Comparing genome scans among species of the stickleback order reveals three different patterns of genetic diversity

James Reeve[1], Qiushi Li[1], Dorothea Lindtke[1], and Sam Yeaman[1]

[1]University of Calgary

September 25, 2021

Abstract

Comparing genome scans among species is a powerful approach for investigating the patterns left by evolutionary processes. In particular, this offers a way to detect candidate genes that drive convergent evolution. We compared genome scan results to investigate if patterns of genetic diversity and divergence are shared among divergent species within the stickleback order (Gasterosteiformes): the threespine stickleback (Gasterosteus aculeatus), ninespine stickleback (Pungitius pungitus) and tubesnout (Aulorhynchus flavidus). Populations were sampled from the southern and northern edges of each species' range, to identify patterns associated with latitudinal changes in genetic diversity. Weak correlations in genetic diversity (F_{ST} and expected heterozygosity) and three different patterns in the genomic landscape were found among these species. Additionally, no candidate genes for convergent evolution were detected. This is a counterexample to the growing number of studies that have shown overlapping genetic patterns, demonstrating that genome scan comparisons can be noisy due to the effects of several interacting evolutionary forces.

Introduction:

Genome scans are useful tools for identifying the effects of evolutionary processes on the genome of a species (Lotterhos and Whitlock, 2015; Fraser and Whiting, 2019). In the past decade they have been used to analyse genomic patterns in many wild species (Alves et al., 2019; Dennenmoser, Vamosi, Nolte, & Rogers, 2017; Jones et al., 2012; Vijay et al., 2016; Westram et al., 2014), as they can provide genetic information about evolution without requiring typically impractical experimental setups. The growth of studies using genome scans has provided a new opportunity to compare results among species to identify common patterns of genetic variation, which may be imprinted on different species through the same evolutionary processes. Ultimately, comparisons of genome scans among species will help to assess the generality of genetic patterns to learn how evolution shapes the genomes of different species.

At the simplest level, genome scans are a comparison of genetic diversity among different populations within a species. Genetic diversity can be split into two main types; diversity within a population and diversity among populations (referred to as genetic divergence). Many statistics represent genetic diversity (e.g. π, H_E, Tajima's D, and Fay & Wu's H) or genetic divergence (e.g. F_{ST}, d_{xy}), and different interpretations of these scores have been discussed at length in other papers (Burri et al., 2015; Ellegren et al., 2012; Reid et al., 2016; Van Doren et al., 2017; Vijay et al., 2016, 2017). A genome scan moves along the genome looking for extreme patterns of these statistics that may be associated with local adaptation (Lotterhos & Whitlock, 2015, Fraser and Whiting 2019), but alternatively could be the product of background selection (Charlesworth et al., 1993, Matthey-Doret & Whitlock, 2019) or demographic events such as range expansions, population bottlenecks or inbreeding (Barton, 1998; Excoffier & Ray 2008; Lotterhos & Whitlock, 2014; Nielsen, Hellmann, Hubisz, Bustamante, & Clark, 2007). These extreme patterns can be identified visually as "peaks" and "troughs" of genetic diversity or divergence, from their distinctive shape on a Manhattan plot. Statistical methods are

used to determine which evolutionary processes most likely generated these peaks and troughs, often as the first step towards identifying candidate genes.

Comparison of genome scan results among species provides insight into how shared ancestry, demography, and environmental conditions can affect the similarity of patterns in their genomes. Commonly, genome scans are compared to detect convergent evolution (Fraser and Whiting 2019), as shared peaks or troughs have the potential to reveal genes that underpin evolution to a shared environmental pressure in many species (Stern 2013). Examples of these convergently evolving genes have already been found such as digestive proteins in primates (Stewart, Schilling, & Wilson, 1987), pigmentation in vertebrates (Gompel & Prud'homme, 2009; Hoekstra, 2006; Manceau et al., 2010) or anthocyanin proteins in flowering plants (Kopp 2009). Outside of convergent evolution, comparing genome scans can also show shared properties of the genome such as recombination landscapes (Samuk *et al.* , 2017) or ancestral population structure (Vijay *et al.* , 2017). On one hand, genomes scans should not be used in isolation to detect convergent evolution, as shared patterns can come from several sources. On the other hand, genome scans offer a useful way to identify broad scale genetic similarities among several species. By comparing patterns in diversity and divergence across many species and environmental gradients, we can better understand how evolutionary processes affect the genome.

Threespine stickleback (*Gasterosteus aculateus*) is a good system for comparative genome scans, as several regions of the genome have been identified that are strongly associated with local adaptation in this species (Colosimo et al., 2005; Hohenlohe et al., 2010; Jones et al., 2012; Schluter & Conte, 2009). Several closely related fish species live in overlapping niches allowing their genomic landscape to be compared to the threespine stickleback's to learn how evolution shapes patterns in their respective genomes. This study aims to compare patterns of genetic diversity and divergence in the threespine stickleback with both the ninespine stickleback (*Pungitus pungitus* ; for simplicity the stickleback species will be referred to as threespines and ninespines) and tubesnout (*Alurhychous flavidus*), as an example of how comparisons of genome scan results can identify common genetic patterns.

Ninespines and threespines diverged 26mya (Varadharajan et al., n.d.) and have already been subjected to comparative genetic studies (Varadharajan *et al.* , no date; Shikano *et al.* , 2013; Nelson and Cresko, 2018), in part because both species have colonised freshwater lakes in similar regions. Interestingly, while targeted genetic studies support convergent evolution to freshwater (Shikano*et al.* , 2013), whole genome data found no genetic signatures of convergent evolution (Raeymaekers *et al.* , 2017). The extent of similarity in genetic patterns among these sticklebacks is still an open question.

