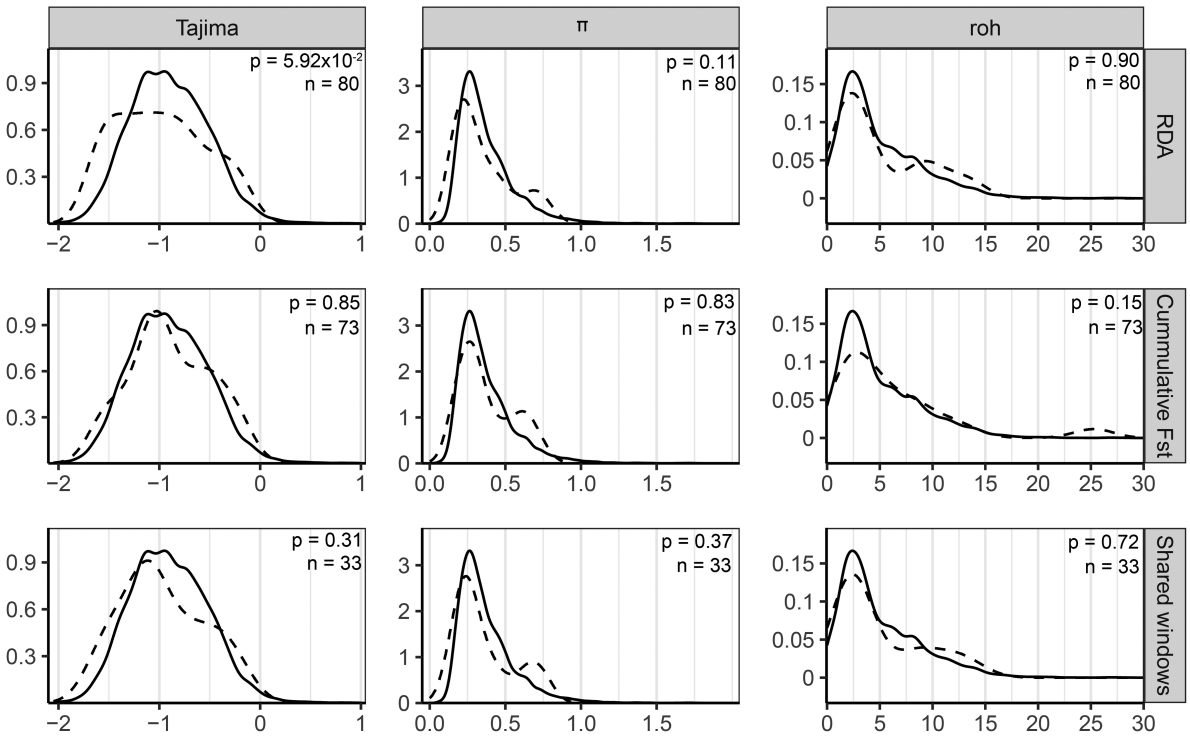

Figure 4. Association between signatures of putative intralocus sexual conflicts and genetic metrics. Distribution of Tajima's *D*, π, and recombination rates (from left to right) for windows detected by each method (dashed line) as significantly associated with sexual conflict compared to the bulk of the genome (solid line); from top to bottom, RDA, cumulative F_{ST} and windows detected by both methods. *p* represents the *p*-value of a Wilcoxon rank-sum test comparing the distribution of significant and nonsignificant windows; *n* represents the number of significant windows.

heterogeneity in recombination rates along the genome is also worth considering before interpretation. While we did not find detection bias toward particular level of recombination for significant regions identified by cumulative F_{ST} method (Wilcoxon rank sum test, $p = .15$ or RDA [$p = .9$]; Figure 4), it remains important to compare genetic diversity among regions of similar recombination rate, as recombination tends to be positively correlated with genetic diversity (Payseur & Nachman, 2002). We performed our analyses again by comparing significant regions and other genomic regions with similar recombination rates. To do this, we divided our dataset into low (≤1 cm/Mb), medium ([1–5] cM/Mb), and high (>5 cM/Mb) recombination rates. Similar results were observed for the cumulative F_{ST} method that showed no association with Tajima's *D* neither in medium ($p = .54$) or high ($p = .62$) recombination regions. However, windows identified by the RDA have a significantly lower Tajima's *D* than the genomic distribution in medium ($p = 2.99 \times 10^{-4}$) and high recombination regions ($p = 3 \times 10^{-2}$) (Figure 5). Results are consistent for nucleotide diversity (Supplementary Figure S7). Low recombination regions did not have enough significant windows to allow testing and are not represented.

We then performed the same analysis focusing on windows identified by the two methods, as they represent more confident targets for potential sex-specific selection. Similarly, these regions are not associated with increased Tajima's *D* ($p = .31$) or nucleotide diversity ($p = .37$), which holds true when comparing these metrics across regions of different recombination levels (medium recombination: Tajima's *D*: $p = .29$, π: $p = .15$; high recombination: Tajima's *D*: $p = .38$, $\pi = 0.21$) (Figure 5, Supplementary Figure S7).

Rare intralocus sexual conflicts are associated with increased genetic diversity

Using the 1% *p*-value threshold for cumulative F_{ST} and RDA, no significant windows overlapped with regions increased genetic diversity as measured by high Tajima's *D*. However, relaxing this threshold to 5%, two genomic regions of high Tajima's *D* also contained at least one window identified by cumulative F_{ST} and one windows identified by RDA (Figure 6). Significant SNPs in those regions were located in regions of positive Tajima's *D* values at a finer scale (25 kb sliding windows), except for the region on chrXIII. Pairwise estimation of r^2 between all pairs of SNPs in those regions showed that significant SNPs were associated with elevated linkage disequilibrium on chrVII ([0.3 Mb–1.05 Mb]), but not for the region on chrXIII. Using the UniProt database, we identified potential function of genes located within 1 kb of a significant SNP in those regions. Concerning the region on chrVII, 4 SNPs fall within 1 kb of coding sequences, with notable genes identified inhibiting bacterial growth (OXLA), TRIM2, potentially involved in pathogen recognition and LEGC involved in the innate immune response and inflammatory response. Other genes are involved in cytoskeletal organization (SH319) and hook protein (FHI1A) On the chrXIII region, one SNP is found within 1 kb of the Msh3 gene involved in DNA repair mechanism. Checking coverage pattern of these genes, they do not present deviation from the 1:1 M:F ratio expected, although genes from the protocadherin alpha family showed high variance in coverage ratio (Fig. S8). They also feature a surprising pattern with a peak of coverage in female followed by an excess of coverage in male, located in the exon of protocadherin alpha-13 and alpha-2 (Supplementary Figure S8).

