Intercontinental genomic parallelism in multiple three-spined stickleback adaptive radiations

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Abstract

Parallelism, the evolution of similar traits in populations diversifying in similar conditions, provides strong evidence of adaptation by natural selection. Many studies of parallelism focus on comparisons of different ecotypes or contrasting environments, defined *a priori*, which could upwardly bias the apparent prevalence of parallelism. Here, we estimated genomic parallelism associated with components of environmental and phenotypic variation at an intercontinental scale across four freshwater adaptive radiations (Alaska, British Columbia, Iceland, Scotland) of the three-spined stickleback (*Gasterosteus aculeatus*). We

combined large-scale biological sampling and phenotyping with RAD-sequencing data from 73 freshwater lake populations and four marine ones (1,380 fish) to associate genome-wide allele frequencies with continuous distributions of environmental and phenotypic variation. Our three main findings demonstrate: 1) quantitative variation in phenotypes and environments can predict genomic parallelism; 2) genomic parallelism at the early stages of adaptive radiations, even at large geographic scales, is founded on standing variation; and 3) similar environments are a better predictor of genome-wide parallelism than similar phenotypes. Overall, this study validates the importance and predictive power of major phenotypic and environmental factors likely to influence the emergence of common patterns of genomic divergence, providing a clearer picture than analyses of dichotomous phenotypes and environments.

Introduction

Adaptive radiations are rapid branchings on the tree of life, associated with adaptation to distinct ecological niches ([1]). As major sources of biodiversity, their study has revealed much about the evolution of phenotypic variation ([1],[2]). Adaptive radiations also highlight what is unknown about biodiversity evolution. For example, despite abundant phenotypic diversity, not all trait combinations evolve in every radiation, yet organisms in different places sometimes arrive at very similar endpoints ([3],[4]). The latter has been observed in cichlid fish ([5]), Anolis lizards ([6]) and Darwin's finches ([7]); famous examples of parallel phenotypic evolution. This suggests Stephen Jay Gould's contention that evolution is contingent and unrepeatable ([8]) cannot be completely true. Extensive prior work has examined the role genetic correlations between traits might play in constraining diversity,

but the answers provided have not been entirely satisfactory ([3],[9],[10]). Alongside these processes, it is probable that repeatable patterns of evolution are channelled in predictable ways by common environments and selection regimes. Often termed parallel evolution (distinguishable from convergence by shared evolutionary 'start' as well as 'end' points, but see ([11],[12])), this process results from environmental similarities within and between radiations. Striking, natural examples of phenotypic parallelism ([1],[6]) support this hypothesis, and the persistent appearance of familiar forms in similar ecological niches demonstrates the significance of selection.

However, a limitation with studies on phenotypic parallelism is that they have concentrated largely on the comparison of pairs of strongly different ecotypes or environments ([13–15]). This approach might upwardly bias the prevalence of parallelism with comparisons known *a priori* to occur repeatedly, effectively constraining the evolutionary 'end' point. Further, similar environments are typically assumed on the basis of comparable, typically morphological or life history, phenotypes, concealing the role of individual components of environmental variation in driving parallelism. This compromises our ability to understand adaptation, much of which is likely to be broadly physiological. Such drawbacks highlight the importance of combining measures of phenotype, environment and genomics in studies of parallelism. Addressing this gap is needed for a complete understanding of adaptation ([16]).

Highlighting consistent signatures of adaptation in the genomes of multiple, independent natural populations has also proven to be a valuable tool for studies on the genetic basis of adaptation ([17–19]). However, again our comprehension of the relationship between genomic parallelism and continuous phenotypic or environmental variation is surprisingly poor. A major barrier to combining genotype, environment and phenotype has

been achieving the necessary biological replication across all three to make broad inferences and shift from description to hypothesis testing. This has rarely been applied (but see [20–22]), and it remains to be shown whether signals of parallelism obtained from continuous measures are comparable to those from ecotypes and previous studies. Here we make use of such methods to test for environmentally and phenotypically associated genomic parallelism across radiations of three-spined stickleback fish (*Gasterosteus aculeatus*, hereafter 'stickleback').

Stickleback provide a powerful natural experiment to test parallelism. They are ancestrally marine but, following colonisation of freshwater across the northern hemisphere, are in the early stages of replicated adaptive radiations (which we define as sets of geographically proximal, closely-related populations). Comparing multiple populations derived from the marine ancestor provides a model for exploring both phenotypic parallelism and its genetic basis ([23,24]) in response to environmental variation. Phenotypic parallelism is well established ([25,26]), and whilst often considered in dichotomous pairings of marine-freshwater, benthic-limnetic or lake-stream ecotypes, there is substantial continuous phenotypic variation among freshwater populations that has rarely been explored ([27]) in this context. Parallel genomic loci under selection have been identified across the contrasting ecotype pairs ([19,28,29]), but the combination of phenotypic and genomic parallelism in one study is rare (although see [20,30,31]) and has not been done at the scale of replicated adaptive radiations across continents.

For this study we sampled 73 freshwater lake populations (1,304 fish) and four marine populations (76 fish) from four adaptive radiations: two from the Pacific coast of North America (Alaska and British Columbia ['BC']), and two from Atlantic Europe (Iceland

and the island of North Uist ['Scotland']) (Fig. 1, Supplementary Table 1). We consider the variation between lakes, within geographic regions, as adaptive radiations. Assessing groups of lakes as adaptive radiations distinguishes the stickleback system from other notable systems of adaptive radiations, such as the comparisons of within-lake cichlid radiations ([5, 14,17]). We quantify parallelism between phenotypes, environments, and genomic loci under selection (using RAD-sequencing data) in each adaptive radiation and examine how they are associated. Rewards to be gained by connecting the evolution of parallelism more explicitly to the environmental and phenotypic variation include a better grasp of why some traits evolve in concert and a predictive understanding of parallelism and repeatability ([4]). This new understanding is essential if we are to reach a consensus on how biodiversity is altered by adaptation.

RESULTS & DISCUSSION

Environmental and phenotypic similarity across radiations

We first quantified environmental and phenotypic parallelism across four adaptive radiations to provide an indication of how much genomic parallelism associated with environments and phenotypes to expect. For environments, we quantified lake area, parasite prevalence (*Gyrodactylus* spp [Gyro] and *Schistocephalus* spp [Schisto]) and water chemistry (pH and metal cation concentrations of calcium [Ca], sodium [Na], zinc [Zn]). For phenotypes, we collected 12 variables associated with shape (3), armour (7) and tropic (2) morphology (Supplementary Table 2). See methods for detailed information on sampling and measures.

I) Environment. A Principal Component Analysis (PCA) on all seven variables across all lakes revealed that the first axis of environmental variance (Env_{PC1}) separated lakes along a predominant gradient of pH, with additional minor loadings reflecting Ca and Gyro (Fig. 2a; Supplementary Table 3). This axis did not separate radiations but emphasised the variation from alkaline to acid present in all of them (the most acidic environments were absent in Iceland). Env_{PC2} separated lakes with high and low zinc, with additional minor loadings of Schisto (positive) and Ca (negative). This axis separated European and North American clusters. Interestingly, BC lakes were completely subsumed within the more environmentally variable Alaskan lake cluster (Fig 2a,b).

II) *Phenotypes - Armour.* All armour traits loaded positively onto $Armour_{PC1}$ (Fig. 2a; Supplementary Table 3). All radiations overlapped on $Armour_{PC1}$ (Fig. 2b), but there were also significant differences in $Armour_{PC1}$ values between radiations (Supplementary Table 4). Scotland and Alaska had populations with extreme armour reduction (low $Armour_{PC1}$), but in Scotland these populations also had complete loss of armour plates (high $Armour_{PC2}$) which were retained in Alaska (low $Armour_{PC2}$). These deviations produce the anomalous relationship between $Armour_{PC1}$ and $Armour_{PC2}$ in Alaska (Fig. 2a). Iceland exhibited minimal variation in armour traits compared to other radiations. Aside from a few populations from BC, there was no overlap in armour traits between marine and freshwater populations. Marine populations have a higher number of lateral plates and generally more exaggerated armour traits. Importantly however, projection of marine armour phenotypes suggests they fall on the same, parallel, axis, but the marine morpho space is beyond freshwater space (Fig. 2a).