We are only beginning to compare the genomes of the threespine and tubesnout (Li et al. in review) and have yet to explore the patterns of genetic diversity. These species diverged approximately 50mya (Betancur*et al.* , 2013), which is a timeframe similar to a study in birds which found similar patterns of genetic diversity maintained across 55 million years (Vijay *et al.* , 2017). In contrast to the ninespine-threespine comparison, tubesnouts are an exclusively marine species that overlaps with the marine threespine along most of its range in the Pacific. Marine threespines are known to have genetic structure along the North American West coast (Morris *et al.* , 2018), which may be the result of gene flow from locally-adapted freshwater populations (Nelson and Cresko, 2018). Thus, we may expect to find patterns in the threespine genome that differ from the tubesnout's, due to differences in their demographic history, selection, and ancestral variation.

Here, we compare patterns of population genomic diversity and divergence in these species to assess how such patterns vary across the stickleback order. Specifically, we study patterns in F_{ST} and genetic diversity from populations at each end of a latitudinal gradient and compare these patterns among species-pairs at a whole-genome and a gene-by-gene level to assess their similarity and test for signatures of convergent evolution. We focus on latitude-related effects (e.g. adaptation in traits related to body size, growth rate, changing breeding times or oxygen binding [Andersen et al., 2009; Bell & Foster, 1994, pp. 155–157; Blanck & Lamouroux, 2007]) instead of the patterns of salinity-driven adaptation more commonly investigated in threespine and ninespine, as the tubesnout has not evolved to live in freshwater systems. By studying broad-scale patterns that covary with the selection pressures associated with latitude, we aim to detect whether patterns of genetic diversity are shared among these species, to learn how evolution may have shaped such

patterns.

Method:

Sampling

Tubesnout and threespine samples were collected between May - August 2017 from the West Coast of North America using dip netting and minnow traps. These fish were euthanised in the field using a mixture of 0.5g/L MS222 (Ethyl 3-aminobenzoate methanesulfonate) in sea water, the carcases were then preserved in 95% ethanol which was replaced after 24 hours. The northern populations of both species and all ninespine samples were donated by collectors. Between 30-52 fish were collected per population (Table 1), the specific details of sampling locations are included in Table S1, and population labels are described in Figure 1.

DNA extractions and sequencing

DNA was extracted from a ˜2mm clip of the pectoral fin of each fish using a Qiagen DNeasy Blood and Tissue Kit. The protocol was modified slightly to increase yield by washing the fins in dH_2O before lysis and by repeating the elution step twice using half the volume of buffer. The DNA samples were checked for fragmentation using gel electrophoresis, quality tested with an Implen N60 Nanophotometer, and concentration was measured using a Qubit 3.0 with three replicates per sample. Samples with low quality (A260/A280 $<$ 1.8; A260/A230 $<$ 2.0) or low quantity (concentration $<$ 8ng/µL) were re-extracted. Any sample that failed three re-extractions was removed. This quality check was repeated after pooling DNA samples (see below).

Individual DNA samples were pooled together by population before library preparation (see Table S2 for quality scores of pools). The DNA pools were sent to Genome Québec (McGill University and Génome Québec Innovation Centre, Montréal, Canada) for library preparation and paired-end whole genome shotgun sequencing on their Illumina HiSeqX platform. The estimated coverage of each pool was set as double the number of individuals in the sample (2Nx), so that ideally each chromosome of each individual was sequenced once. A PCR step was performed, even though it is not advised for Pool-seq protocols (Schlötterer et al. 2014), because the mass of DNA in the pools did not meet Genome Québec's minimum threshold for PCR-free sequencing.

Bioinformatics

Unless otherwise mentioned, the default parameter settings were used for all software mentioned. Sequenced reads were trimmed of adaptors with Trimmomatic (v0.38; Bolger, Lohse, & Usadel, 2014), using the paired-end mode 'PE' and with a minimum length set to 120bp. Further trimming was deemed unnecessary after inspecting read quality with FastQC (v0.11.7; https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Trimmed reads were mapped onto each species genome (**threespine** : Peichel, Sullivan, Liachko, & White, 2017; **tubesnout** : Li & Yeaman, n.d.; **ninespine** : Nelson & Cresko, 2018) with BWA-MEM (v0.7.12; Li & Durbin, 2009). PCR-duplicates were flagged using Picard-MarkDuplicates (v2.18.7; http://broadinstitute.github.io/picard). As a prerequisite before running MarkDuplicates, the reads were sorted and read group information was added with Picard - AddOrReplaceReadGroups. Reads were realigned around indels to adjust quality scores for sites surrounding indels using GATK3 – IndelRealigner (v3.8-1-0; McKenna et al., 2010). Before indel realignment the ninespine reads files, which were sequenced on separate lanes, were combined into a single file per population using samtools – merge (v1.9; Li, 2011; Li et al., 2009). After indel realignment samtools – mpileup was used to combine reads from all populations within a species. Any reads flagged as duplicates were ignored by samtools. VarScan (v2.3.9; Koboldt et al., 2012) was used to call SNPs for each species. The ploidy for each sample was set as double the number of individuals in the pool (2N). Thresholds were set to filter out multiallelic SNPs, low coverage (cov $<$ 50), quality (qual $<$ 20), minor alternative allele frequency (maf $<$ 0.01), and SNPs with less than two reads for the minor allele (min-read-count $<$ 2). The coverage filter was set to ensure that each individual in a sample was represented at least once, assuming DNA pooling was balanced.

Genetic diversity calculations

Genetic diversity was measured as two different values, the genetic diversity within a population (H_E) and the genetic divergence (F_{ST}). F_{ST} was calculated for each species using the R package *poolFstat* (Hivert*et al.* , 2018). H_E was estimated per population from the average expected heterozygosity of all SNPs within a 50,000bp window, including invariant sites as 0s in the calculation. This approach was relatively unbiased by depth of coverage, as H_E did not correlate with average window coverage (Fig. S2). H_E was calculated directly from the VCFs using a custom R script (GitHub: ja-Reeve/CompGenoScan/R_scripts/Heterozygosity).