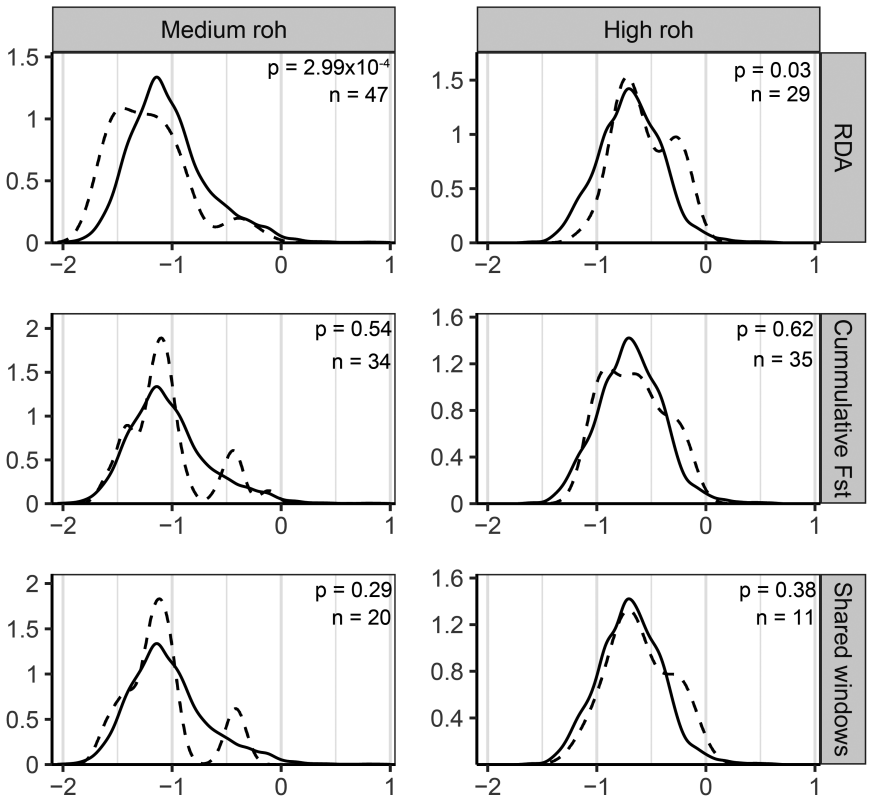

Figure 5. Association between putative intralocus sexual conflict and Tajima's *D* when controlling for recombination rate. Distribution of Tajima's *D* for each detection method (dashed line) compared to the bulk of the genome (solid line) for each category of recombination rate (medium or high). From top to bottom: From top to bottom, RDA, cumulative F_{ST} and windows detected by both methods. As we only identified a few windows in low recombination regions ($n < 10$), they are not reported; *p* represents the *p*-value of a Wilcoxon rank-sum test comparing the distribution of significant and nonsignificant windows; *n* represents the number of significant windows.

Discussion

Intralocus sexual conflicts have the potential to maintain genetic diversity in natural populations. However, because they leave only faint signals in genomic datasets, we still have a weak understanding of their genomic distribution, and thus of their role in the maintenance of genetic diversity. Using whole genome resequencing of 49 male and 50 female three-spined sticklebacks, our study provides a description of the genomic pattern of intralocus sexual conflicts in this species, and directly tests the association between intralocus sexual conflict and genetic diversity. We detected SNPs and regions with signature of putative intralocus sexual conflicts. Following stringent filtering of genomic regions duplicated on sex chromosomes, we combined three methods (SNP-by-SNP, cumulative F_{ST}, and RDA) to detect signals of inter-sex differentiation. Focusing on the windows showing potential enrichment for sex-specific selection, we showed that most intralocus sexual conflicts were not associated with increased genetic diversity. However, we still identified two genomic regions that were potentially associated with signals of balancing selection.

Sexual chromosomes as hotspot of sexual conflict resolution

Our data revealed putative duplications between sex chromosomes and autosomes, which affected 1.35% of SNPs (n = 23,282). Such a signal has often been attributed to the lack of Y reference, which leads to reads belonging to two different copies of a duplicated sequence to map at a single position on an autosome. For the three-spined stickleback, we relied on a high-quality reference of the Y chromosome but still found numerous duplicated regions (56.4% of all potentially duplicated SNPs are associated with the Y chromosome, Figure 1C). These duplicated SNPs are likely to be caused either by high similarity between autosomal and sex-linked copies or by duplications that are not represented in the Y sequence, such as duplications that are specific to our population or that are difficult to assemble. This pattern is consistent with previous studies in the trinidadian guppy (*Poecilia reticulata*) and three-spined stickleback (Bissegger et al., 2020; Lin et al., 2022). We also identified duplications between autosomes and the X chromosome (43.3% of all potentially duplicated SNPs) that were caused by the same artifact. Moreover, we found that potentially duplicated X-chromosome SNPs (i.e., those showing excess of coverage or polymorphism in females) often exhibit increased heterozygosity in females only. However, duplication on the X chromosome would also lead to an increase in male heterozygosity, even if at a lower level, and therefore probably does not explain the observed pattern. Those regions could be duplicated on both the X and the Y chromosomes but correctly assembled on the Y chromosome only, resulting in reads from the X chromosomes mapping on their autosomal copy in females but in their Y copy in males. This would create a female-specific increase in coverage and heterozygosity and would not happen in genomes missing a reference for the Y chromosomes. We believe this is a more likely explanation of the observed heterozygosity bias.

