

1 **Intercontinental genomic parallelism in multiple adaptive radiations**

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3 Isabel S. Magalhaes\*<sup>1,2</sup>, James R. Whiting\*<sup>1,3</sup>, Daniele D'Agostino<sup>1</sup>, Paul A. Hohenlohe<sup>4</sup>,  
4 Muayad Mahmud<sup>1</sup>, Michael A. Bell<sup>5</sup>, Skúli Skúlason<sup>6</sup> and Andrew D.C. MacColl<sup>1</sup>

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6 <sup>1</sup> *School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD,*  
7 *U.K.*

8 <sup>2</sup> *Department of Life Sciences, Whitelands College, University of Roehampton, London SW15*  
9 *4JD, U.K.*

10 <sup>3</sup> *Biosciences, College of Life and Environmental Sciences, Geoffrey Pope, University of Exeter,*  
11 *Stocker Road, EX4 4QD, U.K.*

12 <sup>4</sup> *Institute for Bioinformatics and Evolutionary Studies, Department of Biological Sciences,*  
13 *University of Idaho, Moscow, ID 83844, USA.*

14 <sup>5</sup> *Department of Ecology & Evolution, Stony Brook University, 650 Life Sciences Building,*  
15 *Stony Brook, NY 11794-5245*

16 <sup>6</sup> *Department of Aquaculture and Fish Biology, Hólar University College, Saudárkrókur,*  
17 *Iceland*

18 \*These authors contributed equally to this work

19

20 **Abstract**

21 Parallelism, the evolution of similar traits in populations diversifying in similar conditions,  
22 provides good evidence of adaptation by natural selection. Many studies of parallelism have  
23 focused on comparisons of strongly different ecotypes or sharply contrasting environments,  
24 defined *a priori*, which could upwardly bias the apparent prevalence of parallelism. Here, we  
25 estimated genomic parallelism associated with individual components of environmental and  
26 phenotypic variation at an intercontinental scale across four adaptive radiations of the  
27 three-spined stickleback (*Gasterosteus aculeatus*), by associating genome-wide allele  
28 frequencies with continuous distributions of environmental and phenotypic variation. We  
29 found that genomic parallelism was well predicted by parallelism of phenotype-  
30 environment associations, suggesting that a quantitative characterization of phenotypes  
31 and environments can provide a good prediction of expected genomic parallelism. Further,  
32 we examined the explanatory power of genetic, phenotypic, and environmental similarity in  
33 predicting parallelism. We found that parallelism tended to be greater for geographically

34 proximate, genetically similar radiations, highlighting the significant contingency of standing  
35 variation in the early stages of adaptive radiations, before new mutations accumulate.  
36 However, we also demonstrate that distance within multivariate environmental space  
37 predicts parallelism, after correction for genetic distance. This study thus demonstrates the  
38 relative influences of environment, phenotype and genetic contingency on repeatable  
39 signatures of adaptation in the genome.

40

## 41 **Introduction**

42 Adaptive radiations are well known as rapid branchings on the tree of life <sup>(1)</sup>. They may be  
43 the source of most biodiversity, and their study has revealed a great deal about the  
44 evolution of phenotypic diversity <sup>(1,2)</sup>. However, patterns in adaptive radiations also  
45 highlight some of the unknowns about how biodiversity evolves. For example, although  
46 adaptive radiations are typified by abundant phenotypic diversity, not all trait combinations  
47 evolve in every radiation, while on the other hand organisms in different places sometimes  
48 arrive at very similar endpoints <sup>(3,4)</sup>. This suggests that Stephen Jay Gould's famous  
49 contention that evolution is contingent and unrepeatable <sup>(5)</sup> cannot be completely true. A  
50 good deal of work has focussed on the role that genetic correlations between traits might  
51 play in creating constraints on diversity, but so far the answers provided by this approach  
52 have not been entirely satisfactory <sup>(3,6,7)</sup>. Alongside these processes, it is probable evolution  
53 can be shaped in a predictable way by common environments and shared selective regimes,  
54 and that the emergence of repeatable patterns of evolution, a process predominantly  
55 known as parallelism (which we distinguish from convergence here by the inclusion of  
56 shared evolutionary 'start', as well as 'end', points, although see <sup>(8,9)</sup>), is the direct result of  
57 environmental similarities within and between radiations. Striking examples of phenotypic

58 parallelism (<sup>1,10</sup>) in the natural world support this hypothesis, and the persistent appearance  
59 of familiar forms in similar ecological niches demonstrates the significance of selection in  
60 this process.

61         However, a significant problem with studies that have focused on phenotypic  
62 parallelism is that they have concentrated largely on the comparison of pairs of strongly  
63 different ecotypes or widely different environments (<sup>11-13</sup>). Such studies could upwardly bias  
64 the apparent prevalence of parallelism because the chosen comparisons were known *a*  
65 *priori* to occur repeatedly in different locations, effectively constraining the evolutionary  
66 end point. The approach also conceals the role of individual components of environmental  
67 variation in driving parallelism, as similar environments are often assumed based on  
68 comparable phenotypes. This gap surely needs addressing for a complete understanding of  
69 adaptation (<sup>14</sup>). In addition, traits that are measurable in sufficient numbers from (usually)  
70 wild organisms are generally limited to morphological and life history traits (<sup>13</sup>). This  
71 seriously compromises our ability to understand adaptation, a great deal of which is likely to  
72 be physiological in the broad sense. Such drawbacks highlight the importance of combining  
73 measures of phenotype and environment alongside genomics in studies of parallelism. The  
74 detection of consistent genomic signatures across multiple, independent natural  
75 populations has proven to be a valuable tool for studies of evolutionary patterns and the  
76 discovery of genes involved in adaptation (<sup>15-17</sup>). However, yet again our comprehension of  
77 the relationship between genomic parallelism and continuous phenotypic or environmental  
78 variation is surprisingly poor.

79         Until recently, a major barrier to combining genotype, environment and phenotype  
80 has been the high costs of sequencing, prohibiting large-scale genomic sampling that could  
81 be aligned with large-scale ecological sampling. However with dramatic drops in DNA

82 sequencing costs, such genomic data can nowadays be used alongside ecological data to  
83 associate allele frequencies (<sup>18</sup>), and determine genomic regions associated with individual  
84 components of environment and phenotypes. Such an approach is essential if studies of  
85 parallelism are to shift from description to hypothesis testing, but has rarely been applied  
86 (but see <sup>12,19,20</sup>), and it remains to be shown whether signals of parallelism obtained from  
87 continuous measures are comparable to those from ecotypes and from previous studies.  
88 Here we make use of such methods to test for environmentally and phenotypically  
89 associated genomic parallelism across radiations of three-spined stickleback fish  
90 (*Gasterosteus aculeatus*, hereafter 'stickleback').

91 Stickleback provide a powerful natural experiment to test parallelism. They are  
92 primitively marine but have undergone replicated adaptive radiations across the northern  
93 hemisphere following colonisation of freshwater in widely separated geographical locations.  
94 This allows us to compare multiple populations derived from the marine ancestor, and  
95 provides a model for exploring both phenotypic parallelism and its genetic basis (<sup>21,22</sup>) in  
96 response to environmental variation. Phenotypic parallelism in populations that have  
97 evolved independently in similar habitats is well established (<sup>23,24</sup>), and whilst often  
98 considered in dichotomous pairings of marine – freshwater, benthic – limnetic, lake -stream  
99 ecotypes, there is a huge amount of continuous phenotypic variation among freshwater  
100 populations that has hitherto rarely been explored (<sup>25</sup>) in this context. In addition, genome  
101 scans have identified loci that have come repeatedly under selection across the contrasting  
102 ecotype pairs (<sup>17,26,27</sup>), but the combination of the phenotypic and genomic parallelism in  
103 one study is rare (although see <sup>28</sup>) and has not previously been done at the scale of  
104 replicated adaptive radiations across continents.

