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**Article:**

Lucek, K., Sivasundar, A., Kristjansson, B.K. et al. (2 more authors) (2014) Quick divergence but slow convergence during ecotype formation in lake and stream stickleback pairs of variable age. *Journal of Evolutionary Biology*, 27 (9). pp. 1878-1892. ISSN 1010-061X

<https://doi.org/10.1111/jeb.12439>

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This is the peer reviewed version of the following article: Lucek, K., Sivasundar, A., Kristjánsson, B. K., Skúlason, S. and Seehausen, O. (2014), Quick divergence but slow convergence during ecotype formation in lake and stream stickleback pairs of variable age. *Journal of Evolutionary Biology*, 27: 1878–1892., which has been published in final form at <http://dx.doi.org/10.1111/jeb.12439>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving (<http://olabout.wiley.com/WileyCDA/Section/id-828039.html>)

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1 **Quick divergence but slow convergence during ecotype formation in lake**  
2 **and stream stickleback pairs of variable age**

3

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15

16 **Keywords**

17 Parapatric evolution, P matrix, phenotypic diversification, line of least resistance

**18 Abstract**

19 When genetic constraints restrict phenotypic evolution, diversification can be  
20 predicted to evolve along so-called lines of least resistance. To address the  
21 importance of such constraints and their resolution, studies of parallel  
22 phenotypic divergence that differ in their age are valuable. Here we investigate  
23 the parapatric evolution of six lake and stream threespine stickleback systems  
24 from Iceland and Switzerland, ranging in age from a few decades to several  
25 millennia. Using phenotypic data, we test for parallelism in ecotypic divergence  
26 between parapatric lake and stream populations and compare the observed  
27 patterns to an ancestral-like marine population. We find strong and consistent  
28 phenotypic divergence, both among lake and stream populations and between  
29 our freshwater populations and the marine population. Interestingly, ecotypic  
30 divergence in low dimensional phenotype space (i.e. single traits) is rapid and  
31 seems to be often completed within 100 years. Yet, the dimensionality of  
32 ecotypic divergence was highest in our oldest systems and only there parallel  
33 evolution of unrelated ecotypes was strong enough to overwrite phylogenetic  
34 contingency. Moreover, the dimensionality of divergence in different systems  
35 varies between trait complexes, suggesting different constraints and  
36 evolutionary pathways to their resolution among freshwater systems.

37 **Introduction**

38

39 If natural selection is the principal force governing evolutionary change,  
40 divergence among populations can be considered as the tracking of alternative  
41 adaptive peaks on the underlying fitness landscape (Wright 1932; Lande &  
42 Arnold 1983; Stepan *et al.* 2002; Arnold *et al.* 2008). The degree of divergence is  
43 then expected to depend on the time that has been available for selection to act,  
44 the strength of selection, the topology of the fitness landscape and the amount of  
45 adaptive standing genetic variation. All of these factors may affect both the rate  
46 and the direction of evolution. Additionally, the strength of selection and/or the  
47 fitness landscape itself may fluctuate through time due to environmental  
48 variation (Jones *et al.* 2004; Arnold *et al.* 2008). Genetic drift and selection can  
49 reduce standing genetic variation, which may lead to different evolutionary  
50 outcomes across replicated cases of population divergence, even when selection  
51 is acting in a parallel manner across replicated systems (Barrett & Schluter  
52 2008). Consequently, strong parallel evolution is only expected if the selective  
53 regime, the relative level of standing genetic variation and the segregating alleles  
54 themselves are similar (Langerhans & DeWitt 2004; Kaeuffer *et al.* 2012) and if  
55 selection has enough time to overcome potential historical contingencies (Young  
56 *et al.* 2009).