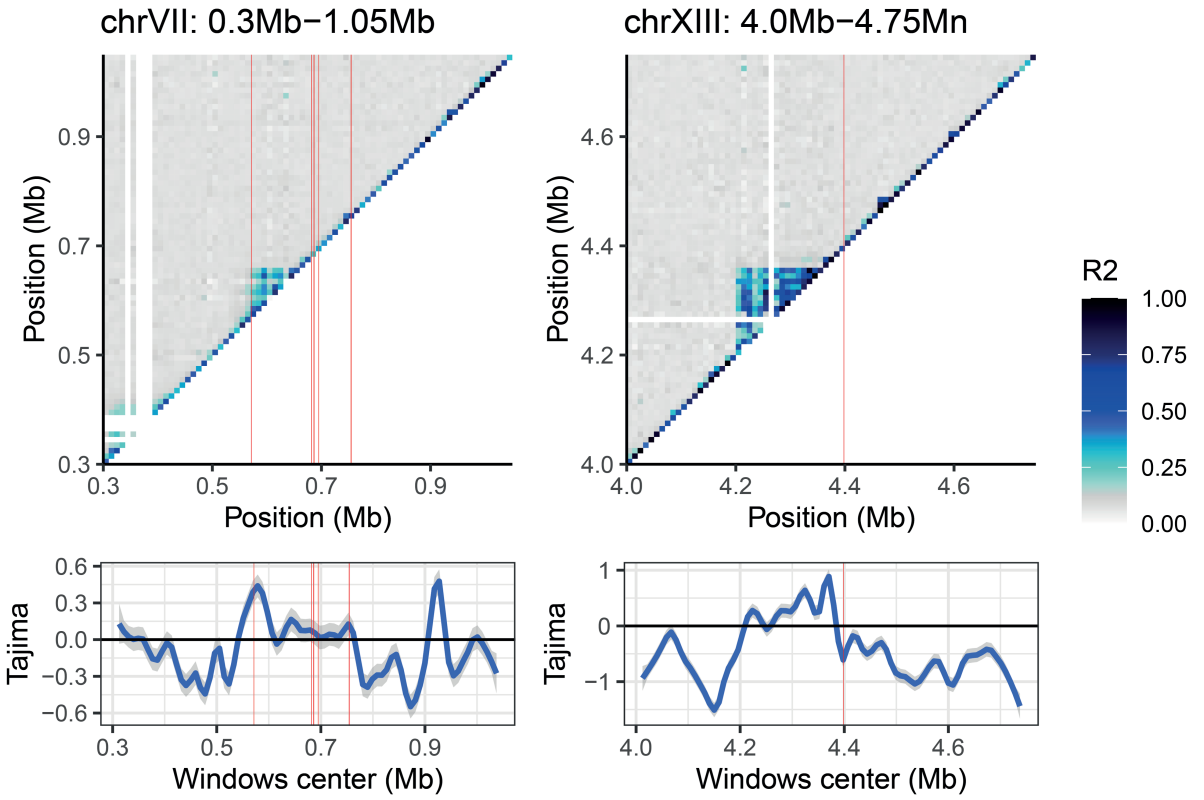

Figure 6. Zoom on regions associated with elevated Tajima's *D*. (A) Pairwise linkage disequilibrium in regions significantly associated with putative intralocus sexual conflict (extended by 250 kb in each direction for visibility) and with a positive Tajima's *D*, averaged over 10 kb windows. (B) Tajima's *D* estimated in 25 kb windows. Vertical lines indicate the location of significant SNPs associated with the signal of intralocus sexual conflict.

Altogether, these results highlight a dynamic process of gene duplication between autosomes and both sex chromosomes that have been interpreted in other context as potential remnant of sexual conflict resolution (Lin et al., 2022; Mank et al., 2020).

Artifactual polymorphism generated by duplicated genomic regions that are collapsed in the assembly represents a challenge for population genomics and this matter is increasingly recognized with any genome alignment-based method and must be addressed through proper filtration (Dorant et al., 2020; Jaegle et al., 2022).

Signature of intralocus sexual conflict as detected by a complementary set of methods

Through differential mortality, intralocus sexual conflicts are predicted to translate into differential allelic frequency between sexes (Cheng & Kirkpatrick, 2016; Lin et al., 2022; Lucotte et al., 2016), which can be detected using various approaches such as inter-sex F_{ST} (Lin et al., 2022; Wright et al., 2018) or genome-wide association studies (GWAS, Kasimatis et al., 2021; Ruzicka et al., 2020). However, substantial mortality is required to generate significant inter-sex differentiation and statistical power to detect this signal is low, especially after accounting for multiple testing in genome-wide datasets. Since many loci under strong intralocus sexual conflict over mortality would likely represent an excessive mortality cost for natural populations, the search for intralocus sexual conflicts in genome-wide datasets mainly focus on finding small-effect variants. This may be done by reducing the false discovery rate corrections at the gene level (Lucotte et al., 2016) or by focusing on genes showing cumulative signals of inter-sex differentiation (Mank et al., 2020; Ruzicka et al., 2020). In this study, we combined stringent genome-wide FDR control (SNP-by-SNP method, Figure 2C) with approaches designed to search for weak, cumulative signals of differentiation (cumulative F_{ST}, and RDA) to scan the three-spined stickleback genome for the presence of intralocus sexual conflict. We found very limited signals of intralocus sexual conflict. Using cumulative F_{ST} or RDA, no signal was found at genome-scale. One reason may be, that sexual conflict is not strong enough to have a broad effect although, a few chromosomes and regions were identified as putatively under sexual conflicts. Another reason might be the low statistical power is low conferred by a sampling size of $N = 50/N = 49$. At a finer scale, a SNP-by-SNP Fisher test detected 40 candidate SNPs with strong inter-sex differentiation. Intriguingly, those SNPs were polymorphic in only one sex and at Hardy–Weinberg disequilibrium. Such a pattern may result from strong selection against the alternative allele in one sex, hence truly reflecting a strong sexual conflict, but may also results from reads from the sex chromosomes erroneously mapped onto autosomal regions, which may have passed unnoticed through our initial, albeit very stringent, filtering. These results are in line with those of Lin et al. (2022) who found limited evidence for intralocus sexual conflict in the Trinidadian guppy, which display sex-specific susceptibility to mortality, most of it being associated to duplications on sex-chromosomes.