105 For this study we sampled 73 freshwater lake populations from four adaptive  
106 radiations in Alaska, British Columbia ('BC'), Iceland and the island of North Uist ('Scotland')  
107 (Fig. 1, Supplementary Table 1), measured a set of six biotic and abiotic environmental  
108 variables and a set of 12 phenotypic traits (measures of body shape, armour traits and gill  
109 rakers) (Supplementary Table 2), and performed a genome-wide scan of a total of 1,304  
110 individual stickleback using restriction-site associated DNA (RAD) sequencing. We first  
111 assessed environmental and phenotypic parallelism among radiations, and their  
112 phylogenetic relationship, which provided a reference for how much genomic parallelism to  
113 expect. We then scanned the genome for associations with continuous environmental and  
114 phenotypic axes of variation within radiations, and identified genomic parallelism by looking  
115 at the presence of allele frequency associations in the same genomic regions across  
116 radiations. As marine-freshwater parallelism is well-documented (<sup>17,27,29</sup>), we compared our  
117 results for parallelism across freshwater radiations with well-studied marine-freshwater  
118 parallelism in this species, and used the results as a positive control for the methods used.  
119 Finally, we examined how the prevalence of parallel genomic regions is associated with the  
120 phylogenetic histories of our adaptive radiations and how well it can be explained by  
121 multivariate quantification of environmental and phenotypic similarity between radiations.  
122 Rewards to be gained by connecting the evolution of parallelism more explicitly to the  
123 environmental and phenotypic variation include a better grasp of why some traits evolve in  
124 concert and a predictive understanding of parallelism and repeatability (<sup>4</sup>). This new  
125 understanding is essential if we are to reach a consensus on how biodiversity is altered by  
126 adaptation.

127

128 **Environmental and phenotypic similarity across radiations**

129 Interpreting inter-radiation patterns of genomic parallelism associated with phenotypic  
130 traits and environmental variables requires that we first assess the environmental and  
131 phenotypic similarity across all four radiations analysed here, as well as the relationship  
132 between the two types of variables. Environmental similarity is predicted to influence both  
133 phenotypic and genomic parallelism, as similar selection regimes are imposed on organisms  
134 (<sup>13,30,31</sup>). These analyses provide insights into some major factors that are likely to influence  
135 the emergence of a common pattern of genomic divergence at such a large geographical  
136 scale, and indicate how much genomic parallelism associated with environments and  
137 phenotypes to expect.

138       *l) Environment.* Adaptation of stickleback to freshwater in these radiations has  
139 happened rapidly, over the course of the past few thousand years, and has most likely been  
140 driven by strong selection favouring pre-existing alleles present at low frequencies in the  
141 ancestral marine population (<sup>32</sup>). Therefore, we might expect to observe shared features of  
142 diversification when multiple groups of organisms experience similar environmental  
143 circumstances, as similar selection regimes are imposed on organisms (<sup>12,33</sup>). A Principal  
144 Component Analysis (PCA) on six measures of water chemistry (pH, calcium (Ca), sodium  
145 (Na) and zinc (Zn), and parasitism (prevalence of the parasites *Gyrodactylus spp.* (Gyro) and  
146 *Schistocephalus solidus* (Schisto)) across all lakes revealed that the first axis of  
147 environmental variance (Env<sub>PC1</sub>) separated lakes along a gradient of pH and calcium  
148 concentration (Fig. 2; Supplementary Table 3). This axis did not separate radiations but  
149 emphasised the variation from alkaline to acid present in all of them, with the widest range  
150 in Alaska. The second axis of environmental variance (Env<sub>PC2</sub>) distinguished mostly between  
151 lakes with high and low zinc and largely separated the European from the North American

152 lakes. The two groups of European lakes overlapped very little environmentally with each  
153 other along the second axis or with the North American lakes, but the variation in BC lakes  
154 was completely subsumed within that of the more environmentally variable Alaskan lakes.

155 An analysis of the similarity of the direction of the major PC vectors ( $\theta$ ) of  
156 environmental variation (PCs that explained at least 10% of the variation) and of the  
157 magnitude of their variation revealed that Alaska and BC exhibited the strongest  
158 environmental similarity in terms of direction ( $\theta = 8.71^\circ$ ,  $p = 0.077$ ) (Supplementary Table 4),  
159 as lower values of  $\theta$  are indicative of more common environmental covariance. However,  
160 they also showed the greatest difference in magnitudes of variation, as BC had the lowest  
161 amount of environmental variation of all radiations while Alaska had the highest. Iceland on  
162 the other hand was the most unique radiation in terms of environmental covariance, a  
163 pattern likely driven by Iceland's uniquely volcanic nature.

164 *II) Phenotypes - Armour.* The major axis of armour variation ( $\text{Armour}_{\text{PC1}}$ ) represented  
165 a correlated axis of all traits, particularly dorsal spine length and pelvic characteristics (Fig.  
166 2; Supplementary Table 3). Although there were significant differences in  $\text{Armour}_{\text{PC1}}$  among  
167 both radiations and lakes (Supplementary Table 5), the direction of this vector was highly  
168 conserved across all radiations ( $4.13^\circ \leq \theta \leq 8.37^\circ$ ;  $0.004 \leq p \leq 0.053$ ) (Supplementary Table  
169 4). Nonetheless, we found populations with extreme armour, such as absence or extreme  
170 reduction of dorsal spines, pelvic spines, pelvis size, and generally low  $\text{Armour}_{\text{PC1}}$ , that occur  
171 in Scotland and Alaska but not elsewhere. However, while in Scotland pelvic armour  
172 reductions were accompanied by complete or almost complete loss of armour plates (high  
173  $\text{Armour}_{\text{PC2}}$ ), Alaskan populations retained those even when other features of armour were  
174 highly reduced. These deviations produce the somewhat anomalous relationship between  
175  $\text{Armour}_{\text{PC1}}$  and  $\text{Armour}_{\text{PC2}}$  in Alaska (Fig. 2). Iceland exhibited particularly low variation in

176 armour traits compared to other radiations, while BC had the highest. These results indicate  
177 that whilst the direction of armour variation occurred predominantly on a shared axis across  
178 adaptive radiations, the amount of armour variation along that axis was variable. When  
179 comparing all freshwater populations with four marine ones (one from each country), we  
180 found that, aside from a few populations from BC, there was no overlap in armour traits  
181 between marine and freshwater populations. This is because marine populations have a  
182 higher number of lateral plates and more exaggerated armour traits in general. Importantly  
183 however, projection of marine armour phenotypes suggests they fall on the same axis but  
184 beyond freshwater space (Fig. 2).

185 *III) Phenotypes -Body shape.* Despite some overlap in body shape across all radiations  
186 suggesting similar morphologies have evolved repeatedly across continents (Fig. 2), there  
187 were significant differences in body shape morphologies among both radiations and lakes  
188 (Supplementary Table 5). The most extreme body shapes were found in Scotland, where  
189 some populations have very elongated and slender bodies with small heads (Shape<sub>PC1</sub> axis),  
190 and in BC where some populations had the deepest bodies and heads and the longest heads  
191 (Shape<sub>PC2</sub> axis). Scotland had the largest amount of variation in body shape, which is a  
192 surprising result given this variation is found within 1000 km<sup>2</sup> of North Uist, a much smaller  
193 area than the other three radiations. The orientation of the major PC vectors ( $\theta$ ) revealed  
194 that North American radiations were the most similar ( $\theta = 3.49^\circ$ ,  $p=0.19$ ), followed by the  
195 European radiations ( $\theta = 8.48^\circ$ ,  $p=0.63$ ) (Supplementary Table 4), which highlights a  
196 potential effect of geography/shared ancestry on the major axis of shape variation. Marine  
197 populations overlapped in body shape with some of the freshwater populations that had  
198 lower ShapePC1 and ShapePC2 scores, most of them found in Iceland (Fig. 2).



199            *IV) Phenotypes - Trophic morphology.* The largest variation in gill raker numbers and  
200 length was found in Alaska, while BC populations contained a subset of that variation (Fig.  
201 2). Across the two dimensions of gill raker number and length, variation within radiations  
202 was generally constrained to a major axis in which populations with more gill rakers also  
203 tended to have longer gill rakers. All freshwater populations generally had shorter gill rakers  
204 for their size relative to marine populations.