57

58 Evolution towards adaptive peaks can be influenced by genetic “lines of least  
59 resistance” or  $g_{\max}$ , which can be quantified as the leading eigenvector of the  
60 genetic variance-covariance matrix  $\mathbf{G}$  (Lande & Arnold 1983; Schluter 1996;  
61 Jones *et al.* 2004; Arnold *et al.* 2008). Biologically, this axis accounts for the

62 largest proportion of genetic variance and is shaped by selection and drift, which  
63 then influence genetic constraints within a population (Lande & Arnold 1983;  
64 Steppan *et al.* 2002; Marroig & Cheverud 2005). Different **G** matrices can be  
65 compared by calculating the angle  $\theta$  between different  $g_{\max}$  (Lande & Arnold  
66 1983; Schluter 1996; Steppan *et al.* 2002). Whereas genetic constraints may  
67 initially bias evolution towards  $g_{\max}$  (Schluter 1996), selection may alter the  
68 direction of  $g_{\max}$  towards an existing or a new optimum on the adaptive  
69 landscape (Lande & Arnold 1983; Jones *et al.* 2004; Arnold *et al.* 2008), e.g.  
70 during the colonization of new environments (Bacigalupe 2009; Eroukhmanoff &  
71 Svensson 2011). Similarly, genetic drift, bottlenecks or mutations may alter the **G**  
72 matrix and hence  $g_{\max}$  (Chapuis *et al.* 2008).

73

74 In the absence of quantitative genetic data, the **G** matrix might be approximated  
75 by the **P** matrix, which is based on phenotypic data from wild populations  
76 (Cheverud 1988), especially when phenotypic traits are highly heritable (Lande  
77 1979), as has been suggested for several taxa (Cheverud 1988; Leinonen *et al.*  
78 2011; Kolbe *et al.* 2011). **P** is defined as the combination of the genetic and  
79 environmental covariance matrices, i.e. **G** + **E** (Lande 1979; Arnold & Phillips  
80 1999), where both effects could also interact (**G** x **E**; Falconer 1989).  
81 Consequently, **P** matrices include phenotypically plastic effects, that are  
82 differentially expressed in distinct environments (Pigliucci *et al.* 1999). The  
83 leading eigenvector of a **P** matrix ( $p_{\max}$ ) therefore serves as an overall measure of  
84 phenotypic variation observed in the wild, combining both genetic and  
85 environmental effects. The changes of **P** and  $p_{\max}$  towards novel adaptive peaks  
86 thus might occur rapidly through phenotypic plasticity (Lande 2009; Draghi &

87 Whitlock 2012) or adaptation from standing genetic variation (Lande & Shannon  
88 1996; Barrett & Schluter 2008). In either case,  $p_{\max}$  of different replicated  
89 systems that vary in age should align, i.e. show a small or zero angle  $\theta$  between  
90 them (Figure S1). Alternatively,  $\theta$  between mainly genetically determined  $p_{\max}$   
91 may evolve over time through selection and drift (Lande & Arnold 1983; Jones *et*  
92 *al.* 2004; Arnold *et al.* 2008).  $\theta$  is thus expected to subsequently increase over  
93 time between an ancestral  $p_{\max}$  and the  $p_{\max}$  of a derived population that evolves  
94 towards a new adaptive peak (Lande & Arnold 1983; Jones *et al.* 2004; Arnold *et*  
95 *al.* 2008).

96

97 In threespine stickleback (*Gasterosteus aculeatus* species complex), the ancestral  
98 marine population repeatedly colonized freshwater throughout its distribution  
99 mainly after the last glacial maximum and subsequently adapted to different  
100 habitats such as streams and lakes. The result was a complex of phenotypically  
101 and ecologically divergent populations and, in some cases, even sympatric or  
102 parapatric species (Bell & Foster 1994; McKinnon & Rundle 2002). Alongside the  
103 marine-freshwater transition, adaptive changes in both the **G** and the **P** matrix  
104 have been recorded (Berner *et al.* 2010b; Leinonen *et al.* 2011), where  $g_{\max}$  and  
105  $p_{\max}$  are correlated (Leinonen *et al.* 2011). The parallel evolution of distinct  
106 parapatric lake-stream pairs within freshwater has made this species complex an  
107 excellent system to investigate the process of ecological speciation. However, on  
108 an evolutionary timescale, most studies use relatively old systems and are often  
109 limited one restricted geographical area (e.g. Reimchen *et al.* 1985; Hendry &  
110 Taylor 2004; Berner *et al.* 2008; Kaeuffer *et al.* 2012; Ravinet *et al.* 2013, but see  
111 Berner *et al.* 2010a; Ravinet *et al.* 2013; Lucek *et al.* 2013; Hendry *et al.* 2013). In