Improving the detection of sexual conflicts thus requires on one hand more accurate references of autosomes and sex chromosomes with the ability to control duplicates, and on the other hand larger sample sizes to increase the power to

detect weak signals of selection. Methods taking advantage of additional information concerning reproductive success, allowing to integrate selection effect over survival and reproduction are also very promising, as Ruzicka & Connallon (2022) demonstrated, but are impractical in most natural systems (see also Ruzicka et al., 2022). Such an approach would also allow to separate with confidence the signals of intralocus sexual conflict from sex differences in the intensity of directional selection or sex-restricted natural selection.

We found two genes involved in the immune system and putatively under sexual conflict. The link between immune response and sexual antagonism would be supported by the fact that different parasite species and abundance have been documented between sexes in three-spined stickleback (Reimchen & Nosil, 2001). Moreover, the immune system can play a role in sexual selection (Folstad et al., 1994) and can affect variation in morphology (De Lisle & Bolnick, 2021), suggesting that sex-specific selection is likely to occur at loci associated with the immune system. Sexual conflict has also been proposed as a driver of immune system evolution (Morrow & Innocenti, 2012). Other interesting findings include the cadhering-18 gene, that have been found in association with salinity adaptation in *Galaxias maculatus* (Delgado et al., 2020), and slc6a4a, which seems to be associated with salinity transport, and have been studied in the context of stickleback behavior (Abbey-Lee et al., 2018). In this population, stickleback reproduce in tidepools in which evaporation induces high salinity (Ward & FitzGerald, 1983). As male stickleback tends to stay longer at the end of the reproductive period (Whoriskey et al., 1986), salinity tolerance may be under stronger selection in males than females.

Intralocus sexual conflicts are not associated with increased genetic diversity

Theory predicts that intralocus sexual conflicts could play a significant role in maintaining genetic diversity in natural populations. This prediction is sometimes taken for granted and some studies have used signature of increased genetic diversity, such as elevated Tajima's *D*, to confirm the identification of intralocus sexual conflict (Wright et al., 2018). However, the extent to which this relationship is valid across the genome still lacks testing. Studies focusing on specific traits or genes have shown that intralocus sexual conflict can maintain genetic diversity in a variety of context (Barson et al., 2015; Foerster et al., 2007; Hawkes et al., 2016; Lonn et al., 2017), but only three studies have tested this relationship directly in large genomic datasets (Dutoit et al., 2018; Lin et al., 2022; Sayadi et al., 2019). These studies led to variable conclusions. Dutoit et al. (2018) found a positive association between signatures of intralocus sexual conflict and genetic diversity, while Lin et al. (2022) did not, and results from Sayadi et al. (2019) suggested that intralocus sexual conflict might maintain genetic diversity through relaxed purifying selection. One explanation for this discrepancy may be that Dutoit et al. (2018) did not account for the effect of sex-chromosome duplications, which can create regions of artificially elevated genetic diversity mistaken for signal of balancing selection. However, their use of inter-species shared polymorphism to test for potential balancing selection should have limited the impact of such duplicated regions, unless they are shared between their focal species and their outgroup. Our results are more in line with those of Lin et al. (2022) as we did not find global associations between signatures of intralocus sexual conflict and increased genetic diversity measured by Tajima's *D* and nucleotidic diversity (Figures 4 and 5).

On the one hand, our weak association between intralocus sexual conflicts and genetic diversity could be caused by our lack of power (due to our sample size of 50 for each sex) causing false positives to hide the signal of increased genetic diversity caused by intralocus sexual conflict. On the other hand, Tajima's *D* and nucleotide diversity are metrics that usually detect medium-to-long term excess in polymorphism (Fijarczyk & Babik, 2015). Hence, we may be missing signals of recent balancing selection or locally relaxed purifying selection instead of long-term balancing selection similar to those identified by Sayadi et al. (2019). Yet, overall, our results suggest that most intralocus sexual conflicts do not play an important role in maintaining genetic diversity in natural populations. This support the hypothesis that most intralocus sexual conflicts could be either resolved over short evolutionary time scales or that they are not stable over time. Resolution of intralocus sexual conflict implies decoupling the genetic bases of the concerned traits between males and females. This can be achieved through gene duplications on sex chromosomes, as discussed before, but also through other processes that can modulate the effect of a genotype in a sex-specific way (van der Bijl & Mank, 2021). In sticklebacks, for instance, both sex-biased patterns of gene expression and alternative splicing have been documented (Kaitetzidou et al., 2022; Naftaly et al., 2021).

However, the conditions for intralocus sexual conflict stability depend on the strength of selection, with conditions for stability narrowing when selective coefficients are lower (Zajitschek & Connallon, 2018). As we mainly detected variants with small inter-sex differentiation, it is possible that a combination of dynamic resolution of intralocus sexual conflicts and instability is one reason why we do not observe association with signal of potential balancing selection.

Because our identification of intralocus sexual conflict was based on significant differences in allelic frequency between sexes, directional selection in one sex could also be detected by our approach. In that case, we would rather expect directional selection to locally reduce genetic diversity. This could explain our finding that genetic diversity was marginally lower in regions of medium recombination rate detected by RDA (Figure 5, Supplementary Figure S7), which is concordant with local directional selection in one sex leading to a soft selective sweep. These results suggest that methods classically used to detect sex differences may likely detect a combination of sex-specific selection and intralocus sexual conflict.