Identifying signatures of local adaptation (within species)

Genes showing signatures of differentiation across the latitudinal gradient were identified for each species using a top-candidate approach (Yeaman *et al.* , 2016). Initially, F_{ST} outliers were identified as any SNPs with scores in the top 999^{th} quantile. Then, the number of F_{ST} outliers within each gene was compared to the expected number that could have arisen by chance, which was estimated from a binomial distribution with a probability of success of 0.001 (i.e. the probability of being an outlier). Any gene that had more observed F_{ST} outliers than the 999^{th} quantile of this binomial distribution was considered a top candidate for local adaptation (using qbinom in R).

Determining orthologs: comparing patterns between species pairs

To assess patterns consistent with convergent evolution between species pairs, candidate genes were matched to orthologs in the other species. Orthologs were identified between threespines and tubesnouts using a table compiled by Li et al. (in review) using OMA (v2.3.0; Altenhoff et al., 2018; Glover, Altenhoff, & Dessimoz, 2019). As the two stickleback species are more closely related and share higher sequence identity, a gapped-alignment program (GMAP; v2017-06-20; Wu & Watanabe, 2005) was used to identify orthologs between threespine and ninespine. For this, any alignments with a mapping quality of < 80 or a percentage identity $< 90\%$ were filtered out. Additionally, any genes with multiple matches (1:many & many:many orthologs) or overlapping positions within a species were removed.

To compare population divergence among species, the average F_{ST} score was calculated per gene. A similar approach could not be used to compare H_E because larger windows were required to obtain sufficiently precise estimates, and multiple genes could be present within a single window. Instead, the score for the whole window was applied to each gene and if a gene's location spanned two windows then it was assigned the score of the window where most of that gene was located. This approach produces some pseudoreplication in the data as a given gene will be present in several neighbouring windows, but this should have only a minor effect, causing an overestimation of the significance of any true correlation. Given that we found less correlation in these metrics than previous studies (see Discussion), this should be a conservative approach.

Identifying signatures of convergent evolution

The simplest approach for detecting of patterns of convergent evolution is to look for genes that are F_{ST} outliers in multiple species, however this approach may miss some true signals as it is very stringent (Storey and Tibshirani, 2003; Fraser and Whiting, 2019). As a more sensitive test, the Null-W approach (Yeaman *et al.* , 2016) was used to detect signatures of convergent evolution, by identifying top candidate genes in one species, and then comparing the F_{ST} scores of orthologs to the top candidate genes to a null distribution of randomly chosen genes from the genome. This was done using a standard set of 10,000 randomly chosen control SNPs and comparing both the orthologs and the null distribution genes to the control SNPs using Wilcoxon ranked sum test W-scores (Wilcoxon, 1945; for more details see Reeve, 2019 or Supp. Mat. of Yeaman et al., 2016). These W-scores were normalized into Z-scores using a formula from Whitlock and Schluter (2009, p. 342), and empirical P-values for the orthologs were calculated based on their position in the null distribution using the *empPvals* function of the *qvalue* R package (Storey *et al.* , 2015). Empirical P-values were corrected to reduce false discoveries using a Bonferroni correction. Any gene pairs that remained significant were considered signatures of convergent evolution.

Results:

Whole genome sequencing yielded 3.9 million threespine SNPs and 3.5 million tubesnout SNPs with consistent coverage and quality after filtering (see Table S3 for summary statistics and Figure S1 for distributions). Only 0.7 million ninespine SNPs were detected after filtering, likely as the result of the low depth of coverage for one ninespine population (NsABm).

Comparison of genome-wide patterns

On a genome-wide level, average intraspecific F_{ST} andH_E were found to be relatively similar between the threespine and tubesnout (Table 1), but ninespineH_E was tenfold lower and F_{ST} was almost four times higher (Table 1). Patterns of variation in these summary statistics involved longer "genomic islands" with elevated F_{ST} and lowerH_E in the threespine compared to the tubesnout (Fig. 2; 99^{th} F_{ST} quantile threespine = 0.67, tubesnout = 0.55; 99^{th} H_E quantile threespine = 0.0070, tubesnout = 0.0056). Patterns of F_{ST} in ninespines were extremely heterogenous to the point that no peaks could be identified, and H_E was noticeably lower than the other two species (Fig. 2) with the exception of the sex chromosome (i.e. chr12; Shapiro et al., 2009; Shikano, Laine, Herczeg, Vilkki, & Merilä, 2013).

Comparison of gene-by-gene level patterns

At a gene-by-gene level, there was no clear relationship among average F_{ST} and H_E for orthologous genes for any species pair (Fig. 3). Average F_{ST} per gene was weakly correlated among all species pairs, with tubesnouts and ninespines having a negative albeit non-significant correlation (Table 2). A lack of similarity was also observed with H_E scores, with a slightly stronger negative correlation between threespines and ninespines (Table 2). Additionally, pairwise comparisons between populations showed less similarity in H_E for among-species comparisons ($\rho < 0.2$) than within-species comparisons ($\rho > 0.4$; Fig. 3B). No clear visual pattern exists inH_E (Fig. 3A) or F_{ST}(Fig. 3C), with the exception of a flattening ofH_E and elongation of F_{ST}towards the ninespine axes. Overall, these patterns show broad-scale similarity between threespines and tubesnouts, which does not extend to the local gene level, or overlap with ninespines.