112 contrast, some lake-stream systems became only recently available to  
113 stickleback, e.g. due to contemporary translocations (Lucek *et al.* 2010; Berner *et*  
114 *al.* 2010a; Moser *et al.* 2012; Lucek *et al.* 2012a) or the creation of artificial lakes  
115 (Kristjánsson *et al.* 2002a; Hendry *et al.* 2013). Hence, stickleback provide a rare  
116 opportunity to study the evolution of parapatric divergence along the lake-  
117 stream habitat axis and the corresponding changes in the **P** matrix and  $p_{\max}$  over  
118 a wide timescale, ranging from decades to millennia.

119

120 Here, we study replicated parapatric lake-stream stickleback from Switzerland  
121 and Iceland that are between 50 and 10,000 years old in relation to their  
122 putative ancestral marine population. Using this temporal gradient, we test if  
123 phenotypic divergence emerges rapidly after the colonization of novel  
124 environments and whether lines of least resistance ( $p_{\max}$ ) diverge over time as  
125 suggested by several authors (Lande & Arnold 1983; Jones *et al.* 2004; Arnold *et*  
126 *al.* 2008). Additionally, the large geographic scale coupled with the very different  
127 colonization histories of Iceland and Switzerland (Ólafsdóttir *et al.* 2007a; Lucek  
128 *et al.* 2010) allows us to test for parallel evolution. Specifically, we can test if  
129 parapatric phenotypic divergence resulted in similar  $p_{\max}$  and if the degree and  
130 the direction of habitat dependent phenotypic divergence are similar among our  
131 studied systems. We predict that habitat dependent phenotypic changes in  
132 similar environments should result in similar  $p_{\max}$  but that the degree of  
133 phenotypic divergence may differ due to different historical contingencies, the  
134 time for selection to act or differences in the selective regimes among our  
135 studied systems.

136

137 **Material and Methods**138 *Sample collection*

139 We studied three Swiss lake-stream systems in the invasive range of stickleback  
140 that differ in their ages of stickleback colonization (Bern (Wohlen): ~50 yrs,  
141 Constance: 140 yrs, Geneva: 140 yrs) and represent either independent  
142 introductions from different freshwater lineages (Constance, Geneva) or a case of  
143 recent admixture of these lineages (Bern; see Lucek *et al.* 2010 for details). In  
144 addition, we studied three Icelandic lake-stream systems that differ in their  
145 geological age (Mývatn: 2500 yrs, Thingvallavatn: 8000-10'000 yrs;  
146 Saemundsson 1992; Einarsson *et al.* 2004) or are man-made (Hraunsfjörður: 50  
147 yrs; Kristjánsson *et al.* 2002b) and have been separately colonized by stickleback  
148 from ancestral marine populations. We also sampled two Icelandic marine  
149 populations (Table S1), presumably resembling the phenotypic marine ancestral  
150 state to most European freshwater stickleback (Mäkinen *et al.* 2006; Jones *et al.*  
151 2012a). In Icelandic lakes, stickleback have been described to diverge in relation  
152 to benthic substrate (Kristjánsson *et al.* 2002b). We thus sampled the largest  
153 potential habitats in each lake (see Figure 1 and Table S1 for sampling locations).  
154  
155 Icelandic samples were obtained between August and September 2010 using  
156 minnow traps and by hand netting. Samples from Switzerland were similarly  
157 collected in 2007 and 2008 (Lucek *et al.* 2010). In all cases, stream stickleback  
158 were obtained from inflowing streams (Table S1). All fish were sacrificed with an  
159 overdose of clove oil and stored in 70% ethanol. A fin clip was additionally taken  
160 for genetic analysis and preserved in absolute ethanol. Sample size per site  
161 ranged from 17 to 62 (mean:  $35 \pm 10$  SD) with a total of 918 individuals from 26



162 sites (Figure 1, Table S1). Altitudinal difference and pairwise waterway distance  
163 between each stream site and the inflow of the stream into the lake were  
164 measured using GOOGLEEARTH (Google, USA).