Rare intralocus sexual conflicts are associated with increased genetic diversity

Two genomic regions with sexual conflict (on chrVII and chrXIII) showed elevated genetic diversity (Tajima's $D \geq 3.84 \times 10^{-2}$), two of which also exhibited increased linkage disequilibrium (Figure 6), both of these statistics being potentially indicative of balancing selection (Fijarczyk & Babik, 2015). While significant SNPs in and chrVII regions fall within regions of increased Tajima's *D* at a finer scale, it is not the case for the chrXIII region, for which the association of potential balancing selection and intralocus sexual conflict does not hold at a finer scale. In three-spined stickleback, balancing selection has previously been reported to be occurring mainly on chrIV, chrVII, and chrXXI (Thorburn et al., 2021). These chromosomes have also been shown to be

involved in controlling the expression of many phenotypic traits (Peichel & Marques, 2017; Rennison et al., 2019; Thorburn et al., 2021) that exhibit differences between sexes, for example, pigmentation, body size, reproduction (for chrIV and chrXXI), and defense (for chrVII, measured as dorsal spine and lateral plate morphology) (Craig & FitzGerald, 1982; Kitano et al., 2007; Poulin & Fitzgerald, 1989). Those results suggest that a small number of intralocus sexual conflicts might be able to generate balancing selection. However, more work is needed to test if intralocus sexual conflicts actually play a causal role in generating balancing selection or if their location within a region of increased Tajima's *D* is caused by other related factors (gene density, polymorphism, recombination, etc.). Moreover, its worth noting that our methods, and particularly the one based on windows, have more power to detect potential intersex differentiation in highly-polymorphic regions including many SNPs of intermediate allele frequency, and thus might have introduced a ascertainment bias toward inside elevated Tajima's *D* regions. Caution is needed while interpreting these results given the high false positive rates associated with our analyses. We find three genes potentially involved in immune response (OXLA, TRIM2, and LEGC). Because immune system genes are often associated with balancing selection (Andres et al., 2009), one can speculate that the intralocus sexual conflict dynamics is one of the processes contributing to genetic diversity in those regions involved in immunity.

Conclusion

The combination of three complementary detection methods (SNP-by-SNP, cumulative F_{ST} and RDA) revealed modest signatures of intralocus sexual conflict of various strengths throughout the three-spined stickleback genome. However, it is possible that sample size of 50 per sex did not offer sufficient power to identify precise targets of those conflicts with confidence. Therefore, we recommend that future similar studies should use higher sample sizes. Yet, we found that most potential intralocus sexual conflicts do not drive long-term balancing selection or increased genetic diversity, suggesting that they are a dynamic process and are either quickly resolved or that selective pressures are varying through time. Those conflicts may however still play a role in maintaining genetic diversity over shorter timescales by locally reducing the effect of background selection. We also showed that false positives can arise from the duplication of genomic regions into the sex chromosomes and are not easily filtered out, highlighting the importance of accounting for group-specific rearrangements in population genomic studies.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpad075).

Data availability

The paired end sequencing reads are available on NCBI under the Bioproject PRJNA935671. Scripts for analyses and figure reproducibility are available at http://github.com/FlorentSylvestre/WGS_intra-locus-sexual-conflict.

Authors contribution

L.B. conceptualized and supervised the project. F.S. generated and filtered the dataset and performed most analysis. E.N. and C.M. provided methodological advises and resources at various steps of the analysis. F.S. wrote the draft of the manuscript, and all coauthors reviewed and approved it before submission.

Conflict of interest: The authors have no conflict of interest to declare.

Funding

This research was funded by a Fonds de recherche du Québec - Nature et technologies (https://frqnet.frq.gouv.qc.ca/) team grant to L.B. and N.A.-H (grant number: 254537). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors are thankful to I. Caza-Allard, E. Reni-Nolin, S. Delaive, and F-A. Deschenes-Picard for their help during fieldwork, as well as S. Bernatchez and R. Bouchard for their occasional help. We also thank A.-L. Ferchaud and M. Laporte for their advice on analysis, Q. Rougemont for its help in SNP calling and filtering, C. Hernandez and A. Perreault for their help with lab work, and A. Xuereb for proofreading the manuscript. We also thank Nadia Aubin-Horth for her advice on field and lab work, various exchanges, and in particular for her help around oral communications of this work. Sequencing was performed at the Centre d'Expertise et de Services Genome Québec (Montréal, QC Canada). The project is part of the Ressources Aquatiques Québec (RAQ) research program.

References

Abbey-Lee, R. N., Uhrig, E. J., Zidar, J., Favati, A., Almberg, J., Dahlbom, J., Winberg, S., & Løvlie, H. (2018). The influence of rearing on behavior, brain monoamines, and gene expression in three-spined sticklebacks. *Brain Behavior and Evolution*, 91(4), 201–213. https://doi.org/10.1159/000489942

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25(22), 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Andres, A. M., Hubisz, M. J., Indap, A., Torgerson, D. G., Degenhardt, J. D., Boyko, A. R., Gutenkunst, R. N., White, T. J., Green, E. D., Bustamante, C. D., Clark, A. G., & Nielsen, R. (2009). Targets of balancing selection in the human genome. *Molecular Biology and Evolution*, 26(12), 2755–2764. https://doi.org/10.1093/molbev/msp190

Barker, T. C. M., & Milinski, M. (1993). The advantages of being red: Sexual selection in the stickleback. *Marine Behaviour and Physiology*, 23(1–4), 287–300. https://doi.org/10.1080/10236249309378870

Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., Jacq, C., Jensen, A. J., Johnston, S. E., Karlsson, S., Kent, M., Moen, T., Niemelä, E., Nome, T., Næsje, T. F., Orell, P., Romakkaniemi, A., Sægrov, H., Urdal, K., … Primmer, C. R. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature*, 528(7582), 405–408. https://doi.org/10.1038/nature16062