205            *V) Relationship between environmental and phenotypic similarity.* We used GLMs to  
206 test for parallel associations between the first two environmental PCs ( $Env_{PC1}$  and  $Env_{PC2}$ )  
207 and armour, shape, and gill raker morphology across radiations. We found that  $Armour_{PC1}$   
208 and  $Armour_{PC2}$  were also significantly associated with  $Env_{PC1}$  ( $Armour_{PC1}$ :  $F=6.13$ ,  $p=0.016$ ;  
209  $Armour_{PC2}$ :  $F=4.11$ ,  $p=0.047$ ), but not with  $Env_{PC2}$  ( $Armour_{PC1}$ :  $F= 1.12$ ,  $p=0.293$ ;  $Armour_{PC2}$ :  
210  $F=1.10$ ,  $p=0.298$ ) (Supplementary Table 6).  $Armour_{PC1}$  correlations with  $Env_{PC1}$  were similar  
211 across radiations but significant slope variation was observed between radiations for  
212  $Armour_{PC2}$  and  $Env_{PC1}$  associations ( $F= 2.76$ ,  $p=0.050$ ). The result highlights parallelism in the  
213 way general skeletal traits ( $Armour_{PC1}$ ) are reduced with lower pH and calcium ( $Env_{PC1}$ ), but  
214 non-parallel associations between these measures and the relationship between pelvic size  
215 and plate number ( $Armour_{PC2}$ ). The latter is consistent with previous findings of a lack of  
216 convergence in plate number in response to Ca between North American and European  
217 radiations (<sup>34</sup>), while the former suggests that common pH and calcium environments,  
218 experienced in all radiations as a shared acid-alkali axis, promotes phenotypic parallelism.

219             $Shape_{PC1}$  and  $Shape_{PC2}$  were significantly associated with both  $Env_{PC1}$  ( $Shape_{PC1}$ :  $F=$   
220  $4.85$ ,  $p=0.031$ ;  $Shape_{PC2}$ :  $F=28.20$ ,  $p<0.001$ ) and  $Env_{PC2}$  ( $Shape_{PC1}$ :  $F= 7.56$ ,  $p=0.008$ ;  $Shape_{PC2}$ :  
221  $F=48.01$ ,  $p<0.001$ ), suggesting body shape variation is strongly linked to specific measures of  
222 water chemistry. Intercepts across radiations varied significantly but slopes did not,

223 indicating parallelism across radiations in the way shape and environment are associated  
224 (Supplementary Table 6). The result highlights parallelism in the way bodies become more  
225 elongated and slender and heads are reduced with lower pH and calcium and higher Zinc.

226 As for gill rakers, we expected trophic morphology to evolve in response to  
227 zooplankton communities, which in turn have been shown to vary in response to measures  
228 of water chemistry (<sup>35,36</sup>), in particular Zn which was the dominant component of Env<sub>PC2</sub>.  
229 Consistent with this, we found gill raker number to be strongly significantly associated with  
230 both Env<sub>PC2</sub> (F=43.74, p<0.001) and Env<sub>PC1</sub> (F=18.17, p<0.001). Slopes varied between  
231 radiations for the association of gill raker number and Env<sub>PC1</sub>, demonstrating non-parallelism  
232 in trophic response to pH and calcium. In contrast, common slopes across radiations  
233 highlight parallelism in trophic association between gill raker number and Zn variation  
234 (Env<sub>PC2</sub>).

235 Corroborating predictions that selection imposed by similar environments can lead  
236 to phenotypic parallelism (<sup>1</sup>), we found that similar variation from alkaline to acid  
237 environments (Env<sub>PC1</sub>) present in all radiations was significantly associated with parallel  
238 variation in body shape and armour. If phenotypic adaptation happens via natural selection  
239 on genetic variation, we would then expect these associations to translate into genomic  
240 parallelism associated with both environmental variables that show a similar range across  
241 radiations (pH and Ca) and phenotypic traits associated with them.

242

### 243 **Phylogenetic relationship among radiations.**

244 The probability of genomic parallelism has been linked to time since lineages split,  
245 highlighting the probable influence of genetic similarity and shared ancestral variants on

246 parallelism (<sup>16</sup>), it is therefore important that we know the genetic relationship across all  
247 populations before interpreting patterns of genomic parallelism. Genomic parallelism across  
248 populations within any one radiation is likely to evolve from shared genetic variation, but  
249 the probability of that should decline in geographically distant radiations as a function of  
250 common ancestry (<sup>16,37</sup>). Indeed, recent studies have highlighted the probable limitation of  
251 parallelism in this system across continental scales (<sup>20,38,39</sup>). A Neighbour Joining (NJ) tree  
252 based on 8,395 unlinked SNPs showed that, with the exception of a likely recently formed  
253 population in Alaska (TERN), which is basal to both the Alaskan and British Columbian  
254 radiations, the four geographic locations form four well-resolved radiations (Fig. 1). A PCA  
255 on the same dataset confirmed that radiations form independent clusters, and also revealed  
256 that the dominant axis of variation (PC1 = 36.0%) separates North American and European  
257 radiations pairs from one another (Supplementary Fig. 1). North American radiations then  
258 separated on PC2 (7.0%) and European radiations on PC3 (5.8%). In addition to being close  
259 together in the NJ tree, we found that the geographically adjacent radiations were also the  
260 most genetically similar (Supplementary Table 7): Alaska and BC (mean pairwise  $F_{ST} = 0.198$ ),  
261 and Scotland and Iceland ( $F_{ST} = 0.194$ ), suggesting that although these form independent  
262 clusters, the lineage split between them is relatively recent, or that any gene flow between  
263 radiations is occurring within the Atlantic and Pacific groups. Genetic divergence between  
264 inter-continental pairings was found to be stronger and deeper ( $0.314 \leq F_{ST} \leq 0.338$ ) than  
265 within continents, which is consistent with previous studies that have estimated the time of  
266 divergence between stickleback from Europe and North America to be approximately  
267 200,000 years (<sup>40,41</sup> but see <sup>42</sup>). Further, between-continent structuring accounts for the  
268 largest proportion of molecular variance in our data (AMOVA:  $\sigma = 889.7$ , 34.7%). Within  
269 continents, populations within radiations ( $\sigma = 366.5$ , 14.3%) were more genetically variable

270 than radiations ( $\sigma = 142.3$ , 5.6%) (Supplementary Table 8). This highlights that molecular  
271 variance isn't structuring according to geographic scale (Continent > Radiation >  
272 Populations), but rather gene flow may be occurring between intra-continental radiations.

273       Following the idea that the probability of parallelism at the genetic level is linked to  
274 time since lineages split (i.e. genetic similarity), we would expect groups with closer  
275 evolutionary histories, or indeed present gene flow, to show the strongest genomic  
276 parallelism if adaptation occurs through shared standing genetic variation or the exchange  
277 of beneficial freshwater alleles between intra-continental radiations via marine populations  
278 (<sup>29,32</sup>).

279

280 **Phenotypically and environmentally associated SNPs and genomic regions within**  
281 **radiations.**

282 We scanned the genome to identify regions associated with individual components of  
283 phenotypic and environmental variation within each of the four adaptive radiations. We  
284 focused on repeated changes within the same genomic regions, rather than on reuse of the  
285 same mutations. This is because the causal mutations are unknown in most cases and may  
286 not be sequenced by reduced representation sequencing methods such as RAD-sequencing.

287 For each radiation separately (N=18 or 19 populations) we used Bayesian linear models  
288 implemented in Bayenv2 (<sup>43</sup>) to identify associations between population SNP allele  
289 frequencies (N=10 to 21 individual fish, mean= 17.8), the set of six biotic and abiotic  
290 environmental variables and the set of 12 phenotypic traits (Supplementary Table 2). As a  
291 positive control for the methods used we compared our results for parallelism across  
292 freshwater radiations with well-studied marine-freshwater parallelism in this species  
293 (<sup>17,27,29</sup>), and then examined genomic differentiation between all freshwater populations

294 pooled within a radiation and four marine populations pooled together (one from each  
295 country, Supplementary Table 1).