Testing for signatures of convergent evolution

Northern and southern populations of each species were analysed for genetic patterns driven by adaptation to some unmeasured factor related to latitude, by searching for genes with abnormally high patterns of F_{ST}. Using the top candidate approach (Yeaman *et al.* , 2016) 73 genes had extreme values of F_{ST} in threespines compared with 65 genes in tubesnouts (Table S4; Fig. 3A). None of the top candidates were directly shared between these species, but a pair of candidate genes encoding proteins in the forkhead box family were detected (Ts: *foxo3b* ; Tu: *foxb2* ; Table S4). No signatures of high F_{ST} could be detected in ninespines because too many scores were close to $F_{ST} = 1$ to identify meaningful outliers. Additionally, comparing all species, only three H_E scores overlapped in the upper 95% of the distribution (Fig. 3A). The Null-W test identified five possible signatures of convergent evolution between threespines and tubesnouts (Fig. 4B), but after adjusting for false discoveries these signatures lost significance (Table S5). The Null-W test did not identify any forkhead box genes as candidates.

Discussion:

Comparing the results of three genome scans we found few similarities in genomic patterns among species. Only the tubesnout and threespine had similar genome-wide average F_{ST} and$H_E$scores, but these similarities did not extend to gene-level patterns, while comparison to the ninespine found no similarities at the genome-wide or gene levels.. Additionally, we found contrasting genome scan patterns for each species and no strong evidence to support convergent evolution. This study highlights how the complexities of evolutionary histories, such as genetic bottlenecks or gene flow from unsampled habitats, can complicate the comparisons of genome scans.

Genetic patterns within each species

The patterns of genetic diversity along the threespine genome have previously been described in studies of divergence between marine and freshwater threespine population pairs (Hohenlohe et al. 2010, Chan et al. 2010, Jones et al. 2013, Roesti et al. 2015). F_{ST}scores typically cluster in several broad peaks in comparisons among freshwater and marine environments, with pronounced peaks around the*Eda* locus (chr4; Hohenlohe et al. 2010) and the *Pitx1*locus (chr7; Chan et al. 2010), which are involved in freshwater adaptation. Additionally, broad peaks found at three inversions (chr1, 11 & 21) have also been associated with freshwater adaptation (Jones et al. 2012; Roesti et al. 2015). Unexpectedly, as we compared two marine populations, we identified some of these characteristic patterns of marine-freshwater divergence in this study (Fig. S3). A possible explanation is that the northern and southern populations differ in the degree to which they receive gene flow from freshwater populations. In the south, threespines were sampled from an isolated stream that drained directly into the ocean, while the northern threespines were sampled from a lake connected to an estuary (Tables S1). Counterintuitively, the patterns we found probably came from freshwater alleles in the southern population, as a previous study of the lake in the north found no evidence of hybridization between 'anadromous' and freshwater populations (Drevecky, Falco and Aguirre, 2013), and a study of marine populations in the North-West Pacific found a higher frequency of freshwater associated alleles at the *EDA* locus in Oregon than Alaska (Morris *et al.* , 2018). However, to test such hypotheses about introgression, we would have to look at the frequency of the low-plate *EDA* allele and the frequencies of inversions in Oregon and Alaska and contrast this with nearby freshwater populations. An alternative explanation is that the some of the patterns of marine-freshwater adaptation may also be pleiotropically connected to thermal regulation, as has been suggested for the *EDA* locus (Morris *et al.* , 2018). Whether it is differential gene-flow or pleiotropic adaption, we have found that the genomic landscape of geographically diverse marine threespines is strikingly similar to the marine-freshwater landscape.

In contrast to the patterns found in threespines, no large peaks of F_{ST} were present along the tubesnout genome (Fig. 2). Instead, there were several small and narrow F_{ST} peaks suggesting that the tubesnout genome has been shaped by processes that do not leave strong genetic signals, such as genetic drift or polygenic adaptation (Rockman 2012, Stinchcombe and Hoekstra 2008, Yeaman 2015). As the Null-W test is designed to detect linked clusters of F_{ST} outliers, this also explains the lack of any signatures of convergent evolution. Since the patterns of F_{ST} were not strongly heterogeneous in tubesnout, it is unsurprising that no significant matches to threespine were found.

The genetic patterns present in the ninespine stickleback were likely the result of a strong genetic bottleneck and isolation between the northern and southern populations, as on average, genetic divergence was high and genetic diversity was low in all four populations (Table 1, Fig. 2). Southern populations were sampled from two prairie lakes, which were formed when a larger post-glacial lake dried up, isolating these ninespine populations and presumably causing a genetic bottleneck (Tufts, 2018), similar to the founder-effect observed in Nordic populations (Shikano *et al.* , 2010). In contrast, the northern populations were sampled from lakes close to the sea, which potentially has provided several opportunities for gene flow from the marine populations. A phylogeographic study separated ninespine populations from the Atlantic coast and Great Lakes regions into two post-glacial lineages, with evidence suggesting that the divergence time among these lineages may be much older than the last glacial maximum (Aldenhoven*et al.* , 2010). Presumably, the prairie lake populations are part of this Great Lakes lineage (Tufts, 2018) and therefore should be highly diverged from the Northern populations. The extreme genetic divergence among these populations is likely to be the result of long-term genetic isolation combined with a strong genetic bottleneck in the southern populations, not adaptation to latitude.

Comparing the genome scans of all species reveals three distinct patterns, suggesting that the balance between the evolutionary processes has differed among these species. The F_{ST} Manhattan plots (Fig. 2A) show different patterns, which can be interpreted as the result of three distinct evolutionary scenarios: local adaptation (threespine), genetic bottlenecks (ninespine) and a weak or polygenic selection and/or drift (tubesnout). This does not imply that the ninespine has not experienced selection or that the threespine has not been affected by drift, just that the patterns of diversity in the genome have been more strongly affected by different processes in each species.