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal*

of the Royal Statistical Society: Series B Statistical Methodology, 57, 289–300.
van der Bijl, W., & Mank, J. E. (2021). Widespread cryptic variation in genetic architecture between the sexes. *Evolution Letters*, 5(4), 359–369. https://doi.org/10.1002/evl3.245
Bissegger, M., Laurentino, T. G., Roesti, M., & Berner, D. (2020). Widespread intersex differentiation across the stickleback genome—The signature of sexually antagonistic selection? *Molecular Ecology*, 29(2), 262–271. https://doi.org/10.1111/mec.15255
Capblancq, T., & Forester, B. R. (2021). Redundancy analysis: A Swiss Army Knife for landscape genomics. *Methods in Ecology and Evolution*, 12(12), 2298–2309. https://doi.org/10.1111/2041-210x.13722
Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaSci*, 4, 7.
Chellappa, S., & Huntingford, F. A. (1989). Depletion of energy reserves during reproductive aggression in male three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, 35(2), 315–316. https://doi.org/10.1111/j.1095-8649.1989.tb02982.x
Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560
Cheng, C., & Kirkpatrick, M. (2016). Sex-specific selection and sex-biased gene expression in humans and flies. *PLoS Genetics*, 12(9), e1006170. https://doi.org/10.1371/journal.pgen.1006170
Connallon, T., & Clark, A. G. (2014). Balancing selection in species with separate sexes: Insights from Fisher's geometric model. *Genetics*, 197(3), 991–1006. https://doi.org/10.1534/genetics.114.165605
Cox, R. M., & Calsbeek, R. (2009). Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *American Naturalist*, 173(2), 176–187. https://doi.org/10.1086/595841
Craig, D., & FitzGerald, G. J. (1982). Reproductive tactics of four sympatric sticklebacks (Gasterosteidae). *Environmental Biology of Fishes*, 7, 369–375.
Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R.; 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.
De Lisle, S. P., & Bolnick, D. I. (2021). Male and female reproductive fitness costs of an immune response in natural populations. *Evolution*, 75(10), 2509–2523. https://doi.org/10.1111/evo.14266
Delgado, M. L., Manosalva, A., Urbina, M. A., Habit, E., Link, O., & Ruzzante, D. E. (2020). Genomic basis of the loss of diadromy in *Galaxias maculatus*: Insights from reciprocal transplant experiments. *Molecular Ecology*, 29(24), 4857–4870. https://doi.org/10.1111/mec.15686
DePristo, M. A., Banks, E., Poplin, R. E., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, 43, 491–498.
Dorant, Y., Cayuela, H., Wellband, K., Laporte, M., Rougemont, Q., Mérot, C., Normandeau, E., Rochette, R., & Bernatchez, L. (2020). Copy number variants outperform SNPs to reveal genotype–temperature association in a marine species. *Molecular Ecology*, 29(24), 4765–4782. https://doi.org/10.1111/mec.15565
Dutoit, L., Mugal, C. F., Bolívar, P., Wang, M., Nadachowska-Brzyska, K., Smeds, L., Yazdi, H. P., Gustafsson, L., & Ellegren, H. (2018). Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome. *Molecular Ecology*, 27(18), 3572–3581. https://doi.org/10.1111/mec.14789
Fijarczyk, A., & Babik, W. (2015). Detecting balancing selection in genomes: Limits and prospects. *Molecular Ecology*, 24(14),3529–3545. https://doi.org/10.1111/mec.13226
Foerster, K., Coulson, T., Sheldon, B. C., Pemberton, J. M., Clutton-Brock, T. H., & Kruuk, L. E. B. (2007). Sexually antagonistic genetic variation for fitness in red deer. *Nature*, 447(7148), 1107–1110. https://doi.org/10.1038/nature05912
Folstad, I., Hope, A. M., Karter, A., & Skorping, A. (1994). Sexually selected color in male sticklebacks: A signal of both parasite exposure and parasite resistance. *Oikos*, 69(3), 511–515. https://doi.org/10.2307/3545863
Gaunt, T. R., Rodríguez, S., & Day, I. N. M. (2007). Cubic exact solutions for the estimation of pairwise haplotype frequencies: Implications for linkage disequilibrium analyses and a web tool "CubeX.". *BMC Bioinformatics*, 8, 428.
Harano, T., Okada, K., Nakayama, S., Miyatake, T., & Hosken, D. J. (2010). Intralocus sexual conflict unresolved by sex-limited trait expression. *Current Biology*, 20(22), 2036–2039. https://doi.org/10.1016/j.cub.2010.10.023
Hawkes, M. F., Gamble, C. E., Turner, E. C. R., Carey, M. R., Wedell, N., & Hosken, D. J. (2016). Intralocus sexual conflict and insecticide resistance. *Proceedings of the Royal Society of London. Series B*, 283(1843), 20161429. https://doi.org/10.1098/rspb.2016.1429
Innocenti, P., & Morrow, E. H. (2010). The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biology*, 8(3), e1000335. https://doi.org/10.1371/journal.pbio.1000335
Jaegle, B., Soto-Jiménez, L. M., Burns, R., Rabanal, F. A., & Nordborg, M. (2022). Extensive gene duplication in Arabidopsis revealed by pseudo-heterozygosity. *bioRxiv*. https://doi.org/10.1101/2021.11.15.468652
Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., Swofford, R., Pirun, M., Zody, M. C., White, S., Birney, E., Searle, S., Schmutz, J., Grimwood, J., Dickson, M. C., Myers, R. M., Miller, C. T., Summers, B. R., Knecht, A. K., … Kingsley, D. M.; Broad Institute Genome Sequencing Platform & Whole Genome Assembly Team. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, 484, 55–61.
Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Research*, 25(6), 918–925. https://doi.org/10.1101/gr.176552.114
Kaitetzidou, E., Gilfillan, G. D., Antonopoulou, E., & Sarropoulou, E. (2022). Sex-biased dynamics of three-spined stickleback (*Gasterosteus aculeatus*) gene expression patterns. *Genomics*, 114(1), 266–277. https://doi.org/10.1016/j.ygeno.2021.12.010
Kasimatis, K. R., Abraham, A., Ralph, P. L., Kern, A. D., Capra, J. A., & Phillips, P. C. (2021). Evaluating human autosomal loci for sexually antagonistic viability selection in two large biobanks. *Genetics*, 217, iyaa015.
Kasimatis, K. R., Ralph, P. L., & Phillips, P. C. (2019). Limits to genomic divergence under sexually antagonistic selection. *G3*, 9, 3813–3824.
Kitano, J., Mori, S., & Peichel, C. L. (2007). Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). *Copeia*, 2007, 336–349.
Klopfenstein, D. V., Zhang, L., Pedersen, B. S., Ramírez, F., Warwick Vesztrocy, A., Naldi, A., Mungall, C. J., Yunes, J. M., Botvinnik, O., Weigel, M., Dampier, W., Dessimoz, C., Flick, P., & Tang, H. (2018). GOATOOLS: A Python library for Gene Ontology analyses. *Scientific Reports*, 8(1), 10872. https://doi.org/10.1038/s41598-018-28948-z
Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics*, 15(1), 356. https://doi.org/10.1186/s12859-014-0356-4
Kratochwil, C. F., & Meyer, A. (2015). Mapping active promoters by ChIP-seq profiling of H3K4me3 in cichlid fish—A first step to uncover cis-regulatory elements in ecological model teleosts. *Molecular Ecology Resources*, 15(4), 761–771. https://doi.org/10.1111/1755-0998.12350
Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, 34(2), 292–305. https://doi.org/10.1111/j.1558-5646.1980.tb04817.x