165

#### 166 *Genetic analysis*

167 We extracted DNA for individuals from the Marine 1 site and all freshwater sites,  
168 except for Mývatn, where only one of the three stream sites (MS1) was available  
169 for genetic analysis ( $N_{\text{Total}} = 727$ , Table S1). DNA was extracted using a 10%  
170 Chelex solution, following the manufacturer's protocol (Biorad, California, USA).

171 In some cases, additional individuals were included for which no phenotypic data  
172 was collected (Table S1). We amplified ten microsatellite markers in one

173 multiplex set following the protocols of Raeymaekers *et al.* (2007). Three of

174 these markers (Stn26, Stn96 and Stn130) have been shown to be associated with

175 known QTLs for spine lengths (Peichel *et al.* 2001). Detailed information on

176 marker identity, the multiplexing setup, and the PCR protocol are provided as

177 supplementary methods. We visualized alleles on an ABI 3130XL and scored

178 them with GENEMAPPER 4.0 (Applied Biosystems, Switzerland). We generated a

179 genetic tree-like relationship among sampling sites based on their Cavalli-Sforza

180 distances of allelic frequencies using a neighbour-joining algorithm implemented

181 in PHYLIP 3.69 (Felsenstein 2012). Significance was estimated using 1000

182 bootstrapped resampling replicates. To test if our markers conformed to neutral

183 expectations, we conducted an  $F_{ST}$  based outlier test using the software LOSITAN

184 1.0 (Antao *et al.* 2008) separately for each lake-stream system. Using GENODIVE

185 2.0 (Meirmans & Van Tienderen 2004), we calculated pairwise  $F_{ST}$  between

186 parapatric lake and stream populations for all systems, pooling all sampling sites

187 within a lake. We estimated significances using 1000 bootstrapped replicates as  
188 implemented in GENODIVE. Finally, we tested for a correlation of the obtained  
189 pairwise parapatric  $F_{ST}$  values with either the altitudinal difference or the  
190 geographical distance between a parapatric stream site and the lake using linear  
191 models. Models were compared using the Akaike information criterion corrected  
192 for small sample sizes (AICc).

193

#### 194 *Morphological data collection & analysis*

195 We measured sixteen linear morphological traits (see Figure S2 for details),  
196 many of which are known to be associated with ecological diversification in  
197 stickleback (see Kristjánsson *et al.* 2002a; Mori & Takamura 2004; Berner *et al.*  
198 2008; Leinonen *et al.* 2011 and references therein), on the left side of each fish to  
199 the nearest 0.01 mm using a digital caliper. These traits were related to either  
200 anti-predator defense (FSL - length of the first dorsal spine; DSL - length of the  
201 second dorsal spine; PSL - length of the pelvic spine; PGL - length of the pelvic  
202 girdle), feeding (HL - head length; UJL - upper jaw length; SnL - snout length;  
203 SnW - snout width; ED - eye diameter), or body shape and swimming  
204 performance (SL - standard length; PGW - width of the pelvic girdle; BD1 - body  
205 depth measured after the first dorsal spine; BD2 - body depth measured after the  
206 second dorsal spine; CPL - caudal peduncle length; BLA - basal length of the anal  
207 fin; BLD - basal length of the dorsal fin; TLP - total length of the pelvic fin). We  
208 measured two additional feeding related traits: the length of the lower gill arch  
209 (AL) and the length of the second gill raker (GRL2), as counted from the joint of  
210 the dorsal arch bone on the first lower gill arch (Berner *et al.* 2008). Both  
211 measurements on the gill arch were done using a micrometer mounted on a

212 dissection microscope. Because all traits were significantly correlated with SL  
213 (results not shown), we size-corrected the data by using the residuals from a  
214 regression of each trait against SL. This regression was either performed pooling  
215 all individuals for the overall comparison of populations or separately (i.e. for  
216 each lake-stream system and for the marine population) for pairwise  
217 comparisons. By pooling all systems and populations, allometric information in  
218 some populations may be retained if the allometric trajectories differ among  
219 them. This allows, however, to estimate system and population specific  
220 components of phenotypic variation, which can be explained by different  
221 historical contingencies or differences in the selective regimes.