Phenotypic change vectors analysis (PCVA - see methods) highlighted phenotypic constraint along a common axis for armour (mean angle (θ) within radiations = 14.5°,

155 between = 19.5°) (Fig. 2c, Supplementary Table 5). Indeed, θ values between vectors from
156 Iceland and Scotland were not significantly larger than θ values between vectors within
157 Iceland or within Scotland ($p > 0.05$), suggesting a highly parallel axis for marine to
158 freshwater transition in Europe. There was some divergence away from this axis, particularly
159 in Alaska, with significant deviations ($p < 0.001$) in trajectory through trait space (Fig. 2d).
160 This likely comes as a result of Alaskan $Armour_{PC2}$ deviations, but these results suggest some
161 non-parallelism between North America and Europe. Despite significant variation among
162 vector trajectories, average θ remained low (≤ 28.3°), suggesting these significant
163 differences in vector angle represent relatively minor idiosyncrasy along an otherwise
164 conserved, parallel axis.
165 *III*) *Phenotypes - Body shape.* Body shape differed significantly between lakes and
166 radiations (Supplementary Table 4), despite overlap within morphospace (Fig. 2a,b).
167 Scotland exhibited the most extreme body shapes (high $Shape_{PC1}$: elongated, slender bodies,
168 small heads). BC populations had particularly deep bodies, and long, deep heads (high
169 $Shape_{PC2}$). Surprisingly Scotland, the smallest region sampled (303 km^2), had the most shape
170 variation. Marine populations had low $Shape_{PC1}$ and $Shape_{PC2}$ scores, overlapping with some
171 freshwater populations mostly from Iceland (Fig. 2a). Shape PCVA highlighted greater non-
172 parallelism compared with armour (mean θ within radiations = 38.3°, between = 59.3°), with
173 numerous orthogonal comparisons (Fig. 2c). All between-radiation comparisons of θ were
174 significantly greater than within-radiation ($p < 0.001$) (Fig. 2d), demonstrating marine-
175 freshwater shape phenotype transitions have occurred along variable, nonparallel
176 trajectories.
177 *IV*) *Phenotypes - Trophic morphology.* Generally, gill raker length increased with gill
178 raker number. Alaskan trophic morphology was the most variable, and BC contained a

subset of that variation (Fig. 2a,b). Marine-freshwater trophic PCVA highlighted parallel changes (mean θ within radiations = 15.7°, between = 16.6°), although we lacked marine trophic data from Scotland (Fig. 2c, Supplementary Table 5). Significant deviations among Alaska, BC and Iceland (Fig. 2d), however suggest some idiosyncrasy among radiations. Freshwater populations had shorter gill rakers for their size relative to marine populations.

V) Relationship between environmental and phenotypic similarity. We used GLMs to test for parallel associations between each environmental variable and morphology (full results in Supplementary Table 6). Between armour traits and environmental variables, only $Armour_{PC1}$ was significantly associated with pH in a parallel way across all radiations ($F_{1,71}$ = 9.20, FDR = 0.006, σ^2 explained = 11.3%). Although there were also nonparallel, radiation-specific associations (slopes varying between radiations) between armour and several other environmental variables, such as the North-American specific associations between $Armour_{PC2}$ and both Ca and Na. This is consistent with previously reported nonparallel associations between armour and Ca between North American and European radiations ([32]). These results highlight parallel reductions of skeletal traits ($Armour_{PC1}$) with lower pH, and suggest pH is a predictor of phenotypic armour parallelism globally. Given Iceland lacks more acidic freshwater habitats, this may explain why Iceland has comparably limited armour variation.

$Shape_{PC1}$ and Na were associated in parallel across our dataset ($F_{1,71}$ = 6.51, FDR = 0.018, σ^2 explained = 46.2%), whereby fish at lower salinity levels were elongated and had small heads. $Shape_{PC1}$ and Gyro were associated but only in Europe ($F_{3,65}$ = 3.01, FDR = 0.045, σ^2 explained = 48.7%). $Shape_{PC2}$ varied in a parallel manner with Schisto ($F_{1,71}$ = 46.4, FDR = <0.001, σ^2 explained = 55.5%), potentially because of body shape distortion that can occur as a result of *S. solidus* infection. Although the causative factors driving the link between

body shape and water chemistry are largely unknown, similar relationships between salinity and stickleback body shape have been found in other lakes ([33]).

We expected trophic morphology to evolve in response to zooplankton communities, which can vary in response to water chemistry ([34,35]), particularly Zn. In agreement, we found parallel, inverse associations between gill raker number and Zn ($F_{1,71}$ = 63.6, FDR = <0.001, σ^2 explained = 53.4%).

The parallel environment - phenotype associations described here (either globally or between specific radiations) might thus be expected to be underwritten by parallel genetic variation. This is particularly true for environmental variables that also overlap between radiations (such as pH and Ca) and phenotypes associated with them. These predictions are contingent on traits having a simple genetic basis however ([28,36,37]), and may not hold for phenotypes with more complex, polygenic architectures.

Phylogenetic relationship among radiations.

Older lineages, or lineages not experiencing gene flow, are expected to share less ancestral variation as a function of common ancestry, which may constrain parallelism ([18,38,39]). Recent studies have highlighted this as a probable limitation of parallelism in adaptive radiations across continental scales ([32,40-42]). Knowing the genetic relationship across all populations is therefore important to interpret patterns of genomic parallelism. A Neighbour Joining (NJ) tree based on 11,266 unlinked SNPs showed that the four geographic locations form four well-resolved radiations (Fig. 1). TERN in Alaska is slightly anomalous, sitting at a shorter evolutionary distance from European lakes compared with the rest of

225 Alaska, suggesting more recent colonisation by the common ancestor. Interestingly,
226 Icelandic and Scottish marine populations clustered separately with their respective
227 freshwater populations, but both North American marine populations clustered together.
228 This may indicate stronger structuring of marine populations in the Atlantic relative to the
229 Pacific, or a re-invasion of Alaskan marine regions by BC marine populations. PCA on
230 freshwater populations confirmed that radiations form independent clusters, with the
231 dominant axis of variation (PC1 = 36.0%) separating Pacific/North American and
232 Atlantic/European radiation pairs (Supplementary Fig. 1). PC2 (7.0%) and PC3 (5.8%)
233 separated North American and European radiations respectively. Geographically adjacent
234 radiations were also the most genetically similar (Supplementary Table 7, Alaska and BC:
235 mean pairwise F_{ST} = 0.198), Scotland and Iceland: F_{ST} = 0.194), suggesting that despite
236 independent clustering, lineage splits may be relatively recent, or that gene flow may be
237 occurring within the Atlantic and Pacific groups. Divergence across continents was stronger
238 and deeper ($0.314 \leqslant F_{ST} \leqslant 0.338$) than within oceans/continents, consistent with previous
239 studies that have estimated the time of divergence between Atlantic and Pacific stickleback
240 at approximately 200,000 years ([43,44] but see [45]).
241 Shared polymorphisms among adaptive radiations were structured predictably
242 (Supplementary Fig. 2), with most shared sites found between Alaska and BC (N = 11,524),
243 and Iceland and Scotland (N = 8,789). A considerable number of SNP polymorphisms were
244 common to all four radiations however (SNP was polymorphic in all radiations, N = 6,339),
245 suggesting some global retention of ancestral variation. Not including globally shared
246 polymorphisms, sharing among inter-continental comparisons was minimal ($N \leq 933$). In
247 agreement, between-continent structuring accounts for the largest proportion of molecular
248 variance in our data (AMOVA: σ = 889.7, 34.7%). Within continents, populations within

radiations (σ = 366.5, 14.3%) were more genetically variable than radiations themselves (σ = 142.3, 5.6%) (Supplementary Table 8). Molecular variance then is not structured according to geographic scale (Continent > Radiation > Populations). This may be due to intra-continental gene flow, bottlenecks at the founding of ancestral Pacific/Atlantic marine lineages ([52]), or older Pacific/Atlantic divergence times relative to modern freshwater, but it is not possible to differentiate between the two scenarios without demographic modelling.

Phenotypically and environmentally associated SNPs and genomic regions within radiations.

We associated allele frequency changes with each environmental/phenotypic variable (N = 19) within each radiation (18-19 populations) using Bayenv2 ([46]) (see methods), and compared outlier genome windows across radiations to identify parallel genome changes.

Several thousand SNPs for each radiation were highly associated (high Bayesfactor [$>\log_{10}(1.5)$] and top 5% of Spearman's ρ, see methods) with environmental and phenotypic variables (Supplementary Table 9). We mapped SNPs onto non-overlapping 50kb windows, consistent with approximate linkage disequilibrium within the stickleback genome ([47,48]), but also examined 75kb, 100kb, 200kb windows (Supplementary Dataset 1) and windows of equivalent genetic distance (0.1 cM), which confirmed our results were robust and not influenced by variable linkage across the genome (Supplementary Information 1).