Laporte, M., Pavey, S. A., Rougeux, C., Pierron, F., Lauzent, M., Budzinski, H., Labadie, P., Geneste, E., Couture, P., Baudrimont, M., & Bernatchez, L. (2016). RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. *Molecular Ecology*, 25(1), 219–237. https://doi.org/10.1111/mec.13466

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, 27(21), 2987–2993. https://doi.org/10.1093/bioinformatics/btr509

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*. https://doi.org/10.48550/ARXIV.1303.3997

Lin, Y., Darolti, I., Furman, B. L. S., Almeida, P., Sandkam, B. A., Breden, F., Wright, A. E., & Mank, J. E. (2022). Gene duplication to the Y chromosome in Trinidadian guppies. *Molecular Ecology*, 31(6), 1853–1863. https://doi.org/10.1111/mec.16355

Liu, S., Ferchaud, A.-L., Grønkjaer, P., Nygaard, R., & Hansen, M. M. (2018). Genomic parallelism and lack thereof in contrasting systems of three-spined sticklebacks. *Molecular Ecology*, 27(23), 4725–4743. https://doi.org/10.1111/mec.14782

Lonn, E., Koskela, E., Mappes, T., Mokkonen, M., Sims, A. M., & Watts, P. C. (2017). Balancing selection maintains polymorphisms at neurogenetic loci in field experiments. *Proceedings of the National Academy of Sciences of the United States of America*, 114(14), 3690–3695. https://doi.org/10.1073/pnas.1621228114

Lucotte, E. A., Albiñana, C., Laurent, R., Bhérer, C., Bataillon, T., & Toupance, B.; Genome of the Netherland Consortium. (2022). Detection of sexually antagonistic transmission distortions in trio datasets. *Evolution Letters*, 6(2), 203–216. https://doi.org/10.1002/evl3.271

Lucotte, E. A., Laurent, R., Heyer, E., Ségurel, L., & Toupance, B. (2016). Detection of allelic frequency differences between the sexes in humans: A signature of sexually antagonistic selection. *Genome Biology and Evolution*, 8(5), 1489–1500. https://doi.org/10.1093/gbe/evw090

Manichaikul, A., Mychaleckyj, J. C., Rich, S. S., Daly, K., Sale, M., & Chen, W.-M. (2010). Robust relationship inference in genome-wide association studies. *Bioinformatics*, 26(22), 2867–2873. https://doi.org/10.1093/bioinformatics/btq559

Mank, J. E., Shu, J. J., & Wright, A. E. (2020). Signature of sexual conflict is actually conflict resolved. *Molecular Ecology*, 29(2), 215–217. https://doi.org/10.1111/mec.15311

McCairns, R. J. S., & Bernatchez, L. (2009). Adaptive divergence between freshwater and marine sticklebacks: Insights into the role of phenotypic plasticity from an integrated analysis of candidate gene expression: adaptation of freshwater sticklebacks. *Evolution*, 64(4), 1029–1047. https://doi.org/10.1111/j.1558-5646.2009.00886.x

Merilä, J., Sheldon, B. C., & Ellegren, H. (1997). Antagonistic natural selection revealed by molecular sex identification of nestling collared flycatchers. *Molecular Ecology*, 6(12), 1167–1175. https://doi.org/10.1046/j.1365-294x.1997.00295.x

Morrow, E. H., & Innocenti, P. (2012). Female postmating immune responses, immune system evolution and immunogenic males. *Biological Review*, 87, 631–638.

Naftaly, A. S., Pau, S., & White, M. A. (2021). Long-read RNA sequencing reveals widespread sex-specific alternative splicing in threespine stickleback fish. *Genome Research*, 31(8), 1486–1497. https://doi.org/10.1101/gr.274282.120

Nath, S., Shaw, D. E., & White, M. A. (2021). Improved contiguity of the threespine stickleback genome using long-read sequencing. *G3*, 11, jkab007.