296 Our analyses identified population allele frequencies of several thousand SNPs for  
297 each radiation as being highly correlated (high Bayesfactor [ $>\log_{10}(1.5)$ ] and top 5% of  
298 Spearman's  $\rho$ , see methods) with the abiotic and biotic environmental variables  
299 ('environmentally associated SNPs') or with phenotypic traits ('phenotypically associated  
300 SNPs' (Supplementary Table 9). SNPs were then mapped onto non-overlapping sliding  
301 windows of 50kb, 75kb, 100kb or 200kb, which allowed us to test the robustness of our  
302 results across different extents of linkage. Further, we repeated our analysis across windows  
303 of equivalent genetic distance (0.1 cM windows), which confirmed that our results were not  
304 influenced by variable linkage across the genome (Supporting Information). Here we report  
305 only results for 50kb windows, given that this is consistent with approximate linkage  
306 disequilibrium within the stickleback genome (<sup>44,45</sup>). Results for window sizes of 75kb,  
307 100kb, 200kb can be found in Supplementary Dataset 1. Our 50kb dataset was composed of  
308 4,868 windows with SNPs in all locations, covering approximately 55% of the 447 Mb  
309 genome, with a further 1,940 windows sequenced in 2 or more locations providing  
310 information on an additional 21.7% of the genome.

311 Windows were classified as 'environmentally associated' and 'phenotypically  
312 associated' if they contained more associated SNPs than expected under a 99% binomial  
313 expectation (<sup>46</sup>). We found 1791 unique 50kb windows associated with an environmental  
314 variable or a phenotypic trait (Supplementary Dataset 1), ranging from 146 windows  
315 associated with pelvic spine length in BC, to 21 associated with Na variation, also in BC. It is  
316 striking how many windows show strong signals of association with phenotypic and

317 environmental variables, even when their variation is modest, clearly supporting the  
318 adaptive nature of these radiations.

319 Out of all the unique windows, 431 were associated with both an environmental and  
320 phenotypic variable in the same radiation, suggesting several regions associated with  
321 phenotypic traits might also be responsible for local adaptation to environments  
322 (Supplementary Dataset 1). It also suggests that directly measuring important aspects of the  
323 environment may provide profitable ways of discerning the genomic basis of adaptation and  
324 of identifying agents of selection, while bypassing the often-difficult measurement of  
325 phenotypes.

326

327 **Genomic parallelism associated with environmental and phenotypic variation across**  
328 **radiations.**

329 To quantify genomic parallelism we identified environmentally- and phenotypically-  
330 associated windows that were shared across two or more of our radiations, i.e. parallel  
331 windows (Supplementary Fig. 2). We then compared the overall observed numbers of  
332 parallel windows to a null distribution of randomly associated windows permuted over  
333 10,000 iterations.

334 We quantified genomic parallelism at three levels: 1) gross, general levels of  
335 parallelism associated across all radiations for groups of phenotypes or environment; 2)  
336 genomic parallelism associated with individual variables across all comparisons to  
337 understand contributions of individual variables; 3) parallelism for individual variables in  
338 specific pairings, to identify pairs of radiations having the highest levels of parallelism, and  
339 for which variables. The latter is important as patterns of parallelism may be lost when we

340 pool more than two radiations or variables together, if for example parallelism is very strong  
341 in one radiation-pairing but not others.

342 When quantifying the overall level of genomic parallelism associated with groups of  
343 environmental or phenotypic variables we found no environmentally- or phenotypically-  
344 associated 50kb windows parallel in all four radiations for individual variables (Randomised  
345 permutations  $N_{\text{Expected-Environmental}} = 0.0002$ ,  $N_{\text{Exp-Pheno}} = 0.0001$ ), but one window was parallel  
346 in a group of three radiations (chrIV: 14400000-14450000 associated with length of pelvis in  
347 BC, Iceland and Scotland) ( $N_{\text{Exp-Env}} = 0.05$ ,  $N_{\text{Exp-Pheno}} = 0.161$ ,  $p = 0.149$ ). Many windows  
348 however exhibited parallelism between pairs of two radiations. A total of 39  
349 environmentally associated windows (pooled across all 6 environmental variables) ( $N_{\text{Exp}} =$   
350 11.9, 95% Upper limit (UL) = 18,  $p < 0.001$ ) and 65 phenotypically associated windows  
351 (pooled across all 12 phenotypic variables) ( $N_{\text{Exp}} = 30.9$ , 95% UL = 40,  $p < 0.001$ ) were  
352 parallel between two radiations (Supplementary Fig. 2). Parallelism was disproportionately  
353 greater for armour and gill raker traits (number mostly) than for shape, with the 65  
354 phenotypically associated windows split into 46, 12 and seven associated windows for  
355 armour, gill raker and shape variables respectively ( $\chi^2 = 6.506$ ,  $P = 0.04$ ). This is consistent  
356 with the fact that skeletal traits, several of which are known to have simple genetic  
357 architectures (<sup>26,47,48</sup>), are particularly likely to show evidence of phenotypic parallelism.  
358 Interestingly, parallel associated windows (mean SNP  $N = 7.13$ ) had on average more SNPs  
359 per window than non-parallel windows (mean SNP  $N = 6.27$ ) (GLM,  $LRT_{1,3867} = 22.1$ ,  $p <$   
360 0.001) and exhibited slightly stronger signals of association with variables (mean residual  
361 SNPs above expected = 1.82 parallel; 1.61 non-parallel; GLM,  $LRT_{1,3867} = 5.05$ ,  $p = 0.025$ ).

362 Random permutations indicated that there were statistically significant levels of  
363 parallelism for the number of windows ('significantly parallel windows') associated with two

364 environmental variables (Ca and pH) (Fig. 3; Supplementary Table 10). Consistent with our  
365 expectations from patterns of environmental parallelism, these were the same variables  
366 that share an axis of variation ( $Env_{PC1}$ ) across freshwater environments in all radiations. In  
367 addition, we did not detect significant genomic parallelism associated with variables that  
368 exhibit variation between radiations, such as salinity, zinc and *S. solidus* prevalence. These  
369 results together confirm that common environmental axes, such as the one experienced in  
370 all radiations as a shared acid-alkali axis, likely promote signals of parallelism in the genome.

371 We also found more genomic parallelism than expected by chance associated with a  
372 total of five phenotypic variables (Fig. 3; Supplementary Table 10): four armour traits (2<sup>nd</sup>  
373 dorsal spine, pelvic spine length, length of pelvis and armour plate number) and gill raker  
374 number. These results are consistent with the high heritability of skeletal traits (<sup>26,47,48</sup>), and  
375 suggest that variation in armour is the result of different genotypes being selected in  
376 different environments. Further, parallel QTLs have been described for gill raker number  
377 (<sup>49</sup>), but not length, which exhibits more plasticity (<sup>50</sup>). Body shape traits were not associated  
378 with any significant genomic parallelism, despite parallelism across radiations in the way  
379 shape is associated with the environment. It is probable the partly plastic nature of body  
380 shape (<sup>51,52</sup>) leads to an association between environment and body shape via the reaction  
381 norm rather than genomic re-use.

382 Our analyses also showed much stronger parallelism across marine-freshwater (MxF)  
383 comparisons than across freshwater variables (Fig. 3), and 44/158 of MxF associated  
384 windows overlapped with previously identified genomic regions contributing to marine-  
385 freshwater divergence (<sup>17,44</sup>) (Supplementary Table 11). Several regions found parallel for  
386 MxF analyses were also parallel for Ca, pH, Na, armour traits and gill raker number  
387 (Supplementary Table 12). These results suggest our methods and sequencing coverage are



388 robust enough to recover known parallel regions, and interestingly that genomic parallelism  
389 associated with freshwater variables is more modest than marine-freshwater parallelism.  
390 The latter likely reflects subtler variation between habitats within radiations in comparison  
391 to stark marine-freshwater contrasts. Further, these results highlight that binary ecotype  
392 pairings, which likely include variation in many environmental and phenotypic traits, lump  
393 together parallelism of many components of fitness without being able to discern which are  
394 parallel and which are non-parallel. Overall, our results suggest that across these four  
395 freshwater adaptive radiations, evolution of these phenotypes and environmentally  
396 associated traits are disproportionately linked to the same genomic regions.