222

223 To estimate the relative contributions of *country* (Iceland or Switzerland), lake-  
224 stream *system* (Bern, Constance, Geneva, Hraunsfjörður, Mývatn,  
225 Thingvallavatn), *habitat* (lake or stream) and the interaction of *system x habitat*  
226 on diversification within freshwater, we calculated the percentage of non-error  
227 variance based on the respective sums of squares using a sequential ANOVA  
228 model (Langerhans & DeWitt 2004; Eroukhmanoff *et al.* 2009; Lucek *et al.* 2013).  
229 Here, *country* should reflect variation due to different historical contingencies,  
230 which include differences in past selection regimes, or differences between the  
231 current selective regimes between Switzerland and Iceland. Similarly, *system*  
232 accounts for the variation among isolated lake-stream systems. The *habitat* term  
233 reflects the component of parapatric phenotypic divergence that is replicated  
234 among systems, i.e. parallel. Finally, the *system x habitat* interaction accounts for  
235 interactions between system differences (colonization history and  
236 environmental differences) and habitat dependent phenotypic divergence within

237 systems (Langerhans & DeWitt 2004; Eroukhmanoff *et al.* 2009). To further  
238 compare the overall phenotypic divergence among all sampled sites, we  
239 constructed a tree like relationship using pairwise Mahalanobis distances based  
240 on the overall size-corrected phenotypic measurements.

241

242 Because local adaptation can lead to phenotypic differentiation between  
243 populations of the same ecotype (i.e. within one habitat type; Hendry & Taylor  
244 2004; Kaeuffer *et al.* 2012; Ravinet *et al.* 2013), all individuals from the same  
245 habitat were pooled if more than one site was sampled in a given lake or in a  
246 given stream system to estimate the overall degree of habitat dependent  
247 phenotypic divergence. We estimated the parapatric phenotypic divergence  
248 using  $P_{ST}$ , an analog to  $Q_{ST}$  (Spitze 1993) based on phenotypic measurements  
249 from wild individuals, which serves as a unit-less proportional measure of  
250 pairwise phenotypic divergence and is analogous to our measure of pairwise  
251 genetic divergence ( $F_{ST}$ ). Following Leinonen *et al.* (2006), we estimated  $P_{ST}$  as  
252  $P_{ST} = \delta^2_{GB} / (\delta^2_{GB} + 2 * (h^2 * \delta^2_{GW}))$ , where  $\delta^2_{GB}$  and  $\delta^2_{GW}$  are the between population  
253 and within population variance components for a specific trait and  $h^2$  is  
254 heritability. For stickleback, only few heritability estimates are available, which  
255 differ among the studied populations (e.g. Baumgartner 1995; Leinonen *et al.*  
256 2011). We thus assumed a full trait heritability ( $h^2=1$ ), which provides a  
257 conservative estimate for  $P_{ST}$  (Leinonen *et al.* 2006). For each  $P_{ST}$ , the 95%  
258 confidence interval was established using a resampling approach of 1000  
259 replicates.  $P_{ST}$ s were either based on the residuals of the leading principal  
260 component (PC) axis, combining all traits or a combination of traits, related to  
261 functionally different groups (defense, feeding, body shape and swimming

262 performance) as well as separately for each trait. For cases where the 95%  
263 confidence interval exceeded zero, the directionality of a  $P_{ST}$  was further  
264 assessed by comparing the mean trait values between the different ecotypes.  
265  
266 We calculated the leading eigenvector ( $p_{max}$ ) of the phenotypic variance-  
267 covariance matrix  $\mathbf{P}$  based on a PC analysis for each habitat and freshwater  
268 system using traits that were separately size corrected for each system and the  
269 marine population. For the marine populations, we pooled both sites to obtain a  
270 better estimate of the putative ancestral state of stickleback. In addition, we  
271 calculated the overall  $p_{max}$  for each freshwater system, where we pooled fish  
272 from lake and stream sites. By pooling individuals from distinct populations and  
273 habitats, the calculated  $\mathbf{P}$  matrix may differ from the  $\mathbf{P}$  matrices of each habitat  
274 population as traits may covary between populations from different habitats  
275 even if they do not covary within either of the populations.  $p_{max}$  of such overall  $\mathbf{P}$   
276 matrices may however be compared among each other to test if parapatric  
277 phenotypic divergence results in similar and hence predictable patterns. We  
278 compared  $p_{max}$  of two  $\mathbf{P}$  matrices by calculating the angle  $\theta$  between them  
279 following Schluter (1996), where  $\theta$  is the inversed cosine of the dot product of  
280 two leading eigenvectors that is divided by the summed length of both  
281 eigenvectors. We estimated  $\theta$  between parapatric lake and stream ecotypes for  
282 all ecotype pairs, between the freshwater ecotypes and our marine populations,  
283 as well as between our lake-stream systems. In the latter case, we pooled all  
284 populations (lake and stream) from each lake-stream system. The significance of  
285  $\theta$  between  $p_{max}$  of all comparisons was estimated using a bootstrap procedure  
286 with 1000 replicates following Berner (2009). The obtained values for  $\theta$  were