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., ... Weedon, J. (2022). vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Payseur, B. A., & Nachman, M. W. (2002). Gene density and human nucleotide polymorphism. *Molecular Biology and Evolution*, 19(3), 336–340. https://doi.org/10.1093/oxfordjournals.molbev.a004086

Peichel, C. L., & Marques, D. A. (2017). The genetic and molecular architecture of phenotypic diversity in sticklebacks. *Philosophical Transactions of the Royal Society B*, 372(1713), 20150486. https://doi.org/10.1098/rstb.2015.0486

Peichel, C. L., McCann, S. R., Ross, J. A., Naftaly, A. F. S., Urton, J. R., Cech, J. N., Grimwood, J., Schmutz, J., Myers, R. M., Kingsley, D. M., & White, M. A. (2020). Assembly of the threespine stickleback Y chromosome reveals convergent signatures of sex chromosome evolution. *Genome Biology*, 21(1), 177. https://doi.org/10.1186/s13059-020-02097-x

Poulin, R., & Fitzgerald, G. J. (1989). Early life histories of three sympatric sticklebacks in a salt-marsh. *Journal of Fish Biology*, 34(2), 207–221. https://doi.org/10.1111/j.1095-8649.1989.tb03303.x

R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/

Rastas, P., Calboli, F. C. F., Guo, B., Shikano, T., & Merilä, J. (2016). Construction of ultradense linkage maps with Lep-MAP2: Stickleback F_2 recombinant crosses as an example. *Genome Biology and Evolution*, 8, 78–93.

Reimchen, T. E., & Nosil, P. (2001). Ecological causes of sex-biased parasitism in threespine stickleback. *Biological Journal of the Linnean Society London*, 73, 51–63.

Rennison, D. J., Stuart, Y. E., Bolnick, D. I., & Peichel, C. L. (2019). Ecological factors and morphological traits are associated with repeated genomic differentiation between lake and stream stickleback. *Philosophical Transactions of the Royal Society B*, 374(1777), 20180241. https://doi.org/10.1098/rstb.2018.0241

Rezvoy, C., Charif, D., Gueguen, L., & Marais, G. A. B. (2007). MareyMap: An R-based tool with graphical interface for estimating recombination rates. *Bioinformatics*, 23, 2188–2189.

Rice, W. R. (1984). Sex chromosomes and the evolution of sexual dimorphism. *Evolution*, 38(4), 735–742. https://doi.org/10.1111/j.1558-5646.1984.tb00346.x

Rowe, L., Chenoweth, S. F., & Agrawal, A. F. (2018). The genomics of sexual conflict. *American Naturalist*, 192(2), 274–286. https://doi.org/10.1086/698198

Ruzicka, F., & Connallon, T. (2022). An unbiased test reveals no enrichment of sexually antagonistic polymorphisms on the human X chromosome. *Proceedings of the Royal Society of London. Series B*, 289, 20212314.

Ruzicka, F., Dutoit, L., Czuppon, P., Jordan, C. Y., Li, X., Olito, C., Runemark, A., Svensson, E. I., Yazdi, H. P., & Connallon, T. (2020). The search for sexually antagonistic genes: Practical insights from studies of local adaptation and statistical genomics. *Evolution Letters*, 4(5), 398–415. https://doi.org/10.1002/evl3.192

Ruzicka, F., Hill, M. S., Pennell, T. M., Flis, I., Ingleby, F. C., Mott, R., Fowler, K., Morrow, E. H., & Reuter, M. (2019). Genome-wide sexually antagonistic variants reveal long-standing constraints on sexual dimorphism in fruit flies. *PLoS Biology*, 17(4), e3000244. https://doi.org/10.1371/journal.pbio.3000244

Ruzicka, F., Holman, L., & Connallon, T. (2022). Polygenic signals of sex differences in selection in humans from the UK Biobank. *PLoS Biology*, 20(9), e3001768. https://doi.org/10.1371/journal.pbio.3001768

Sayadi, A., Martinez Barrio, A., Immonen, E., Dainat, J., Berger, D., Tellgren-Roth, C., Nystedt, B., & Arnqvist, G. (2019). The genomic footprint of sexual conflict. *Nature Ecology and Evolution*, 3(12), 1725–1730. https://doi.org/10.1038/s41559-019-1041-9

Thorburn, D. -M., Sagonas, K., Lenz, T., Chain, F., Feulner, P., Bornberg-Bauer, E., Reusch, T., Milinski, M., & Eizaguirre, C. (2021). Balancing selection in structured populations of threespined sticklebacks. *Researchsquare*. https://doi.org/10.21203/rs.3.rs-1092984/v1

Van Doorn, G. S. (2009). Intralocus sexual conflict. *Annals of the New York Academy of Sciences*, 1168, 52–71.

Van Rossum, G., & Drake, F. L. (2009). *Python 3 reference manual*. CreateSpace.

Ward, G., & FitzGerald, G. J. (1983). Macrobenthic abundance and distribution in tidal pools of a Quebec salt marsh. *Canadian Journal of Zoology*, 61(5), 1071–1085. https://doi.org/10.1139/z83-141

Whoriskey, F. G., Fitzgerald, G. J., & Reebs, S. G. (1986). The breeding-season population structure of three sympatric, territorial sticklebacks (Pisces: Gasterosteidae). *Journal of Fish Biology*, 29, 635–648.

Wright, A. E., Fumagalli, M., Cooney, C. R., Bloch, N. I., Vieira, F. G., Buechel, S. D., Kolm, N., & Mank, J. E. (2018). Male-biased gene expression resolves sexual conflict through the evolution of sex-specific genetic architecture. *Evolution Letters*, 2(2), 52–61. https://doi.org/10.1002/evl3.39

Wright, A. E., Rogers, T. F., Fumagalli, M., Cooney, C. R., & Mank, J. E. (2019). Phenotypic sexual dimorphism is associated with genomic signatures of resolved sexual conflict. *Molecular Ecology*, 28(11), 2860–2871. https://doi.org/10.1111/mec.15115

Zajitschek, F., & Connallon, T. (2018). Antagonistic pleiotropy in species with separate sexes, and the maintenance of genetic variation in life-history traits and fitness. *Evolution*, 72(6), 1306–1316. https://doi.org/10.1111/evo.13493