397       Within specific pairings, we found the greatest number of significantly parallel  
398 windows in the comparison between Alaska and BC (two environmental variables: Ca and  
399 *Gyrodactylus spp.*, and three phenotypic traits: pelvic spine length, plate number and gill  
400 raker number), followed by Iceland and Scotland (two environmental variables: Ca and pH,  
401 and one phenotypic trait: dorsal spine length) (Supplementary Fig. 2; Supplementary Table  
402 10). Two environmental variables and one phenotypic trait were also associated with  
403 significantly more parallel windows in comparisons between Iceland and the North  
404 American radiations: Ca and length of the pelvis (Alaska and Iceland) and *Schistocephalus*  
405 *solidus* (BC and Iceland). Pelvic spine length was the only variable of any category found to  
406 be parallel between Scotland and BC, and no significant parallelism was detected between  
407 Scotland and Alaska (suggesting observed overlap may be the result of chance).  
408 Phylogenetic patterns and the segregation of molecular variance strongly support the  
409 notion that radiations within continents share similar genetic variation, making parallelism  
410 through shared standing variation the most parsimonious explanation for our intra-

411 continental parallelism biases. Experimental studies with stickleback have demonstrated  
412 rapid morphological adaptation from standing genetic variation, even recovering diverse  
413 morphologies from variation found within phenotypically-derived freshwater populations  
414 (<sup>53</sup>). Coancestry patterns, centred at the focal, causative loci, can discern between whether  
415 parallel evolution occurs on *de novo* mutations, standing variation, or introgressed alleles,  
416 however we lack the sequencing resolution in our current data to make these comparisons.

417

#### 418 **Linkage and the genomic location of parallel regions.**

419 As with any reduced-representation genomic approach, the power of RAD  
420 sequencing to detect loci associated with phenotypic or environmental variables depends  
421 on linkage disequilibrium (LD) between markers and functional loci(<sup>44,54,55</sup>). The scale of LD  
422 varies widely across organisms and within genomes, but has been relatively well-  
423 characterized in stickleback (<sup>56,57</sup>), and RAD sequencing has been used successfully in this  
424 species specifically to test for genomic parallelism (<sup>30,44,53,56,57</sup>). One explanation for variable  
425 linkage across the genome is the negative relationship it shares with local recombination  
426 rate. To examine its influence on our results, we estimated recombination rate using a  
427 previously published genetic map (<sup>31</sup>) for our 50kb windows, and marked windows that were  
428 associated with any variable and associated windows that were also parallel across  
429 radiations. Recombination was significantly reduced in associated windows and parallel  
430 windows compared with non-associated windows (Supplementary Fig. 3; Kruskal-Wallis,  $\chi^2 =$   
431 121.43,  $p < 0.001$ ), but did not differ significantly between outlier and parallel windows ( $p =$   
432 0.55). Reduced recombination can be an important process in adaptation through

433 maintaining adaptive alleles, as has been demonstrated in this species (<sup>58</sup>) and others (eg.  
434 <sup>59</sup>). However, we cannot rule out that these patterns are produced by an increased ability to  
435 detect selection in low-recombination windows as a product of increased linkage with  
436 causative SNPs. The latter suggests that our estimates of association, and by extension  
437 parallelism, may be conservative if false-negatives are pervasive in high recombination  
438 regions. Importantly however, our signatures of parallelism cannot be explained by variable  
439 recombination.

440 Windows based on genetic distance (0.1 cM) corroborated 50kb results, returning  
441 strong signals of parallelism for calcium, pH, pelvic spine length, pelvis length, plate number  
442 and gill raker number. Interestingly, we also recovered weakly significant parallelism for  
443 several other environmental and armour variables (Supplementary Fig. 4, Supplementary  
444 Information), suggesting potentially stronger parallelism than we report at 50kb windows.

445 To assess wider, linked parallel regions, we plotted 50kb windows across the genome  
446 to examine clustering of all associated windows (Supplementary Fig. 5). Adjacent windows  
447 (two or more, Supplementary Table 13) were pooled together to inspect putative causative  
448 genes (Supplementary Dataset 2). Using these two methods we identified a number of  
449 wider genomic regions that exhibited parallelism across multiple radiations. An example,  
450 and good positive control for our method, involves the pooling of windows associated with  
451 plate number in three radiations (Alaska, BC and Iceland) on chromosome IV around the  
452 well-known *Eda* gene, which has a well-established role in producing variation in the  
453 number of armour plates (<sup>17,26,27</sup>). This pattern emerges despite the limited variation in plate  
454 number across freshwater populations.

455 Pooling adjacent windows also identified a large cluster (250 kb) on chromosome I  
456 containing genes *igfbp2a*, *stk11ip* and *atp1a1*, and strongly associated with calcium, sodium

457 and pH in several radiations (Supplementary Data 2). The clustering of windows in this  
458 region is perhaps unsurprising given it contains a known inversion (<sup>60</sup>), which as discussed  
459 should be beneficial for adaptive haplotypes by reducing local recombination (<sup>61</sup>). Within  
460 this region specifically, it is likely that the *atpa1a1* gene causes large effects on fitness, given  
461 its previously detected association with the major ecological transition from marine to  
462 freshwater (<sup>62</sup>) and functional role in metal ion management (<sup>17,60</sup>). Its apparent role in  
463 adaptation to much smaller cation variation between freshwater environments in this study  
464 is interesting in light of Fisher's geometric model of adaptation, since this predicts that only  
465 alleles of smaller effect should fix as a fitness optimum is approached (<sup>63</sup>). Thus, it may be  
466 that different mutations in this gene have a spectrum of effect sizes, or that changes cause  
467 subtler differences in expression, rather than larger coding differences.

468 Our results support the existence of genomic regions of physically linked genes that  
469 are hitch-hiking in separate radiation pairs, and may contain genes that are parallel across  
470 all radiations but undetected by our genomic methods. Extensive linkage disequilibrium in  
471 freshwater populations is consistent with what is expected under strong directional  
472 selection after colonization from marine populations and has been reported for stickleback  
473 populations from Alaska (<sup>44</sup>), but it had not previously been observed for the same regions  
474 across several independent adaptive radiations. These results are also consistent with  
475 previous findings of large numbers of SNPs highly divergent between marine and freshwater  
476 stickleback aggregating in just 19 short genomic regions, including three known inversions  
477 (<sup>60</sup>), one of which we also detected and highlighted above.

478

479 **Relationships between genomic parallelism and phylogenetic, phenotypic and**  
480 **environmental similarity.**

481 Freshwater populations have radiated from marine common ancestors (<sup>1,16</sup>), thus the  
482 parallelism patterns described in this study are putatively the result of multiple marine -  
483 freshwater transitions. Based on this assumption, and using our SNP data from a marine  
484 population within each radiation, we performed genome-wide  $F_{ST}$  outlier analyses  
485 comparing each freshwater population with the marine population within that radiation.  
486 We then took the top 5% of 50kb windows according to  $F_{ST}$  in each marine – freshwater  
487 comparison and looked for overlapping outlier windows across all comparisons ( $N = 2,628$ ).  
488 This quantified the extent of repeated genome-wide differentiation for MxF transitions  
489 within and across all radiations (Fig. 4a).

490 We then used Mantel tests to test statistically the effects on MxF genomic  
491 parallelism of relative genetic divergence and environmental/phenotypic similarity  
492 alongside one another. We compared the matrix of overlapping marine - freshwater outlier  
493  $F_{ST}$  windows to equivalent matrices for environmental dissimilarity (Euclidean distances),  
494 phenotypic dissimilarity (Euclidean distances), and genetic dissimilarity ( $F_{ST}$  values) (Fig. 4).  
495 Across all population comparisons the number of parallel windows was strongly negatively  
496 correlated with genetic dissimilarity ( $r = -0.61$ ,  $p < 0.001$ ), and also negatively correlated  
497 with environmental dissimilarity ( $r = -0.42$ ,  $p < 0.001$ ) and phenotypic dissimilarity ( $r = -0.11$ ,  
498  $p = 0.022$ ). These results highlight that genomic parallelism increases in populations that are  
499 more genetically, environmentally and phenotypically similar, though to varying extents.

500 Parallel windows were more common in intra- rather than inter-continental  
501 comparisons. This highlights the significance of our European and North American pairings  
502 as the major contributors towards pairwise signals of genomic parallelism, as we discussed  
503 previously, and strongly suggests that genomic parallelism at large geographic scales is  
504 contingent on shared genetic variation. Further, at an intra-continental scale, there exists  
505 the possibility of haplotype sharing between radiations by gene flow through marine  
506 populations, which may be facilitated in North America, despite the greater geographic  
507 distance, by a shared coastline connecting Alaska and BC (<sup>64</sup>). Recent research has also  
508 highlighted a probable genetic bottleneck in the founding of Atlantic marine populations  
509 that restricts shared freshwater alleles between Atlantic and Pacific freshwater populations  
510 (<sup>39</sup>).