287 then correlated with the time since stickleback colonized each system using  
288 linear models. All statistical analyses were performed in R 2.15.1 (R Core Team,  
289 2012).

290

## 291 **Results**

### 292 *Genetic divergence*

293 The genetic tree indicates differentiation among our studied freshwater systems,  
294 where Iceland and Switzerland form distinct clades (Figure 2a). In the Icelandic  
295 clade, the Marine 1 population falls next to the Hraunsfjörður branch, which is  
296 consistent with the very recent origin of this system (Kristjánsson *et al.* 2002b;  
297 Ólafsdóttir *et al.* 2007b), whereas both Mývatn and Thingvallavatn form distinct  
298 branches. Genetic substructure among the different sampling sites was  
299 furthermore indicated in all Icelandic systems. In the Swiss clade, the Bern  
300 system falls next to the Constance system, which are both distinct from the  
301 Geneva system, reflecting their introduction and admixture history (Lucek *et al.*  
302 2010).

303

304 The outlier tests performed separately for each lake-stream system suggested  
305 that none of the markers deviated from neutral expectations (results not shown).  
306 Therefore all markers were retained for the population genetic analyses. Habitat  
307 dependent parapatric genetic differentiation was highest in the Lake Geneva  
308 system in Switzerland ( $F_{ST} = 0.053$ ,  $p < 0.001$ ), which also showed the greatest  
309 differences in altitude ( $\Delta_{\text{Altitude}}: 108$  m) and distance to the lake (61 km). All  
310 parapatric ecotypes except Bern ( $F_{ST} = 0.000$ , n.s.) showed genetic differentiation  
311 (Constance:  $F_{ST} = 0.018$ ,  $p = 0.017$ ; Hraunsfjörður:  $F_{ST} = 0.009$ ,  $p = 0.006$ ; Mývatn:

312  $F_{ST} = 0.028, p < 0.001$ ; Thingvallavatn:  $F_{ST} = 0.018, p = 0.009$ ). Pairwise  $F_{ST}$   
313 between parapatric lake and stream populations was significantly correlated  
314 with both altitudinal differences between sites ( $R^2 = 0.823, F_{1,4} = 18.6, p = 0.013$ )  
315 and distance to the lake ( $R^2 = 0.784, F_{1,4} = 14.5, p = 0.019$ ). These explanatory  
316 factors were significantly correlated with each other ( $R^2 = 0.922, F_{1,4} = 47.2, p =$   
317  $0.002$ ) and fitted the linear model equally well ( $\Delta AICc = 1.22$ ).

318

### 319 *Historical contingency and divergence in freshwater*

320 The trait based ANOVA models all explained a significant amount of phenotypic  
321 variation (all  $p < 0.001$ , results not shown; Table 1). The highest proportion of  
322 non-error variation was explained by historical contingency or differences in the  
323 current selective regimes between Iceland and Switzerland (*country*:  $37.4\% \pm$   
324  $23.0\%$ ; *system*:  $35.1\% \pm 18.4$ ; Table 1), where Swiss and Icelandic stickleback  
325 differed most strongly in defense related traits and to a lesser extent in feeding  
326 related traits. Variation explained by *system* was highest for body shape related  
327 traits. System specific components of parapatric lake-stream divergence  
328 occurred especially for feeding and to a lesser extent for body shape related  
329 traits as indicated by the *system* x *habitat* interaction. *Habitat* alone explained  
330 only a small fraction of the variance ( $4.4\% \pm 5.4\%$ ), where the traits TLP and BLA  
331 had the largest amount of variance explained.