We found 1836 unique 50kb windows associated with an environmental variable or a phenotypic trait (Supplementary Dataset 1), ranging from 146 windows associated with pelvic spine length in BC, to 11 associated with lake area variation in Scotland. These strong signals of association, even across modest variation, support the adaptive nature of these radiations. Across unique windows, 454 were associated with both an environmental and

phenotypic variable in the same radiation, suggesting some phenotypically-associated regions are also responsible for local adaptation to environments (Supplementary Dataset 1). This result also suggests measuring important aspects of the environment may provide profitable ways of identifying candidate regions for adaptation and cryptic phenotypes, such as physiology.

Genomic parallelism associated with environmental and phenotypic variation across radiations.

To assess genomic parallelism, we compared observed windows associated in two or more radiations (parallel windows) against randomly permuted (10,000 iterations) null distributions. We first quantified the overall level of genomic parallelism associated with groups of environmental or phenotypic variables. There were no environmentally- or phenotypically- associated 50kb windows parallel in all four radiations for individual variables (Randomised permutations $N_{Expected\text{-}Environmental}$= 0, $N_{Exp\text{-}Pheno}$ = 0.0002), but one window was parallel in a group of three radiations (chrIV: 14400000-14450000 associated with length of pelvis in BC, Iceland and Scotland) ($N_{Exp\text{-}Env}$ = 0.05, $N_{Exp\text{-}Pheno}$ = 0.149, p = 0.133). Many windows however exhibited parallelism between pairs of radiations. A total of 39 environmentally-associated windows (pooled across all seven variables) (N_{Exp} = 12.4, 95% Upper limit (UL) = 18, $p < 0.001$) and 65 phenotypically-associated windows (pooled across all 12 variables) (N_{Exp} = 30.7, 95% UL = 40, $p < 0.001$) were parallel between two radiations (Supplementary Table 10). Parallelism was disproportionately greater for armour (46/65) and gill raker traits (12/65) (number mostly) than for shape (7/65) (χ^2= 6.506, P = 0.04). This is consistent with skeletal traits, with simple genetic architectures ([28,36,37]), being more likely

296 to show evidence of phenotypic parallelism. Interestingly, parallel associated windows
297 (mean SNP N = 7.13, sd = 4.4) had on average more SNPs per window than nonparallel
298 windows (mean SNP N = 6.27, sd = 4.3) (GLM, $LRT_{1,3867} = 22.4$, $p < 0.001$) and exhibited
299 slightly stronger signals of association with variables (mean residual SNPs above expected =
300 1.82 parallel; 1.60 non-parallel; GLM, $LRT_{1,3867} = 5.48$, $p = 0.019$).

301 We next explored parallelism associated with individual environmental/phenotypic
302 variables. We observed significantly more parallel windows than expected for two
303 environmental variables (Ca and pH) (Fig. 3; Supplementary Table 10). Reflecting
304 expectations, these were the same variables that load onto the shared Env_{PC1} across all
305 radiations (Fig. 2b), and were involved in parallel environment x phenotype interactions in
306 all (pH) or some (Ca) radiations. Further, we did not detect significant genomic parallelism
307 associated with variables that varied between radiations, such as salinity, zinc and *S. solidus*
308 prevalence. These results highlight that common environmental axes, such as the shared
309 acid-alkali axis, promote signals of parallelism in the genome, although parallelism appears
310 limited to specific pairs rather than extending to all radiations. However, not all
311 environmental variables with parallel environment x phenotype interactions produced
312 signals of genomic parallelism, such as Na-$Shape_{PC1}$ and Zn-Gill Raker number.

313 Five phenotypic variables were associated with more genomic parallelism than
314 expected by chance (Fig. 3; Supplementary Table 10): four armour traits (2^{nd} dorsal spine,
315 pelvic spine length, length of pelvis and armour plate number) and gill raker number. These
316 results are consistent with the observed, constrained marine-freshwater armour phenotype
317 trajectory. This also suggests that variation in armour trajectories (e.g. in Alaska) are the
318 result of different genotypes being selected in different environments. Additionally, parallel
319 QTLs have been described for gill raker number ([49]), but not length, which exhibits more

plasticity ([50]). Shape traits were not associated with any significant genomic parallelism, despite parallel environment x phenotype interactions. The probable polygenic architecture of shape phenotypes, as has similarly been described in cichlids ([51]), may result in greater redundancy in the genotype-phenotype map, reducing the likelihood of genetic parallelism. Moreover, the partly plastic nature of body shape ([52,53]) may lead to environment x shape associations via the reaction norm rather than genomic re-use.

Marine-freshwater (MxF) associated windows were more parallel than those associated with specific variables (Fig. 3), and overlapped well with previously identified MxF QTLs (proportions overlapping: Alaska = 0.85, BC = 0.85, Iceland = 0.81, Scotland = 0.69) ([19,47,54]) (Supplementary Table 11). Several parallel MxF windows were also parallel for Ca, pH, Na, armour traits and gill raker number (Supplementary Table 12). These results suggest our methods and sequencing coverage are robust enough to recover known parallel regions, and that, unsurprisingly, genomic parallelism associated with freshwater variables is more modest than marine-freshwater parallelism. The latter may reflect subtler variation between lakes compared with stark marine-freshwater contrasts but demonstrates that reduced parallelism for individual fitness components is likely biological rather than methodological. Marine-freshwater comparisons lump together many selective agents without being able to discern which are parallel and which are not. Overall, our results suggest that across a number of comparisons involving two or three (but not all four) freshwater adaptive radiations analysed, evolution of these phenotypes and environmentally associated traits are disproportionately linked to the same genomic regions.

342 Finally, we explored genomic parallelism between specific comparisons, to identify
343 pairs of radiations with the highest levels of parallelism. We found the greatest number of
344 significantly parallel windows in the comparison between Alaska and BC (Ca, Gyro, pelvic
345 spine length, plate number and gill raker number), followed by Iceland and Scotland (Ca, pH,
346 dorsal spine length) (Supplementary Table 10). Inter-continental parallelism was weaker:
347 Alaska-Iceland (Ca, pelvis length); BC-Iceland (Schisto); BC-Scotland (pelvic spine length);
348 Alaska-Scotland (none). Indeed, restricting permutations exclusively to Alaska-BC and
349 Iceland-Scotland was enough to recover the significant parallelism observed when all
350 radiations were analysed together (Supplementary Fig. 3). Phylogenetic patterns strongly
351 support the notion that radiations within continents share similar genetic variation, making
352 parallelism through shared standing variation the most parsimonious explanation for these
353 intra-continental biases. Marginal evidence for this was observed as parallel associated
354 regions had a greater enrichment of shared sites (mean sum of $\log_{10}$ enrichment scores =
355 1.3) compared to random expectations relative to associated regions that did not overlap
356 among radiations (mean = 1.16, $p = 0.14$) and neutral regions (mean = 1.02, $p < 0.001$).
357 However, comparisons between parallel and outlier regions were not significant. This
358 suggests that radiations may be exploiting pools of ancestral variation for adaptation, but
359 the same ancestral variation is not always adapted in parallel.

360 Experimental studies of parallelism have increasingly implicated standing genetic
361 variation in the genesis of parallelism in stickleback ([55]) and other species ([56,57,58]).
362 Coancestry patterns, centred at the focal, causative loci, can discern between parallelism via
363 *de novo* mutations, standing variation, or introgressed alleles ([59]), however we lack the
364 sequencing resolution to make these comparisons here. Further, the source of parallelism is

365 likely to vary locus-by-locus and trait-by-trait, making it difficult to assess with a genome-
366 wide approach. Indeed, all three modes of repeated gene-reuse have been observed in the
367 radiation of a wild tomato clade ([60]).