A major caveat to these results is that very few populations were sampled per species. Pool-seq mixes alleles across a population, which means that the basic sampling unit is a population, in effect each species had only 2-4 data points. The comparisons made in this study may have been underpowered to detect any shared genetic patterns. However, the presence of threespine peaks in previously identified regions undergoing adaptation (Fig. S3) shows that strong genetic patterns were detectable, thus only subtle patterns of genetic diversity were lost. The lack of this pattern in tubesnout may be due to the lack of an evolutionary history of repeated colonization followed by gene-flow from freshwater populations, which can lead to complex genomic architecture for adaptive traits (Tigano and Friesen, 2016; Faria *et al.* , 2019). All things considered; this study demonstrates the diversity of genetic patterns that can be identified from genome scans of wild species, even with a limited number of populations.

Comparative genome scans in a broader context

In many cases, similarity in patterns revealed by genome scans among species decreases with phylogenetic distance. Divergent populations of the same species, and sister species that have recently diverged, often have more strongly shared genetic patterns (Fischer *et al.* , 2013; Renaut *et al.* , 2013; Westram *et al.* , 2014; Burri *et al.* , 2015; Ravinet *et al.* , 2016; Vijay *et al.* , 2016). At greater phylogenetic distances, species that diverged long ago often show less similarity in their genetic patterns, with most of the residual patterns being attributed to convergent evolution (Alan Le Moan, Gaggiotti, Henriques, & Martinez, n.d.; Raeymaekers et al., 2017; Vijay et al., 2017) . Henderson and Brelsford (2020) studied this contrast explicitly in three hummingbird species-pairs, showing that more distantly related species pairs had reduced correlations in genetic diversity and increased F_{ST} across the genome. Similarly, a meta-analysis (Conte *et al.* , 2012) demonstrated a negative relationship between the proportion of shared signatures of trait variation and the time since divergence of both species and population pairs. Shared patterns of genome scan variation is not a universal outcome, as Ræymaekers et al. (2017) showed no shared genetic patterns among species despite significant phenotypic sharing. Our study fits in with this latter category, without any signatures of convergent evolution and widespread differences in genetic patterns along the genome.

An interesting contrast to the results of this study is Vijay's (et al. 2017) study of the long-term conservation of genomic patterns among three species of birds. They compared species that had similar generation and divergence times to the fishes used in this study (Bird clades in Vijay et al. = 23-55mya; threespine to ninespine = 26mya [Varadharajan et al., n.d.]; threespine to tubesnout = 50mya [Betancur et al., 2013]); suggesting that patterns of genetic diversity are conserved long past speciation. Vijay found stronger correlations in genetic diversity among their species pairs (range of Pearson's r = 0.08-0.27) than were found in this study (range Spearman's ρ = -0.07 – 0.09). However, Manhattan plots of F_{ST} and genetic diversity also did not show any clear overlapping peaks or troughs (Fig. 2). Other studies looking at fewer genetic markers have also identified more conserved levels of genetic diversity in birds than fishes (Johns and Avise, 1998; Adams and Hadly, 2013), possibly as the result of a faster genome-averaged mutation rate, which has been observed between teleosts and mammals (Ravi and Venkatesh, 2008). Alternatively, fish genomes may evolve faster than birds due to differences in their recombination map or gene densities. Investigating the differences in the rates of evolution among broad taxonomic groups is an interesting question, which is now possible with the increase in publicly available whole genome data.

Conclusion:

In some ways the lack of shared genetic patterns among species is not surprising, as evolution is a balance of several forces that leave a complex mosaic of patterns in the genome. Finding any common patterns among species would require very strong evolutionary forces to consistently shift this balance in the same way for every species. When comparing genome scans divergence in such patterns may be the norm and conservation may be a comparatively rare exception. Our results demonstrate that genome scans can be noisy, due to the effects of demographic shifts, genomic architecture or selective sweeps. Yet these noisy results help in the development of a general theory on how evolutionary forces shape the genome, by showing when similarities do not arise and some of the oddities that one may see when performing a genome scan.

Acknowledgements:

We thank all the organisations and people that have assisted in the collection of samples. The Canadian Department of Fish and Wildlife and Fish and Wildlife Departments of Alaska and Oregon assisted us by providing information about sampling locations. In particular we thank Dr. Darcie Neffs of the Alaskan Department of Fish and Wildlife for collecting tubesnouts from Juneau (TuAK), Prof. Mike Bell for collecting the Alaskan threespine stickleback (TsAK) and Ms. Treasa Tufts who collected the ninespine sticklebacks. Dr. Sarah Smith-Wuitchik is thanked for her advice for sampling fish. We also thank Dr. Jon Mee and the members of the Rogers Lab at the University of Calgary who provided advice on finetuning the DNA extraction protocol. Finally, we would like to thank Ms. Hazel Cameron-Inglis for her assistance during a month and a half of field work. Funding for this work was provided by Alberta Innovates (20150252) and NSERC Discovery (RGPIN/03950-2017).

Data Accessibility:

The raw sequencing data for each population has been uploaded to GenBank (DOI. . . ..) and all code used in the analysis is uploaded to GitHub: ja-Reeve/CompGenoScan.

Author Contributions:

JR collected the data and with the help of SY designed the project and wrote the manuscript. QL and DL provided advice on the analysis and created the list of orthologs to compare species. All authors have read and approved this paper.

Competing Interests:

None of the authors have competing interests.

References:

Adams, R. I. and Hadly, E. A. (2013) 'Genetic diversity within vertebrate species is greater at lower latitudes', *Evolutionary Ecology* , 27, pp. 133–143. doi: 10.1007/s10682-012-9587-x.

Aldenhoven, J. T. *et al.* (2010) 'Phylogeography of ninespine sticklebacks (Pungitius pungitius) in North America : glacial refugia and the origins of adaptive traits', *Molecular Biology Evolution* , 19, pp. 4061–4076. doi: 10.1111/j.1365-294X.2010.04801.x.