511 We also conducted partial mantel tests for the effects of environmental and  
512 phenotypic similarity whilst controlling for genetic similarity, given this is likely to correlate  
513 with environment and phenotype in some cases due to geographic proximity. Effects of  
514 environmental dissimilarity were marginally reduced when controlling for genetic similarity,  
515 but were still strongly negative ( $r = -0.35$ ,  $p < 0.001$ ), suggesting that similar environments  
516 promote genomic parallelism irrespective of genetic similarity. Phenotypic dissimilarity was  
517 no longer associated with genomic parallelism after controlling for genetic similarity ( $r = -$   
518  $0.10$ ,  $p = 0.097$ ). This latter result suggests that environmental similarity is a better predictor  
519 of genomic parallelism than phenotypic similarity (at least in terms of observable  
520 morphometric phenotypes) in this system.

521 In conclusion, our study is the largest to date in this system addressing the relative  
522 effects of environment, phenotype and genetics in predicting parallel evolution. Genetic

523 similarity is the best predictor of genomic parallelism here, in line with recent results and  
524 expectations regarding sharing of ancestral variants and introgression between populations  
525 (<sup>29,39</sup>). However, even whilst controlling for this, environmental variation (most likely Ca and  
526 pH variation in particular) is a good predictor of genome-wide parallelism among freshwater  
527 populations on a wide geographic scale. In particular, the higher environmental similarity  
528 among North American radiations, compared to European populations, provides a good  
529 explanation for the strong phenotypic and genomic parallelism among those freshwater  
530 populations, alongside the probable genetic bottleneck in the founding of Atlantic marine  
531 populations (<sup>39</sup>). The fact that, despite the larger geographic distance between the two  
532 North American radiations, they have more similar phenotypes and exhibit stronger  
533 genomic parallelism demonstrates that environment, and by extension selection, can  
534 counteract the effects of distance to some extent. Explicit measurements of environment  
535 are thus important in predicting parallel evolution even across large geographic scales, and  
536 distance alone can be misleading. Phenotypic parallelism was observed for several traits  
537 across continents, and populations with similar phenotypes also exhibit stronger genomic  
538 parallelism. However, this relationship is weaker than for commonality of environment, and  
539 is likely confounded by variable heritability and genetic architecture among phenotypes.  
540 Most importantly, our results highlight that quantitative analyses of phenotypes and  
541 environments and of their relationship can provide a good prediction of expected genomic  
542 parallelism, and provide a much clearer picture of major factors that are likely to influence  
543 the emergence of a common pattern of genomic divergence than analyses of dichotomous  
544 phenotypes and environments.

## 545 **Methods**

546 **Sampling and environmental data collection.**

547 We sampled 18 lakes in North Uist, Scotland between April and June 2013, 18 lakes  
548 from Iceland between May and June 2014, 18 lakes from British Columbia (BC) between  
549 April and May 2015 and 19 lakes from the Cook Inlet basin, Alaska in June 2015. Lake  
550 names, geographic coordinates and numbers of samples used in the study are shown in  
551 Supplementary Table 1. We measured the pH, concentrations of metallic cation  
552 concentrations sodium (“Na”), calcium (“Ca”) and zinc (“Zn”) of each lake, and calculated  
553 population prevalence of *Gyrodactylus spp.* and *Schistocephalus solidus*. Concentrations of  
554 cations, pH and parasite prevalence per lake are shown in Supplementary Table 2. Details of  
555 the fish collection and quantification of abiotic and abiotic variables can be found in  
556 Supplementary Information.

557

558 **DNA extractions, RAD library preparation and sequencing.**

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

563 In 2014 we conducted RAD sequencing on samples from Scotland and from Iceland.  
564 Sequencing libraries were prepared and processed into RAD following the modified libraries  
565 according to <sup>(65)</sup>. In 2016 we conducted RAD sequencing on samples from British Columbia  
566 and from Alaska. Sequencing libraries were prepared following the modified single-digest  
567 RAD protocol of <sup>(66)</sup>. The two RADseq protocols interrogate the same set of loci across the  
568 genome, so that the SNP data are compatible across all four radiations. See Supplementary  
569 Information for details of the RAD library preparation and sequencing.

570

571 **Population genetics statistics and phylogenetic tree.**

572 Raw sequence reads were demultiplexed using Stacks – 1.35 <sup>(67)</sup>. Number of reads  
573 per individual are shown in Supplementary Dataset 3 (see Supplementary Information for  
574 details on the alignment of reads and Stacks pipeline used). For Bayenv2 data, autosomal  
575 SNPs were called as being in >7 populations, >50% of the individuals within a population,  
576 and with a MAF-filter of 0.05. After filtering we retained 26,990, 26,937, 29,111, 26,169  
577 SNPs for Scotland, Iceland, British Columbia and Alaska respectively. For analyses of  
578 population structure across all radiations, a subset of unlinked SNPs were generated. Here,  
579 autosomal SNPs were called that were present in all radiations and in >50% of individuals  
580 within a radiation, with a MAF-filter of 0.05 (within a radiation). Only the first SNP per RAD  
581 locus was retained.  $F_{ST}$  was bootstrapped and calculated in POPULATIONS. This set of SNPs  
582 were then pruned for linkage disequilibrium in plink using indep-pairwise 50 5 0.2. The set  
583 of unlinked SNPs were used to construct a neighbour-joining tree for all fish in the R  
584 package ‘adegetnet’, using a distance matrix computed from the SNP data <sup>(68)</sup>. The tree was  
585 bootstrapped 100 times and nodes with less than 80% support were collapsed. The tree was  
586 plotted using the ‘ape’ package in R <sup>(69)</sup>. PCA analysis of population structure was conducted  
587 using plink <sup>(70)</sup>.

588

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



591 All morphological measurements (body shape, body armour and spine traits) were  
592 done following (<sup>25</sup>). Details of the quantification of phenotypic traits can be found in  
593 Supplementary Information.

594 We performed three Principal Component Analyses (PCAs): one on the armour traits,  
595 another on body shape, and another on the 6 environmental variables. Body shape and  
596 armour PCAs were performed on regression residuals of all individuals from all radiations  
597 pooled together to extract the common PCs of body shape and armour variation (Shape\_PCs  
598 and ArmourPCs) and environmental variation and retained axes that explained more than  
599 10% of the total variance. Armour and environmental PCAs were conducted with scaled  
600 inputs due to different units of measurement between variables. Shape PCAs were  
601 conducted on morphometric residuals, and as such were not scaled. All phenotypic  
602 analyses, including ANOVAs and ANCOVAs and plotting were done in R version 3.4.3.

603

#### 604 **Genotype-Environment/Phenotype Associations.**

605 For each radiation separately (N=18 to 19 populations) we used Bayenv2 (<sup>43</sup>) to  
606 identify associations between genomic allele frequencies (N=10 to 21 individual fish, mean=  
607 17.8), the set of six biotic and abiotic environmental variables (Ca, Na, pH, Zn, prevalence of  
608 *Gyrodactylus spp.* and *Schistocephalus solidus*) and the set of 12 phenotypic traits  
609 (Shape\_PC1, Shape\_PC2, Shape\_PC3, DS1, DS2, PS, LP, HP, BAP, Plate\_N, Gill\_Raker\_L and  
610 Gill\_Raker\_N) mentioned above. For each radiation, a matrix of genetic covariance was  
611 calculated using a subset of SNPs limited to a single SNP per RAD-locus and pruned for  
612 linkage disequilibrium ( $R^2 < 0.4$ ) in plink (<sup>70</sup>). This cut-off was selected to balance the trade-  
613 off between SNPs retained and minimising the effects of linkage. Covariance matrices were  
614 therefore calculated using 9619, 7983, 7300 and 5705 SNPs for Alaska, BC, Iceland and  
615 Scotland respectively. Covariance matrices were calculated across 100,000 iterations and  
616 averaged across 5 independent runs. Bayenv2 was run independently 8 times and final  
617 results were averaged across runs.