368

369 **Linkage and the genomic location of parallel regions.**

370 As a reduced-representation approach, selection scans with RAD-sequencing depend
371 on linkage disequilibrium (LD) between markers and functional loci ([47,61,62]). LD varies widely
372 across organisms and within genomes but has been relatively well-characterized in
373 stickleback ([63,64]), and RAD-sequencing has been used successfully in this species to test for
374 genomic parallelism ([36,47,59,63-65]). LD associates inversely with recombination rate across the
375 genome, so we estimated how recombination may affect our results using a previously
376 published genetic map ([65]). Recombination was significantly reduced in associated windows
377 and parallel windows compared with non-associated windows (Supplementary Fig. 4;
378 Kruskal-Wallis, $\chi^2 = 122.21$, $p < 0.001$), but did not differ significantly between associated
379 and parallel windows ($p = 0.55$). Reduced recombination can be an important mechanism in
380 adaptation through maintaining adaptive alleles, as seen in stickleback ([66]) and cichlids ([67]).
381 These patterns may also reflect an increased ability to detect selection in low-
382 recombination windows through increased linkage with causative SNPs. If true, our
383 estimates of association, and by extension parallelism, may be conservative if false-
384 negatives are pervasive in high-recombination regions. Importantly, our signatures of
385 parallelism cannot be explained by variable recombination. Background selection can
386 produce false-positive signatures of parallelism by reducing local diversity in shared low-

387 recombination regions ([68]). This is less of an issue however when associating allele
388 frequencies with environmental/phenotypic clines as done here.
389 Genetic distance (0.1 cM) windows corroborated 50kb results, returning significant
390 parallelism for Ca, pH, pelvic spine length, pelvis length, plate number and gill raker number.
391 Interestingly, we also recovered weakly significant parallelism for several other
392 environmental and armour variables (Supplementary Fig. 5, Supplementary Information 1),
393 suggesting our 50kb results may be conservative.
394 We plotted parallel 50kb windows (Supplementary Fig. 6) and merged adjacent
395 windows (Supplementary Table 13) to inspect putative causative genes (Supplementary
396 Dataset 2). Merging adjacently prior to permutations did not change which variables were
397 significantly parallel (Fig. 3). Doing this we identified some wider genomic regions with
398 parallelism across multiple radiations. One example was the pooling of plate number-
399 associated windows in three radiations around the well-known *Eda* gene, with known
400 functional role in armour plate evolution ([19,28,29]), which emerged despite the limited plate
401 number variation across freshwater populations.
402 We also observed adjacent windows around a known inversion ([69]) region (250kb) on
403 chromosome I, which contains the genes *igfbp2a*, *stk11ip* and *atp1a1*, and was strongly
404 associated with calcium, sodium and pH in several radiations (Supplementary Data 2).
405 Removing these windows prior to permutations did not change which variables were
406 significantly parallel (Fig. 3). Inversions can be beneficial for adaptive haplotypes by reducing
407 local recombination, and have been implicated in genomic parallelism in other systems such
408 as parallel crab/wave ecotypes of *Littorina saxatillis* ([70]). Within this region *atpa1a1* is
409 particularly interesting, given its previously detected association with the major ecological

410 transition from marine to freshwater ([71]) and functional role in metal ion management
411 ([19,69]).

412 Extensive LD in freshwater populations could result from drift, but is consistent with
413 strong directional selection after freshwater invasion and has been reported for stickleback
414 populations from Alaska ([47]). It had not however previously been observed for the same
415 regions across several independent adaptive radiations. These results are also consistent
416 with many diverged marine-freshwater SNPs aggregating in just 19 short genomic regions,
417 including three known inversions ([69]). Overall these results suggest physically linked genomic
418 regions are hitch-hiking in separate radiation pairs, which may contain parallel genes across
419 all radiations but are undetected by our methods.

420

421 **Relationships between genomic parallelism and phylogenetic, phenotypic and**
422 **environmental similarity**

423 Based on the assumption that freshwater populations radiated from common
424 marine ancestors ([1,18]), and to leverage statistical power from our biological sampling, we
425 also compared parallel F_{ST} outliers between all marine-freshwater comparisons to examine
426 the relative effects of genetic, phenotypic, and environmental similarity on genome-wide
427 parallelism at large geographic scale. To do this, we calculated the top 5% of 50kb windows
428 based on F_{ST} for each freshwater population and its relevant marine population and
429 assessed all pairwise overlaps (N = 2,628) (Fig. 4a). Genome-wide F_{ST} outliers are more
430 susceptible to random parallelism through recurrent drift or background selection although,
431 as discussed above, these processes are unlikely to influence previous results obtained by

comparing allele frequency gradients across all populations within a radiation. Nevertheless, broad patterns inferred over all 2,628 comparisons should be apparent despite noise.

Parallel windows were more common in intra- rather than inter-continental comparisons, again highlighting importance of these pairings as the major contributors towards pairwise genomic parallelism signals, as discussed previously. This strongly suggests that genomic parallelism at large geographic scales must be partly contingent on shared genetic variation, although exceptions exist such as recurrent *de novo* mutation at *Pitx1* ([72]) during freshwater pelvis evolution. There is also the possibility of haplotype sharing between radiations within continents by gene flow through marine populations, which may be facilitated in North America, despite the greater geographic distance, by a shared coastline connecting Alaska and BC ([38]).

We used Mantel tests to compare a parallel F_{ST} overlap matrix with genetic, environmental, and phenotypic distance matrices (Fig. 4b-d). Across all comparisons the number of parallel windows was strongly negatively correlated with genetic (r = -0.67, p < 0.001) and environmental dissimilarity (r = -0.45, p < 0.001), but only weakly with phenotypic dissimilarity (r = -0.07, p = 0.060) (Fig. 4e-g). Thus, genomic parallelism increases in populations that are more genetically or environmentally similar, but not phenotypically similar. Associations between environmental dissimilarity and F_{ST} parallelism were still strongly negative (r = -0.41, p < 0.001), after correcting for genetic similarity in partial Mantel tests, suggesting correlations between local environment and local ancestry do not drive this effect. Phenotypic dissimilarity remained un-associated with F_{ST} parallelism after controlling for genetic similarity (r = -0.026, p = 0.341), suggesting environmental similarity is a better predictor of genomic parallelism than phenotypic similarity, at least in terms of our measured environmental variables and the observable morphological phenotypes in this

system. The genotype-phenotype map may be simpler than the equivalent genotype-environment map, potentially leading to over-correction when including genetic distance matrices, but this would not explain why phenotypic distance associations were weaker prior to correction.

Early studies of marine and freshwater stickleback ([19,29,47]) were some of the first to demonstrate the repeatability of the genetic basis of adaptation in natural populations, and that it might be pervasive. These results drove researchers to examine other systems for genomic parallelism, such as cichlids ([67,73]), periwinkles ([74]), stick insects ([15]) and *Arabidopsis* ([75]). These diverse systems have highlighted that genomic parallelism is highly variable, and more recent studies of stickleback from outside the original Eastern Pacific populations have similarly shown variability within the system itself ([69]).The field has since matured to question how and why this variability persists, and the stickleback system remains at the forefront of this research. Studies on 16 lake-stream stickleback pairs from British Columbia demonstrated that continuous phenotypic and environmental variation predicts genomic parallelism (F_{ST} regions) ([20]) and suggested that ecotype genomic parallelism may be stronger for certain phenotypic traits than others ([76]). These findings are recapitulated and extended here, to demonstrate that similarity of specific environmental and phenotypic variables is a good predictor for signatures of genomic parallelism. Our results also confirm these results extend beyond only BC. Further, our data provides additional statistical power (73 marine-freshwater comparisons vs 16 lake-stream, albeit with reduced independence) to elucidate that environmental similarity is a better predictor of genomic parallelism than phenotypic parallelism.

A major question of interest concerns the geographic scale to which patterns of genomic parallelism extend. Whilst we found marine-freshwater genomic parallelism to be

480 constrained across all four radiations, we do observe genomic parallelism at a continental
481 scale, and within freshwater populations founded from both Eastern Pacific and Atlantic
482 marine populations. Agreeing with our results, a comparison of 'regional' lake-stream
483 parallelism in BC to 'global' lake-stream parallelism in a collection of lake-stream pairs from
484 Europe and North America (one pair per region) highlighted a global constraint on
485 parallelism at the genetic level and for some phenotypes ([40]). However, comparable
486 watersheds of multiple lake-stream ecotype pairs are less common beyond BC ([40, but see 77,78]),
487 restricting global comparisons of lake-stream adaptive radiations such as those presented
488 here for marine-freshwater.