Altenhoff, A. M. *et al.* (2018) 'The OMA orthology database in 2018: retrieving evolutionary relationships among all domains of life through richer web and programmatic interfaces', *Nucleic Acids Research* , 46(November 2017), pp. D477–D485. doi: 10.1093/nar/gkx1019.

Alves, J. M. *et al.* (2019) 'Parallel adaptation of rabbit populations to myxoma virus', *Science* , 363(6433), pp. 1319–1326.

Andersen, Ø. *et al.* (2009) 'Haemoglobin polymorphisms affect the oxygen- binding properties in Atlantic cod populations',*Proceedings of the Royal Society B: Biological Sciences* , 276, pp. 833–841. doi: 10.1098/rspb.2008.1529.

Bell, M. A. and Foster, S. A. (1994) *The evolutionary biology of the threespine stickleback* . Oxford: Oxford University Press.

Betancur, -R. R. *et al.* (2013) 'The Tree of Life and a New Classification of Bony Fishes', *PLOS Currents Tree of Life* , 1. doi: 10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288.Revisions.

Blanck, A. and Lamouroux, N. (2007) 'Large-scale intraspecific variation in life-history traits of european freshwater fish', *Journal of Biogeography* , 34(5), pp. 862–875. doi: 10.1111/j.

Burri, R. *et al.* (2015) 'Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of Ficedula flycatchers', *Genome Research* , 25(11), pp. 1656–1665. doi: 10.1101/gr.196485.115.

Conte, G. L. *et al.* (2012) 'The probability of genetic parallelism and convergence in natural populations', *Proceedings of the Royal Society B: Biological Sciences* , 279(1749), pp. 5039–47. doi: 10.1098/rspb.2012.2146.

Deagle, B. E. *et al.* (2013) 'Phylogeography and adaptation genetics of stickleback from the Haida Gwaii archipelago revealed using genome-wide single nucleotide polymorphism genotyping', *Molecular Ecology* , 22, pp. 1917–1932. doi: 10.1111/mec.12215.

Dennenmoser, S. *et al.* (2017) 'Adaptive genomic divergence under high gene flow between freshwater and brackish-water ecotypes of prickly sculpin (Cottus asper) revealed by Pool-Seq', *Molecular Ecology* , 26, pp. 25–42. doi: 10.1111/mec.13805.

Drevecky, C. J., Falco, R. and Aguirre, W. E. (2013) 'Genetic divergence of a sympatric lake-resident-anadromous three-spined stickleback Gasterosteus aculeatus species pair', *Journal of Fish Biology* , 83(1), pp. 111–132. doi: 10.1111/jfb.12154.

Faria, R. *et al.* (2019) 'Evolving inversions', *Trends in Ecology and Evolution* . Elsevier Ltd, 34(3), pp. 239–248. doi: 10.1016/j.tree.2018.12.005.

Fischer, M. C. *et al.* (2013) 'Population genomic footprints of selection and associations with climate in natural populations of Arabidopsis halleri from the Alps', *Molecular Ecology* , 22, pp. 5594–5607. doi: 10.1111/mec.12521.

Fraser, B. A. and Whiting, J. R. (2019) 'What can be learned by scanning the genome for molecular convergence in wild populations ?',*Annals of the New York Academy of Sciences* , pp. 1–20. doi: 10.1111/nyas.14177.

Glover, N. M., Altenhoff, A. and Dessimoz, C. (2019) 'Assigning confidence scores to homoeologs using fuzzy logic', *PeerJ* , 6, p. e6231. doi: 10.7717/peerj.6231.

Haenel, Q. *et al.* (2019) 'Predictable genome-wide sorting of standing genetic variation during parallel adaptation to basic versus acidic environments in stickleback fish', *Evolution Letters* , 3(1), pp. 28–42. doi: 10.1002/evl3.99.

Henderson, E. C. and Brelsford, A. (2020) 'Genomic differentiation across the speciation continuum in three hummingbird species pairs',*BMC Evolutionary Biology* . BMC Evolutionary Biology, 20(1), pp. 1–11. doi: 10.1186/s12862-020-01674-9.

Hivert, V. *et al.* (2018) 'Measuring genetic differentiation from pool-seq data', *Genetics* , 210, pp. 315–330.

Hohenlohe, P. A. *et al.* (2010) 'Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags',*PLoS Genetics* , 6(2), p. e1000862. doi: 10.1371/journal.pgen.1000862.

Johns, G. C. and Avise, J. C. (1998) 'A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene',*Molecular Biology Evolution* , 15(11), pp. 1481–1490.

Jones, F. C. *et al.* (2012) 'The genomic basis of adaptive evolution in threespine sticklebacks', *Nature* , 484(7392), pp. 55–61. doi: 10.1038/nature10944.

Koboldt, D. C. *et al.* (2012) 'VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing',*Genome Research* , 22, pp. 568–576. doi: 10.1101/gr.129684.111.568.

Lawniczak, M. K. N. *et al.* (2010) 'Widespread divergence between incipient Anopheles gambiae species revealed by whole genome sequences',*Science* , 330, pp. 512–515.

Li, H. *et al.* (2009) 'The Sequence Alignment/Map format and SAMtools', *Bioinformatics* , 25(16), pp. 2078–2079. doi: 10.1093/bioinformatics/btp352.

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

Lindtke, D., Li, Q. and Yeaman, S. (no date) *Co-evolution of local adaptation and genome architecture in threespine stickleback* .

Lotterhos, K. E. and Whitlock, M. C. (2015) 'The relative power of genome scans to detect local adaptation depends on sampling design and statistical method', *Molecular Ecology* , 24, pp. 1031–1046. doi: 10.1111/mec.13100.

McKenna, A. *et al.* (2010) 'The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data',*Genome Research* , 20, pp. 1297–1303. doi: 10.1101/gr.107524.110.20.