618 Environmentally and phenotypically associated SNPs were selected as having a log<sub>10</sub>-  
619 BayesFactor > 1.5 and an absolute Spearman's rank coefficient above the 95th percentile.  
620 The combination of BayesFactor and non-parametric measure of correlation helps to avoid  
621 selecting SNPs with high BayesFactors due to spurious populations (<sup>18</sup>). SNPs were grouped  
622 into 50kb, 75kb, 100kb, 200kb and 0.1 cM windows (Supplementary Table 14) to test the  
623 robustness of our results across different extents of linkage. To evaluate whether windows  
624 were environmentally or phenotypically associated, we adapted the methodology of (<sup>46</sup>).  
625 We calculated the upper 99% binomial expectation for the number of associated SNPs given  
626 the total number of SNPs in a specific window, and selected windows that had a greater  
627 number of associated SNPs than this expectation. This method controls for variation in SNP  
628 density across windows and ensures that significant windows exhibit consistent allele  
629 frequency correlations across multiple SNPs. We visualised the genomic locations of  
630 associated windows using Manhattan plots (Supplementary Fig. 5) and plotting the residual  
631 number of outlier SNPs above the binomial expectation (Supplementary Fig. 6). Linkage  
632 groups I-XXI were visualised with the exception of XIX; windows on scaffolds were not  
633 visualised. Finally, we compared these associated windows across radiations to examine  
634 those that were parallel.

635

#### 636 **Parallelism statistics.**

637 For all radiation groupings (11 combinations in total: one four radiation grouping, four three  
638 radiation groupings, and six two radiation groupings), we calculated the significance of  
639 parallel window counts using a permutation method. For each environmental or phenotypic  
640 variable, we randomly drew N windows from each radiation's total pool where N was  
641 equivalent to the associated window count for each radiation. We then assessed the overlap  
642 of randomly associated windows across radiations and pooled the results over 10,000  
643 iterations. The output from all permutations was used as a null distribution to infer p-values,  
644 which were then FDR-corrected using the R package *qvalue* (<sup>71</sup>).

645

#### 646 **Grouping of adjacent windows and expanding parallelism regions.**

647 Windows of 50kb and above were based on a linkage assumption and to minimise non-  
648 independence between windows. There were, however, occasionally adjacent windows  
649 associated with the same variable across different groupings. Large regions of relatively  
650 strong linkage are plausible if recombination is reduced through processes such as genomic  
651 rearrangements. To investigate these, we grouped associated windows that were adjacent  
652 as well as those that were direct matches across radiations based on the likelihood of  
653 adjacent associated windows resulting independently being low, suggesting non-  
654 independence and probable linkage. These windows are available in Supplementary Table  
655 13.

656

#### 657 **Multivariate vector comparison of environments and phenotypes.**

658 Vectors for environmental, shape and armour PCs (>10% variance) and gill raker data were  
659 calculated as the difference between the linearly predicted maximum and minimum values  
660 per radiation. Angles ( $\theta$ ) and difference in length ( $\Delta L$ ) were calculated for each vector  
661 between radiations. Significance of vectors was determined through permutations by  
662 simulating random traits from a normal distribution with mean and s.d. equivalent to the  
663 observed data and assessing vectors of random traits/variation as above (Supplementary  
664 Information).

665

#### 666 **Comparing relative influences of environment, phenotype and genetics.**

667  $F_{ST}$  was calculated between each freshwater population and its relevant marine population  
668 (Alaska = MUD1, BC = LICA, Iceland = NYPS, Scotland = OBSM) in 50kb windows using the R  
669 package 'PopGenome' (REF). For each MxF comparison, windows above the 95% quantile  
670 were classed as outliers. Outlier windows were compared across all pairwise freshwater  
671 comparisons (2,628 comparisons among 73 populations), with overlapping outliers  
672 representing MxF  $F_{ST}$  parallelism. Dissimilarity matrices of environment and phenotype were  
673 calculated as Euclidean distance in PCA space for the 6 and 12 environmental and phenotypic  
674 variables respectively. The genetic dissimilarity matrix was composed of genome-wide pairwise  $F_{ST}$   
675 estimates between freshwater populations. The matrix of MxF parallelism was associated to  
676 environmental, phenotypic and genetic dissimilarity matrices using Mantel tests with 9,999  
677 permutations. Partial Mantel tests were performed with genetic distance as the conditional matrix  
678 for environmental and phenotypic effects on MxF parallelism, again with 9,999 permutations.

679

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688

### 689 **Contributions**

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

694

### 695 **Competing financial interests**

696 The authors declare no competing financial interests.

697

### 698 **Data Accessibility**

699 Bam files of aligned reads for each individual and corresponding sample information have  
700 been deposited in the European Nucleotide Archive database under the project  
701 PRJEB20851, with the sample accession numbers ERS1831811-ERS1833111, and run  
702 accession numbers ERR2055459-ERR2056759. Scripts used for all analyses are available at  
703 [https://github.com/JimWhiting91/stickleback\\_adaptive\\_radiations/](https://github.com/JimWhiting91/stickleback_adaptive_radiations/)