489 A recent comparison of levels of genomic parallelism in North American (Pacific-
490 founded) and European populations suggested that a founding event of Atlantic marine
491 populations limited standing freshwater variation, leading to lower parallelism in European
492 populations ([42]). Consistent with the idea that even minor differences in selection may limit
493 genomic parallelism of standing genetic variation ([38]), this study also speculated that
494 differences in selection homogeneity between North American and European environments
495 could explain variable genomic parallelism. The data to test this hypothesis however has
496 been hitherto lacking. Our results on segregating variants and molecular variance confirm
497 distinct North American and European clusters of standing genetic variation, which are
498 consistent with a founding bottleneck, or older divergence time between inter-continental
499 radiations compared to within each ocean. However, genetic variation within North
500 American and European radiations was broadly comparable, suggesting similar potential
501 within Europe to produce freshwater parallelism as in North America. This result, combined
502 with our stronger observed environmental homogeneity in North America vs Europe, and
503 strong associations between environmental distance and genome-wide parallelism (Fig.

4b,e), suggests environmental homogeneity is a valid explanation for some of the differences in parallelism observed between the two continents. Overall, our study thus highlights that variable levels of genomic parallelism observed within marine-freshwater stickleback comparisons at the global scale are likely the result of an interplay between environmental heterogeneity (but not physical distance) at continental scales, and a history of founding bottlenecks and segregating genetic variation among founding populations at a global scale. Further, genomic parallelism of specific phenotypes is predictable on the basis of common phenotypic trajectories, likely underwritten by simple vs complex genetic architectures.

Methods

Sampling and environmental data collection.

We sampled 18 (19 in Alaska) freshwater lakes and one marine coastal population in North Uist, Scotland (April and June 2013), Iceland (May and June 2014), British Columbia (BC) (April and May 2015) and the Cook Inlet basin, Alaska (June 2015). Lake names, geographic coordinates and numbers of samples used in the study are shown in Supplementary Table 1. Each adaptive radiation analysed comprises a variety of different adaptive forms that most likely evolved from closely related ancestral marine lineages in each region. We measured a set of seven biotic and abiotic environmental variables and a set of 12 phenotypic traits (measures of body shape, armour traits and gill rakers). We measured the pH, concentrations of metallic cation concentrations sodium ("Na"), calcium ("Ca") and zinc ("Zn") of each lake, lake area, and calculated population prevalence of *Gyrodactylus spp.* and *Schistochephalus solidus*. Concentrations of cations, pH, lake area and parasite prevalence per lake are shown in Supplementary Table 2. The environmental variables included in our analyses were selected on the basis of presumed fitness effects and that could be precisely measured (Supplementary Table 2). For abiotic environmental variables we chose pH, ionic concentrations of calcium (Ca), sodium (Na) and zinc (Zn), and lake area (Area) which have been associated previously with the evolution of body shape, size and armour in stickleback ([27,33,79,80]). Many biotic variables are difficult to quantify precisely so we used the prevalence of two parasites *Gyrodactylus* sp. (ectoparasitic trematodes) (Gyro) and *Schistocephalus solidus* (endoparasitic cestode) (Schisto) that are likely to have an impact on the reproduction and life cycle of stickleback ([81–83]). It is important to note that measured variables might actually be proxies for other, unmeasured variables and not the primary causes of selection. Details of the fish collection and quantification of abiotic and abiotic variables can be found in Supplementary Methods. As

marine-freshwater parallelism is well-documented ([19,29,84]), we compared our results for parallelism across freshwater radiations with well-studied marine-freshwater parallelism in this species, and used the results as a positive control for the methods used (Supplementary Information 2). Some marine-freshwater associated regions detected by our use of Bayenv2 could be the result of differences in allele frequencies between only a few freshwater populations and marine populations (within-freshwater variation rather than explicit marine-freshwater divergence). Such false-positives are however unlikely as a combination of high Bayes factor and high Spearman's ρ requires many freshwater populations to display consistent allele frequency changes relative to marine populations. Some variables that vary among freshwater populations may not vary consistently between marine and freshwater, such that our parallel MxF regions are not expected to be a sum of our single variable, parallel, freshwater regions.

DNA extractions, RAD library preparation and sequencing.

Genomic DNA was purified from 10 to 21 individuals from each of the populations, chosen to represent a widely distributed subset of the most environmentally and phenotypically variable lakes (Supplementary Dataset 3). Extracted genomic DNA was normalized to a concentration of 25 ng /μL in 96-well plates.

In 2014 we conducted RAD sequencing on samples from Scotland and from Iceland. Sequencing libraries were prepared and processed into RAD following the modified libraries according to ([85]). In 2016 we conducted RAD sequencing on samples from British Columbia and from Alaska. Sequencing libraries were prepared following the modified single-digest RAD protocol of ([86]). The two RADseq protocols interrogate the same set of loci across the genome, so that the SNP data are compatible across all four radiations. See Supplementary Methods for details of the RAD library preparation and sequencing.

Population genetics statistics and phylogenetic tree.

Raw sequence reads were demultiplexed using Stacks – 1.35 ([87]). Number of reads per individual are shown in Supplementary Dataset 3 (see Supplementary Methods for details on the alignment of reads and Stacks pipeline used). For Bayenv2 and association analyses, autosomal SNPs were called using the Populations within Stacks as being present in >7 populations (-p 8), >50% of the individuals within a population (-r 0.5), and with a MAF-filter of 0.05 (--min-maf 0.05). After filtering we retained 26,169, 29,111, 26,937, 26,990 SNPs for Alaska, BC, Iceland, and Scotland, Iceland, British Columbia and respectively.

For analyses of population structure and phylogenetics across all radiations, a subset of unlinked SNPs were generated. Here, autosomal SNPs were called that were present in all radiations (-p 4) and in >50% of individuals within a radiation (-r 0.5), with a MAF-filter of 0.05 (within a radiation) (--min-maf 0.05). Only the first SNP per RAD locus was retained (--write-single-snp). F_{ST} was bootstrapped and calculated in POPULATIONS. This set of SNPs were then pruned for linkage disequilibrium in plink using indep-pairwise 50 5 0.2. We also produced an additional linkage-pruned dataset with marine populations (-p 5) with 11,266 SNPs. The unlinked SNP dataset with marine fish was used to construct a neighbour-joining tree for all fish in the R package 'ape', using a distance matrix (*bionj*) computed from the

SNP data ([88]). The tree was bootstrapped 100 times and nodes with less than 80% support were collapsed. PCA analysis of population structure was conducted using plink ([89]).

Phenotypic and environmental variation - body shape, armour, gill rakers and environmental data analyses.

All morphological measurements (body shape, body armour and gill raker traits) were done following ([27]). Details of the quantification of phenotypic traits can be found in Supplementary Methods.

We performed three Principal Component Analyses (PCAs): one on the armour traits, another on body shape, and another on the 6 environmental variables. Body shape and armour PCAs were performed on regression residuals of all individuals from all radiations pooled together to extract the common PCs of body shape, armour, and environmental variation, and retained axes that explained more than 10% of the total variance. Armour and environmental PCAs were conducted with scaled inputs due to different units of measurement between variables. Shape PCAs were conducted on morphometric residuals, and as such were not scaled. All phenotypic analyses, including ANOVAs and ANCOVAs and plotting were done in R version 3.4.3.

For ANCOVAs, we compared each phenotype (PC1 and PC2 for shape and armour, actual values for gill raker N and L) with each of our seven environmental variables. We explored five possible GLMs: 1) A null model; 2) phenotype varies by environment (linear slope); 3) phenotype varies by radiation (intercepts vary); 4) phenotype varies by environment and intercept varies by radiation (parallel slope, intercepts vary); 5) phenotype varies by environment in a radiation-specific manner (nonparallel slopes). The best model was chosen by backwards model selection using AIC, with simpler models (model 1 simplest) preferred if the change in AIC > -2. We then calculated F-statistics for the resulting GLM. For variable slopes, we used post-hoc Tukey tests to compare radiations.

Genotype-Environment/Phenotype Associations.

For each radiation separately (N=18 to 19 populations) we used Bayenv2 ([46]) to identify associations between genomic allele frequencies (N=10 to 21 individual fish, mean = 17. 8), the set of seven biotic and abiotic environmental variables (Ca, Na, pH, Zn, lake area, prevalence of *Gyrodactylus spp.* and *Schistochephalus solidus*) and the set of 12 phenotypic traits ($Shape_{PC1}$, $Shape_{PC2}$, $Shape_{PC3}$, DS1, DS2, PS, LP, HP, BAP, Plate N, Gill Raker L and Gill Raker N) mentioned above. For each radiation, a matrix of genetic covariance was calculated using a subset of SNPs limited to a single SNP per RAD-locus and pruned for linkage disequilibrium ($R^2 < 0.4$) in plink ([89]). This cut-off was selected to balance the trade-off between SNPs retained and minimising the effects of linkage. Covariance matrices were therefore calculated using 9619, 7983, 7300 and 5705 SNPs for Alaska, BC, Iceland and Scotland respectively. Covariance matrices were calculated across 100,000 iterations and averaged across 5 independent runs. Bayenv2 was run independently 8 times and final results were averaged across runs. The purpose of the covariance matrix is to rule out spurious associations between drifting allele frequencies associated with population structure and environmental/phenotypic variation. Some of these correlations did exist in our data, but poorly explain signals of genomic parallelism (Supplementary Fig. 7).