Le Moan, A. *et al.* (no date) 'Beyond parallel evolution: when several species colonize the same environmental gradient'. doi: http://dx.doi.org/10.1101/662569.

Le Moan, A., Gagnaire, P.-A. and Bonhomme, F. (2016) 'Parallel genetic divergence among coastal–marine ecotype pairs of European anchovy explained by differential introgression after secondary contact',*Molecular Ecology* , 25, pp. 3187–3202. doi: 10.1111/mec.13627.

Morris, M. R. J. *et al.* (2018) 'Contemporary ancestor? Adaptive divergence from standing genetic variation in Pacific marine threespine stickleback', *BMC Evolutionary Biology* . BMC Evolutionary Biology, 18, p. 113.

Nelson, T. C. and Cresko, W. A. (2018) 'Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations', *Evolution Letters* , 2(1), pp. 9–21. doi: 10.1002/evl3.37.

Raeymaekers, J. A. M. *et al.* (2017) 'Adaptive and non-adaptive divergence in a common landscape', *Nature Communications* . Springer US, 8, p. 267. doi: 10.1038/s41467-017-00256-6.

Ravi, V. and Venkatesh, B. (2008) 'Rapidly evolving fish genomes and teleost diversity', *Current Opinion in Genetics and Development* , 18, pp. 544–550. doi: 10.1016/j.gde.2008.11.001.

Ravinet, M. *et al.* (2016) 'Shared and nonshared genomic divergence in parallel ecotypes of Littorina saxatilis at a local scale', *Molecular Ecology* , 25, pp. 287–305. doi: 10.1111/mec.13332.

Reeve, J. (2019) *The genetic basis of convergent evolution among three species of fishes* . University of Calgary.

Reid, N. M. *et al.* (2016) 'The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish',*Science* , 354(6317), pp. 1305–1309.

Renaut, S. *et al.* (2013) 'Genomic islands of divergence are not affected by geography of speciation in sunflowers', *Nature Communications* , 4, p. 1827. doi: 10.1038/ncomms2833.

Samuk, K. *et al.* (2017) 'Gene flow and selection interact to promote adaptive divergence in regions of low recombination',*Molecular Ecology* , 26(17), pp. 4378–4390. doi: 10.1111/mec.14226.

Shapiro, M. D. *et al.* (2009) 'The genetic architecture of skeletal convergence and sex determination in ninespine sticklebacks',*Current Biology* . Elsevier Ltd, 19, pp. 1140–1145. doi: 10.1016/j.cub.2009.05.029.

Shikano, T. *et al.* (2010) 'History vs. habitat type: explaining the genetic structure of European nine-spined stickleback (Pungitius pungitius) populations', *Molecular Ecology* , 19, pp. 1147–1161. doi: 10.1111/j.1365-294X.2010.04553.x.

Shikano, T. *et al.* (2013) 'Genetic architecture of parallel pelvic reduction in ninespine sticklebacks', *G3* , 3, pp. 1833–1842. doi: 10.1534/g3.113.007237.

Soria-Carrasco, V. *et al.* (2014) 'Stick insect genomes reveal natural selection's role in parallel speciation', *Science* , 344, pp. 738–743.

Storey, J. D. *et al.* (2015) 'qvalue: Q-value estimation for false discovery rate control.' R package version 2.8.0. Available at: http://github.com/jdstorey/qvalue.

Storey, J. D. and Tibshirani, R. (2003) 'Statistical significance for genomewide studies', *Proceedings of the National Academy of Sciences* , 100(16), pp. 9440–9445.

Terekhanova, N. V. *et al.* (2014) 'Fast evolution from precast bricks: genomics of young freshwater populations of threespine stickleback Gasterosteus aculeatus', *PLoS Genetics* , 10(10), p. e1004696. doi: 10.1371/journal.pgen.1004696.

Tigano, A. and Friesen, V. L. (2016) 'Genomics of local adaptation with gene flow', *Molecular Ecology* , 25(10), pp. 2144–2164. doi: 10.1111/mec.13606.

Tufts, T. (2018) *Assessing temperature tolerance in ninespine Stickleback (Pungitus pungitus) in response to climate change* . University of Calgary.

Turner, T. L., Hahn, M. W. and Nuzhdin, S. V. (2005) 'Genomic islands of speciation in Anopheles gambiae', *PLoS Biology* , 3(9), p. e285. doi: 10.1371/journal.pbio.0030285.

Varadharajan, S. *et al.* (no date) *Genome sequencing of the nine-spined stickleback (Pungitius pungitius) provides insights into chromosome evolution* .

Vijay, N. *et al.* (2016) 'Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex', *Nature Communications* . Nature Publishing Group, 7, p. 13195. doi: 10.1038/ncomms13195.

Vijay, N. *et al.* (2017) 'Genomewide patterns of variation in genetic diversity are shared among populations, species and higher-order taxa', *Molecular Ecology* , 26, pp. 4284–4295. doi: 10.1111/mec.14195.

Westram, A. M. *et al.* (2014) 'Do the same genes underlie parallel phenotypic divergence in different Littorina saxatilis populations?',*Molecular Ecology* , 23, pp. 4603–4616. doi: 10.1111/mec.12883.

Whitlock, M. C. and Schluter, D. (2009) *The analysis of biological data* . 1st edn. Greenwood Village, Colorado: Roberts and Company.

Wilcoxon, F. (1945) 'Individual comparisons by ranking methods',*Biometrics Bulletin* , 1(6), pp. 80–83.

Wu, T. D. and Watanabe, C. K. (2005) 'Sequence analysis GMAP: a genomic mapping and alignment program for mRNA and EST sequences',*Bioinformatics* , 21(9), pp. 1859–1875. doi: 10.1093/bioinformatics/bti310.