704

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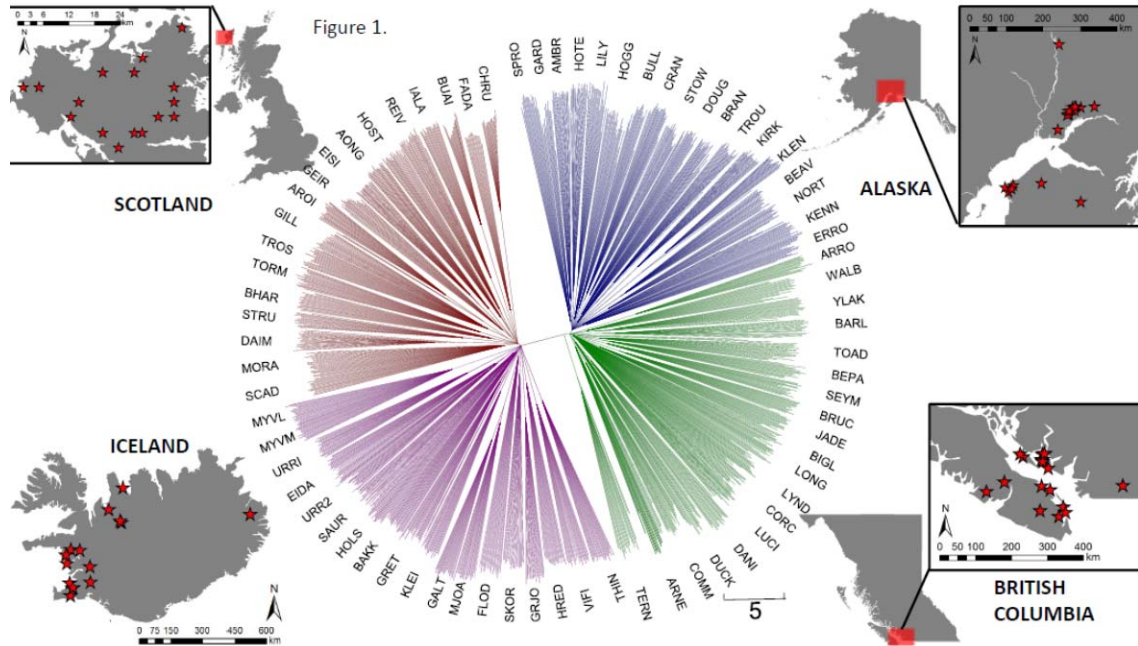
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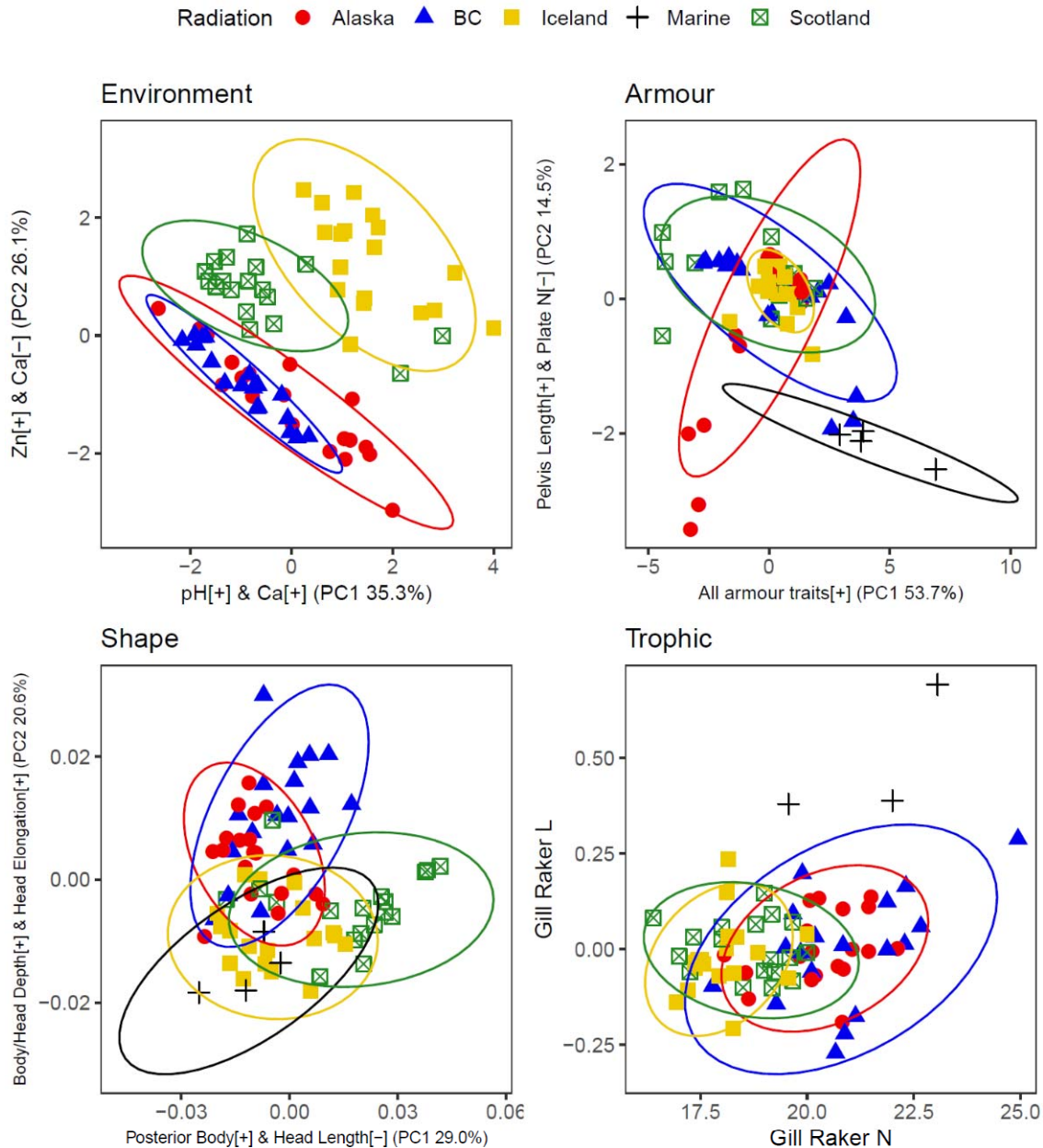
864 Fig. 1. Sampling sites and bootstrapped NJ tree for stickleback from 73 freshwater populations from  
865 four countries on two continents, based on 8,395 genetic markers for 1,304 individuals. All nodes  
866 shown have bootstrap support of at least 80 (other nodes were collapsed). Branches are coloured by  
867 the radiation to which they belong. Tips represent individual fish, which were generally tightly  
868 clustered by population (small labels). Stars represent lakes sampled.  
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873 Fig. 2. Principal Component Analyses of environmental variables (Environment); regression residuals  
874 of Procrustes coordinates against log centroid of body shape (Shape); armour traits (Armour: length  
875 of dorsal spines 1 (DS1) and 2 (DS2), length of pelvic spine (PS), length and height of pelvis (LP and  
876 HP), length of biggest armour plate (BAP) and number of armour plates (Plate\_N); and gill raker  
877 numbers (Gill\_Raker\_N) vs gill raker length (Gill\_Raker\_L) (Trophic). Each point represents a  
878 population and ellipses are 95% confidence ellipses. Names of variables with the highest positive (+)  
879 or negative (-) loadings on each axes are on legends of each axes. All loadings of variables on the first  
880 3 PCs are in Supplementary Table 3. Marine populations (+) are projected where data was available  
881 using PC loadings calculated with freshwater populations only.

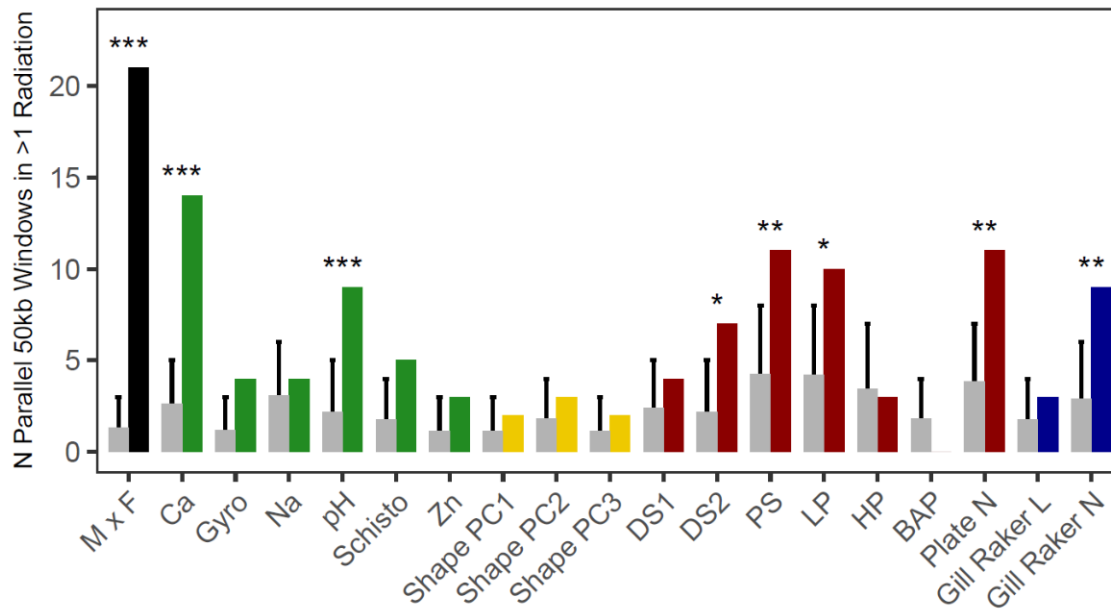


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885 Fig. 3. Expected and observed counts of 50kb windows containing an above 99% binomial  
886 expectation number of SNPs associated with marine x freshwater (MxF), environmental variables  
887 and phenotypic traits in at least 2 radiations. Expected bars (grey) represent mean counts across  
888 10,000 simulated outcomes with 95% confidence intervals per a one-tailed hypothesis. Asterisks  
889 denote significance of FDR-corrected one-tailed tests between the observed counts and the 100,000  
890 simulated counts at the <0.05 (\*), <0.01 (\*\*), <0.001 (\*\*\*) levels.



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893 Fig. 4. Associations between genome-wide marine - freshwater  $F_{ST}$  and environmental, phenotypic  
 894 and genetic distance across all pairwise comparisons of 73 freshwater populations. A) Proportion of  
 895 Mx $F_{ST}$  50kb outlier windows that overlap among freshwater replicates. Freshwater populations are  
 896 ordered as Alaska, British Columbia, Iceland and Scotland, with these location distinguishable as four  
 897 clear clusters. B) Environmental distance between freshwater populations, recorded as euclidean  
 898 distance in PCA space for all 6 environmental variables. C) Phenotypic distance between freshwater  
 899 populations, recorded as for environmental distance for the 12 phenotypic variables. D) Genetic  
 900 distance between freshwater populations, recorded as genome-wide pairwise  $F_{ST}$  based on 8,395  
 901 unlinked SNPs. E-G) Associations between environmental (E), phenotypic (F) and genetic (G) distance  
 902 and Mx $F_{ST}$  overlap (log-transformed). Points are coloured according to whether pairwise  
 903 comparison is being made within a radiation or across radiations.