Environmentally and phenotypically associated SNPs were selected as having a $\log_{10}$-BayesFactor > 1.5 and an absolute Spearman's rank coefficient above the 95th percentile.

The combination of BayesFactor and non-parametric measure of correlation helps to avoid selecting SNPs with high BayesFactors due to single or few outlier populations with extreme allele frequencies and environmental/phenotypic variation ([90]). SNPs were grouped into 50kb, 75kb, 100kb, 200kb and 0.1 cM windows (Supplementary Table 14) to test the robustness of our results across different extents of linkage. Our 50kb dataset was composed of 4,868 windows with SNPs in all radiations, covering approximately 55% of the 447 Mb genome, with a further 1,940 windows sequenced in 2 or more radiations providing information on an additional 21.7% of the genome. To evaluate whether windows were environmentally or phenotypically associated, we adapted the methodology of ([91]). We calculated the upper 99% binomial expectation for the number of associated SNPs given the total number of SNPs in a specific window, and selected windows that had a greater number of associated SNPs than this expectation. We focused on repeated changes within the same genomic regions, rather than on reuse of the same mutations. This is because the causal mutations are unknown in most cases and may not be sequenced by reduced representation sequencing methods such as RAD-sequencing. This method also controls for variation in SNP density across windows and ensures that significant windows exhibit consistent allele frequency correlations across multiple SNPs. We visualised the genomic locations of associated windows using Manhattan plots (Supplementary Fig. 6) and plotted the residual number of outlier SNPs above the binomial expectation (Supplementary Fig. 8). Linkage groups I-XXI were visualised with the exception of XIX; windows on scaffolds were not visualised. Finally, we compared these associated windows across radiations to examine those that were parallel.

As a positive control for the methods used we compared our results for parallelism across freshwater radiations with well-studied marine-freshwater parallelism in this species ([19,29]), and then examined genomic differentiation between all freshwater populations pooled within a radiation and four marine populations pooled together (one from each geographic region, Supplementary Table 1, Supplementary Information 2).

Parallelism statistics

We use the term genomic parallelism when referring to repeated changes within the same genomic regions, rather than the strict definition of genomic parallelism that refers to reuse of the same mutations. The use of 'parallelism' terminology is highly variable within the literature ([12]), but our usage is consistent with stickleback literature ([19,47,77]) and reflects parallelism of phenotypes. For all radiation groupings (11 combinations in total: one four radiation grouping, four three radiation groupings, and six two radiation groupings), we calculated the significance of parallel window counts using a permutation method. For each environmental or phenotypic variable, we randomly drew N windows from each radiation's total pool where N was equivalent to the associated window count for each radiation. We then assessed the overlap of randomly associated windows across radiations and pooled the results over 10,000 iterations. The output from all permutations was used as a null distribution to infer p-values, which were then FDR-corrected using the R package *qvalue* ([92]).

Grouping of adjacent windows and expanding parallelism regions.

Windows of 50kb and above were based on a linkage assumption and to minimise non-independence between windows. There were, however, occasionally adjacent windows associated with the same variable across different groupings. Large regions of relatively

strong linkage are plausible if recombination is reduced through processes such as genomic rearrangements. To investigate these, we grouped associated windows that were adjacent as well as those that were direct matches across radiations based on the likelihood of adjacent associated windows resulting independently being low, suggesting non-independence and probable linkage. We repeated the above permutations assuming adjacent windows to be single associated regions. These windows are available in Supplementary Table 13.

Multivariate vector comparison of environments and phenotypes.

Using the average trait values from marine and freshwater populations, we calculated the vectors of phenotypic change for armour, shape, and gill raker variables (3 vectors per population) separately between each freshwater population and the marine population from the same radiation. We then calculated the angles (θ) between all vectors from the same radiation (one distribution of θ values per radiation), and the angles between vectors from different radiations (one distribution of θ values per between-radiation comparison (N=6): for example, one distribution of θ values comprising of angles between each vector of Iceland vs each vector of Scotland). We then compared radiations pairwise, asking whether the distribution of angles between vectors from different radiations differed significantly from the distribution of angles calculated within a radiation (for example, comparing whether the distribution of θ values between Scottish vectors was significantly different from the distribution of θ values between Scottish and Icelandic vectors). We performed this analysis for all pairwise comparisons, comparing the "between-radiation" distribution to both "within-radiation" distributions separately (Fig. 2c, d). The analysis was performed separately for armour, shape, and gill raker variables. We lacked data for Scottish marine gill rakers, so these comparisons were not possible.

Comparing relative influences of environment, phenotype and genetics.

F_{ST} was calculated between each freshwater population and its relevant marine population (Alaska = MUD1, BC = LICA, Iceland = NYPS, Scotland = OBSM) in 50kb windows using the R package 'PopGenome' ([93]). For each MxF comparison, windows above the 95% quantile were classed as outliers. Outlier windows were compared across all pairwise freshwater comparisons (2,628 comparisons among 73 populations), with overlapping outliers representing MxF F_{ST} parallelism. Dissimilarity matrices of environment and phenotype were calculated as Euclidean distance in PCA space for the seven and 12 environmental and phenotypic variables respectively. The genetic dissimilarity matrix was composed of genome-wide pairwise F_{ST} estimates between freshwater populations. The matrix of MxF parallelism was associated to environmental, phenotypic and genetic dissimilarity matrices using Mantel tests (Spearman's) with 9,999 permutations. Partial Mantel tests were performed with genetic distance as the conditional matrix for environmental and phenotypic effects on MxF parallelism, again with 9,999 permutations.

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Authors Contributions

I.S.M, A.D.C.M and J.R.W. conceived the project, interpreted the data, and wrote the manuscript. I.S.M, D.D., and A.D.C.M performed field work. I.S.M, M.M. and D.D. generated the phenotypic data. I.S.M. and P.A.H. generated RAD data and J.R.W., I.S.M. and P.A.H. analysed it. P.A.H., M.A.B. and S.S. helped with the sampling and revised the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Ethical compliance

Ethical approval for sampling in the UK was under Home Office licence 40/3486, in British Columbia under Professor Dolph Schluter's UBC animal care certificate A11-0402, and in Alaska under University of Alaska Anchorage IACUC protocol 739596-1. No ethical approval was required in Iceland.

Data Accessibility

Bam files of aligned reads for each individual and corresponding sample information have been deposited in the European Nucleotide Archive database under the project PRJEB20851, with the sample accession numbers ERS1831811-ERS1833111, and run accession numbers ERR2055459-ERR2056759. Scripts used for all analyses are archived through Github/Zenodo (DOI:10.5281/zenodo.4024117).