Yeaman, S. *et al.* (2016) 'Convergent local adaptation to climate in distantly related conifers', *Science* , 353(6306), pp. 23–26.

Tables:

Table 1: Average genetic diversity, standard error (SE) and population size (N) per population. Genetic diversity is represented by both the average F_{ST} per SNP and averageH_E per window for each population and each species. The population labels are explained in the caption of Fig. 1.

Population	N	Number of windows	$\mathbf{H_E}$	SE
Threespine stickleback	*Threespine stickleback*	*Threespine stickleback*	*Threespine stickleback*	*Threespine stickleback*
TsAK	52	8,764	2.65×10^{-3}	1.52×10^{-5}
TsOR	51		3.41×10^{-3}	1.75×10^{-5}
Species average	Species average	-	3.03×10^{-3}	1.43×10^{-5}
Tubesnout	*Tubesnout*	*Tubesnout*	*Tubesnout*	*Tubesnout*
TuAK	44	8,925	2.93×10^{-3}	1.73×10^{-5}
TuBC	50		2.86×10^{-3}	1.21×10^{-5}
Species average	Species average	-	2.90×10^{-3}	1.18×10^{-5}
Ninespine stickleback	*Ninespine stickleback*	*Ninespine stickleback*	*Ninespine stickleback*	*Ninespine stickleback*

Population	N	Number of windows	$\mathbf{H_E}$	SE
NsNUn	46	15,058	$0.41x10^{-3}$	$0.67x10^{-5}$
NsNUd	42		$0.37x10^{-3}$	$0.83x10^{-5}$
NsABk	30		$0.18x10^{-3}$	$0.44x10^{-5}$
NsABm	41		$0.13x10^{-3}$	$0.40x10^{-5}$
Species average	Species average	-	$0.27x10^{-3}$	$0.57x10^{-5}$

Table 2: Spearmen's ρ correlations among species. Correlations are made between the average F_{ST} andH_E of interspecific gene pairs.H_E scores are averaged across all populations before comparing species. See Fig. 3B forH_E correlations among populations.

Comparison	F_{ST}	F_{ST}	$\mathbf{H_E}$	$\mathbf{H_E}$	Number of genes
	ρ	**P-value**	**ρ**	**P-value**	
Threespine vs Ninespine	0.01	0.10	-0.07	$2.2x10^{-6}$	20,155
Threespine vs Tubesnout	0.02	0.04	0.09	$2.2x10^{-6}$	9,155
Ninespine vs Tubesnout	-0.04	$2.5x10^{-4}$	-0.02	0.08	8,086

Figures:

Figure 1: The sampling location for each species of fish. Threespine locations are represented by green triangles, ninespines by blue squares and tubesnouts by orange circles. Labels for each population are used consistently throughout this paper; the first half of the label denotes the species (Ts = thresspine stickleback, Tu = tubesnout and Ns = ninespine stickleback). The second half of the label denotes the state or province where the population was collected (AK = Alaska, USA; BC = British Columbia, Canada; OR = Oregon, USA; AB = Alberta, Canada; NU = Nunavut, Canada). The two Albertan ninespine populations are combined into a single point (NsABm & NsABk) for visual clarity. The base map is projected in Azimutahl equal distances (datum = WGS84) orientated to centre on Canada (latitude = 90 & longitude = -98.4). Ocean water is coloured by the annual range in sea surface temperature (°C) taken from the Bio-ORACLE database (https://www.bio-oracle.org/). Threespine and tubesnout photos were taken by Hazel Cameron-Inglis and used with permission, the ninespine photo was taken by Piet Spanns and used under an open license. The final plot was compiled in R using the sf, ggplot, raster and grid packages.

Figure 2: Genome wide patterns of genetic diversity within the threespine stickleback, ninespine stickleback and tubesnout. **A**) F_{ST} per SNP and **B**)H_E per 50Kb window for each species, excluding windows in intergenic regions. Ninespine scores were mapped onto their position on the threespine genome. Threespine and tubesnout F_{ST} was downsized by sampling every 100^{th} SNP along the genome, and approximately 70 windows were filtered out of the $H_E$plots for visual clarity. The red-dashed lines show the 999^{th} F_{ST} and $99^{th}H_E$ quantiles. This plot was generated in R using the ggplot and gridExtra packages.

Figure 3: Comparison of genomic patterns among species.**A)** shows the relationship in average genetic diversity (H_E) among genes for each species pair. Each point is a gene which is orthologous among the species. The dashed lines represent the 95^{th} and 5^{th}quantile of H_E in each species. Any points on the bottom left or top right segments of a panel are genes with extreme H_E that are shared among species. **B)** is a matrix of H_ESpearman's correlations among all population pairs, where the colour represents Spearman's ρ and the text shows the significance level of a correlation test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). **C)** shows the relationship and between the average F_{ST} per gene for each species. Colored points are signatures of local adaptation for each species; red for threespine sticklebacks and blue for tubesnouts. Gray points are

genes not associated with local adaptation; they are partially transparant to show overlapping genes. No signatures of selection overlaped among species.

Figure 4: Detecting genes with elevated divergence and testing for signatures of convergent evolution. **A-B)** Show the top-candidate approach where each point is a separate gene. The total number of SNPs is compared to the number of SNP outliers in each gene, with top candidates identified as those genes that exceed the number of outliers expected under a binomial distribution, represented by the jagged red line. **C-D)** Null-W test results between **C)**tubesnout orthologs of threespine top candidates and **D)**threespine orthologs of tubesnout top candidates. The grey curve is the null-distribution of Z-scores from all orthologs of candidate genes in the focal species (i.e. tubesnout orthologs in **C** and threespine orthologs in **D**). The blue points are top-candidate-orthologs, whose values on the y-axis have been jittered for visual clarity. The red dashed line is the 95th quantile of Z-scores. FDR corrections are not shown.