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Figure Captions

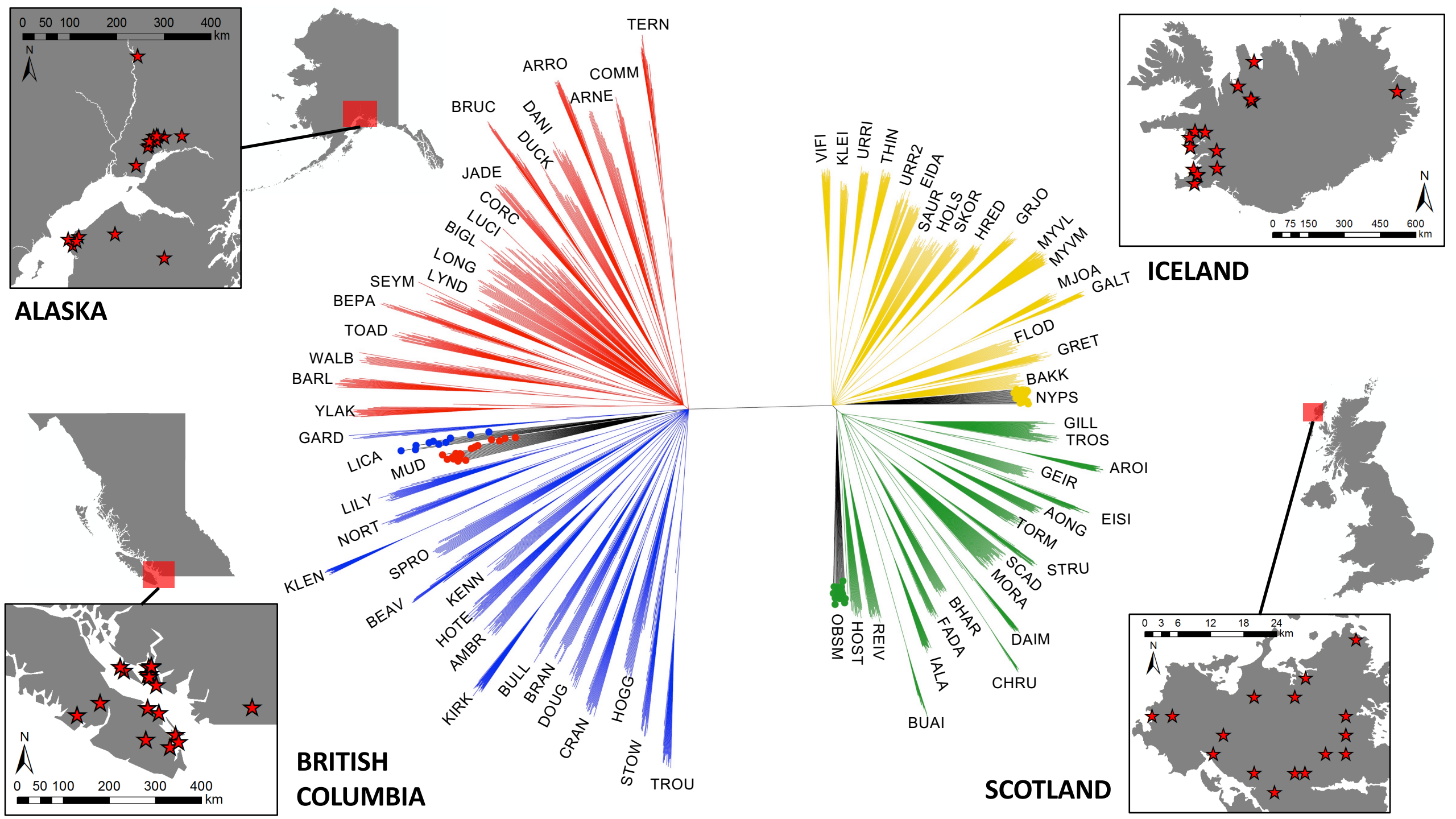
Fig. 1. Sampling sites and bootstrapped unrooted NJ tree for stickleback from 73 freshwater lake populations and 4 marine ones from four countries on two continents, based on 11,266 genetic markers for 1,380 individuals. All nodes shown have bootstrap support of at least 80 (other nodes were collapsed). Freshwater branches are coloured by the radiation to which they belong. Marine branches are black with the tips coloured according to radiation. Tips represent individual fish, which were generally tightly clustered by population (small labels). Stars represent lakes sampled.

Fig. 2. Comparisons and analyses of environmental and phenotypic parallelism across adaptive radiations: (**a**) Principal Component Analyses of environmental variables (Environment); regression residuals of Procrustes coordinates against log centroid of body shape (Shape); armour traits; and trophic traits gill raker numbers and length. Each point represents a population and ellipses are 95% confidence ellipses. Names of variables with the highest positive (+) or negative (-) loadings in subtitle. Marine populations (+) are projected where data was available using PC loadings calculated with freshwater populations only; (**b**) Density distributions of populations along each PC axis grouped by adaptive radiation; (**c**) Angles between marine-freshwater vectors for armour, shape, and gill raker traits. For each category of traits, density distributions are coloured according to whether vectors were compared within, or between different radiations; (**d**) Heatmaps of comparisons of vectors between specific radiation pairings coloured inversely according to average angle. Each panel includes the p-value denoting whether between-radiation vectors differ significantly from within-radiation vectors (defined as radiation on y-axis). If between-radiation vectors do not differ significantly from within-radiation vectors, a common trajectory of marine-freshwater phenotypes is assumed.

Fig. 3. Expected and observed counts of 50kb windows containing an above 99% binomial expectation number of SNPs associated with marine x freshwater (MxF), environmental variables and phenotypic traits in at least 2 radiations. Expected bars (grey) represent mean counts across 10,000 simulated outcomes with 95% confidence intervals per a one-tailed hypothesis. Asterisks denote significance of FDR-corrected one-tailed tests between the observed counts and the 100,000 simulated counts at the <0.05 (*), <0.01 (**) and <0.001 (***) levels.

Fig. 4. Associations between genome-wide marine - freshwater F_{ST} and environmental, phenotypic and genetic distance across all pairwise comparisons of 73 freshwater populations: **a)** Proportion of MxF F_{ST} 50kb outlier windows that overlap among freshwater replicates. Freshwater populations are ordered as Alaska, British Columbia, Iceland and Scotland, with these location distinguishable as four clear clusters; **b)** Environmental distances between freshwater populations, recorded as Euclidean distances in PCA space for all 7 environmental variables; **c)** Phenotypic (Euclidean) distances between freshwater populations for the 12 phenotypic variables; **d)** Genetic distances between freshwater

998 populations, recorded as genome-wide pairwise F_{ST} based on 8,395 unlinked SNPs; **e-g)**
999 Associations between environmental (**e**), phenotypic (**f**) and genetic (**g**) distances and MxF
1000 F_{ST} overlap (log-transformed). Points are coloured according to whether pairwise
1001 comparison is being made within a radiation or across radiations.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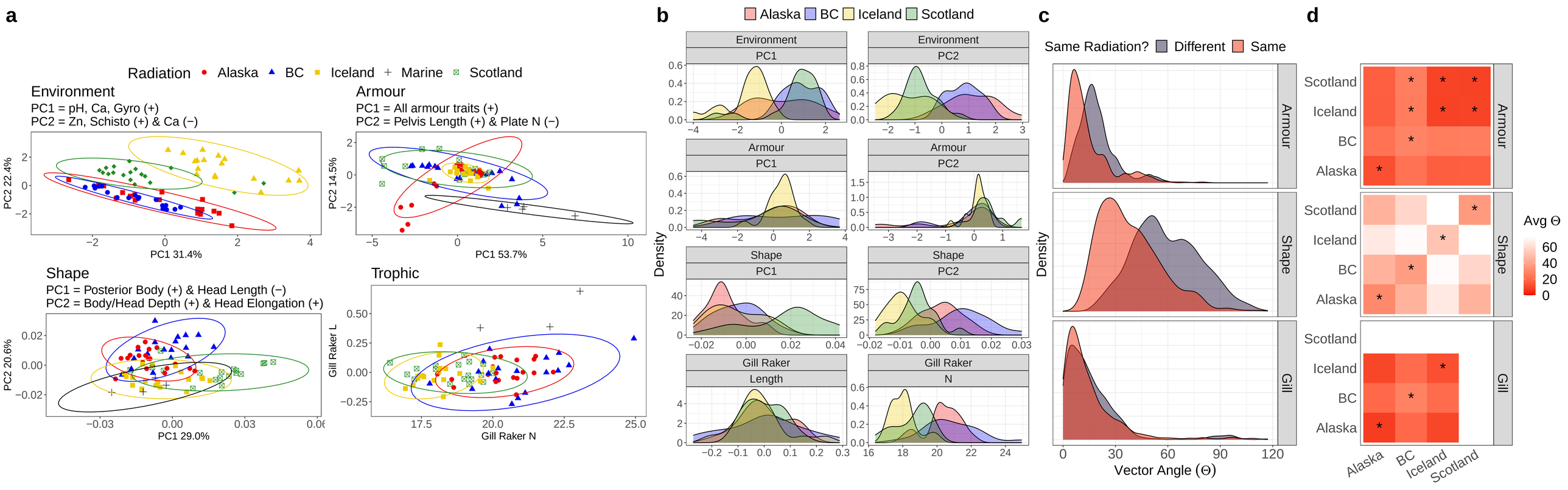
a
Radiation Alaska BC Iceland Marine Scotland
Environment
PC1 = pH, Ca, Gyro (+)
PC2 = Zn, Schisto (+) & Ca (−)
PC2 22.4%
PC1 31.4%
Armour
PC1 = All armour traits (+)
PC2 = Pelvis Length (+) & Plate N (−)
PC2 14.5%
PC1 53.7%
Shape
PC1 = Posterior Body (+) & Head Length (−)
PC2 = Body/Head Depth (+) & Head Elongation (+)
PC2 20.6%
PC1 29.0%
Trophic
Gill Raker L
Gill Raker N
b
Alaska BC Iceland Scotland
Environment PC1
Environment PC2
Armour PC1
Armour PC2
Shape PC1
Shape PC2
Gill Raker Length
Gill Raker N
Density
c
Same Radiation? Different Same
Armour
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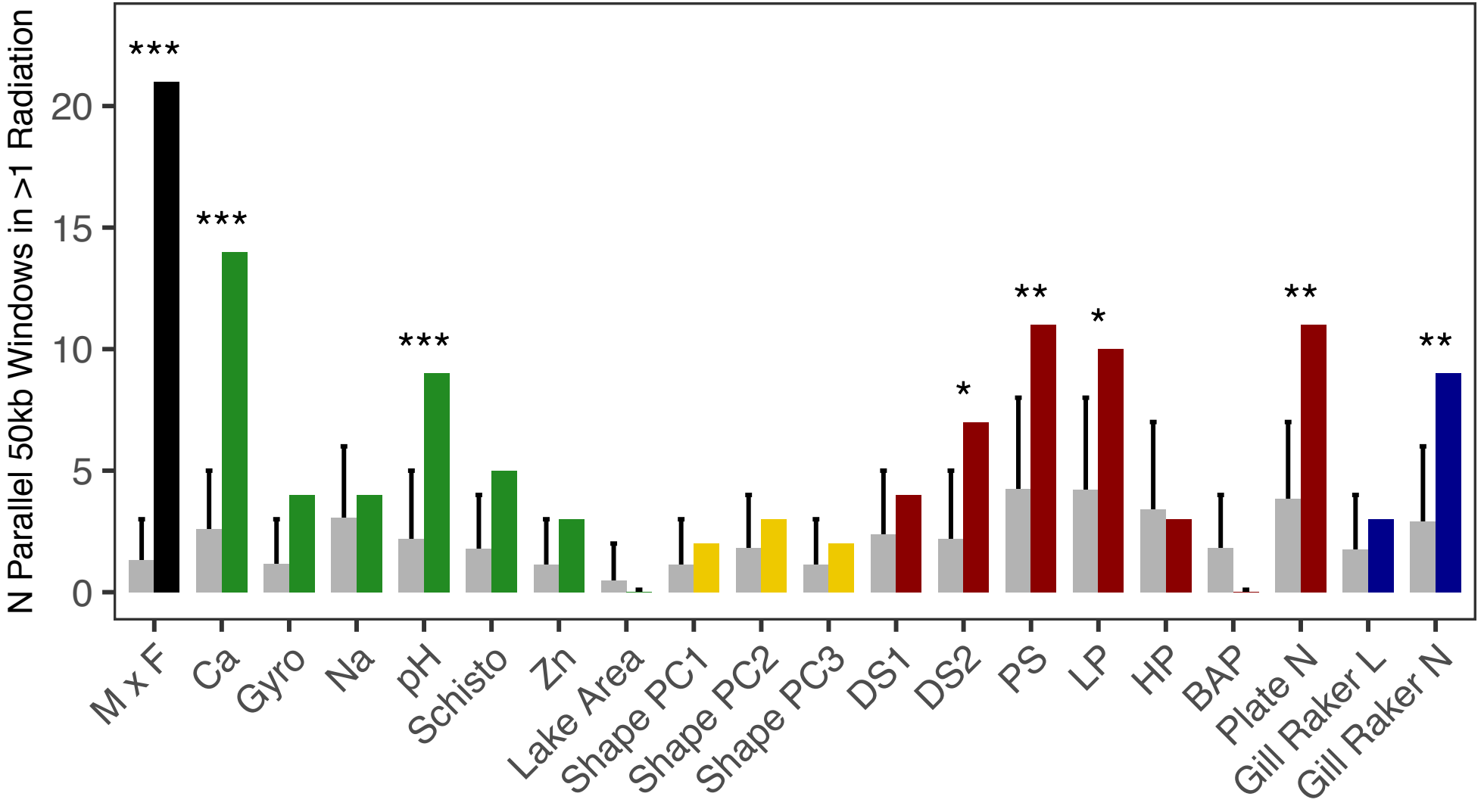
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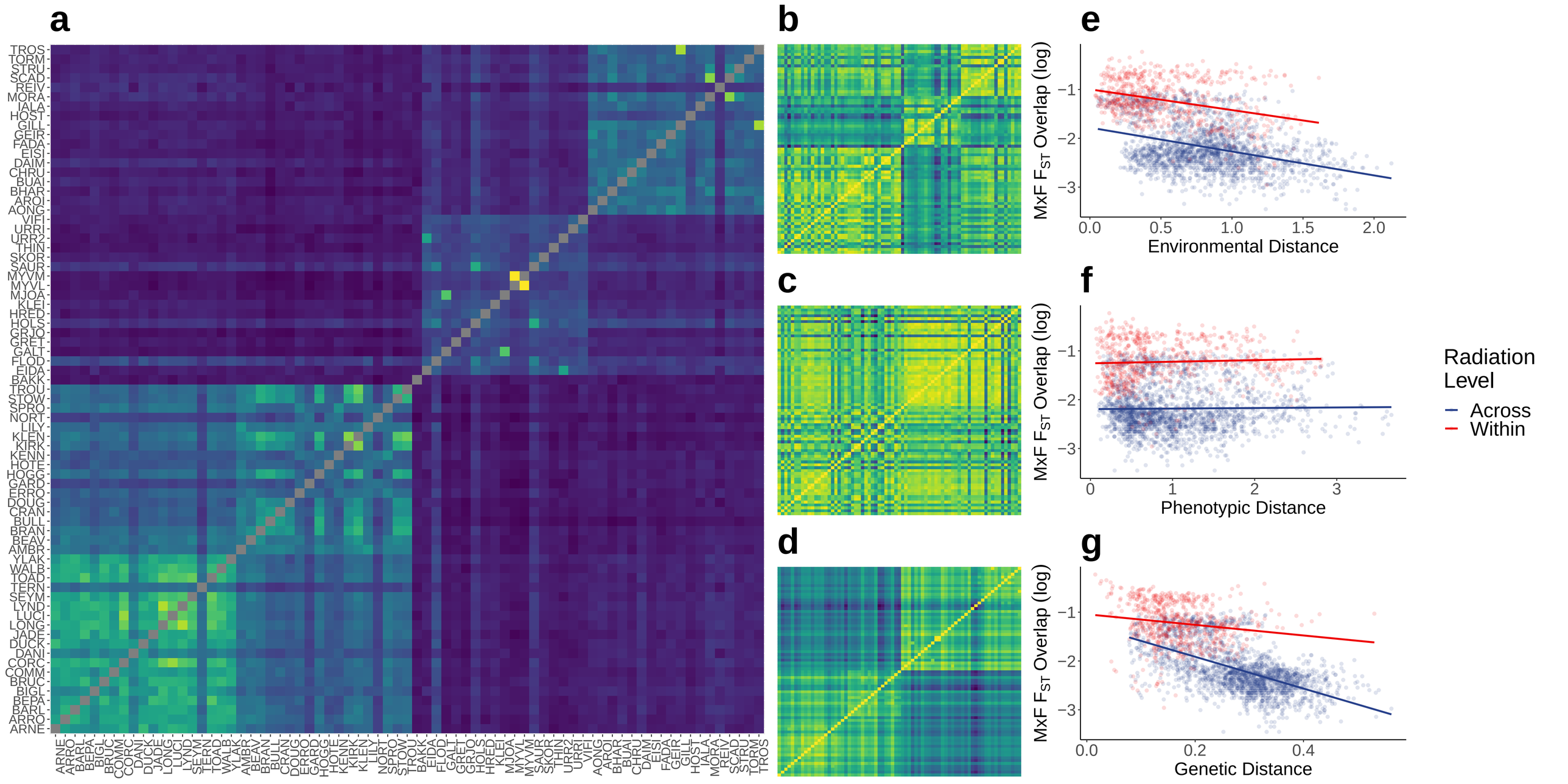
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LUCI
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JADE
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BARL
ARRO
ARNE
MxF F_{ST} Overlap (log)
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1.0
1.5
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Environmental Distance
Phenotypic Distance
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Genetic Distance
0.2
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Radiation Level
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