332

333 The occurrence of individual trait based parapatric phenotypic divergence ( $P_{ST}$ )  
334 and the overall dimensionality of parapatric divergence, measured as the  
335 number of traits with significant parapatric  $P_{ST}$ , differed among systems and  
336 countries. Similarly, the directionality of the trait divergence between lake and

337 stream differed among Swiss and Icelandic ecotype pairs, and to a lesser extent  
338 also between ecotype pairs in different lake-stream systems within either  
339 country (Figure 3). In the two oldest Icelandic lakes,  $P_{ST}$  exceeded zero for 14  
340 (Thingvallavatn) and 16 (Mývatn) out of 18 traits. However, even in the 50 year  
341 old Hraunsfjörður system, this was true for 6 traits. In Switzerland, significant  
342 trait specific  $P_{ST}$ s were observed only in the slightly older Constance and Geneva  
343 systems, especially for defense traits (4 and 7 traits respectively), whereas  $P_{ST}$   
344 did not exceed zero for any of the traits in the Bern system which stickleback  
345 colonized 50 years ago. The PC based  $P_{ST}$  combining either all traits or only  
346 defense related traits, exceeded zero in all but the two youngest systems (Figure  
347 3). In contrast,  $P_{ST}$  exceeded zero only in Mývatn for feeding related morphology  
348 and in the Icelandic systems for body shape and swimming performance related  
349 traits. The magnitude of  $P_{ST}$  among parapatric ecotypes was not statistically  
350 associated with the altitudinal difference, with the waterway distance between  
351 sites, or with the age of a system for any trait combinations (all  $p > 0.1$ , results  
352 not shown).

353

354 The angle  $\theta$  between  $p_{max}$  from parapatric lake and stream populations based on  
355 all phenotypic traits differed from zero in all cases except Thingvallavatn (Figure  
356 4, Table 2), whereas it was greatest in the two other Icelandic systems (Table 2),  
357 whose  $p_{max}$  were significantly differentiated from all other freshwater systems in  
358 the pairwise comparisons (Table 3). In the Swiss systems, the parapatric  $\theta$ s were  
359 significantly different from zero (Table 2), whereas  $p_{max}$  did not differ among the  
360 systems (Table 3). When traits were analyzed by functional categories, the angle  
361  $\theta$  between parapatric lake-stream  $p_{max}$  differed across traits and systems (Figure



362 4, Table 2).  $\theta$  between parapatric ecotypes differed especially for feeding related  
363 traits, albeit to a small degree (average  $\theta$ :  $9.3^\circ \pm 2.3^\circ$  SD), whereas parapatric  $p_{max}$   
364 differed less commonly for defense, body depth and swimming performance  
365 related traits (Table 2).  $p_{max}$  were furthermore comparable among lake-stream  
366 systems for feeding and defense related traits as suggested by the non-significant  
367 angle  $\theta$  between them (Table 3). None of the angles between parapatric ecotypes  
368 were statistically correlated with the time since stickleback colonization, the  
369 altitudinal difference or the geographical distance between the lake and the  
370 stream populations (all  $p > 0.1$ , results not shown).

371

372 *Parallel adaptation trumps historical contingency late but not early in ecotype*  
373 *formation*

374

375 Mahalanobis distances showed overall consistent morphological differentiation  
376 between Swiss and Icelandic freshwater stickleback populations (Figure 2b).  
377 Despite the aforementioned evidence for consistent parapatric divergence, all  
378 populations of young lake-stream pairs, i.e. Hraunsfjörður in Iceland and all the  
379 Swiss systems, clustered by historical lineage rather than by ecotype. In contrast,  
380 the populations from the two oldest lake systems, i.e. Mývatn and Thingvallavatn,  
381 clustered strongly by ecotype despite being genetically more strongly  
382 differentiated than the lineages with young lake-stream pairs (Figure 2a).

383

384 *Phenotypic divergence during the marine-freshwater transition*

385 The angle  $\theta$  between  $p_{max}$  of the different freshwater lake populations differed  
386 significantly in all replicates when pooling all traits (average  $\theta$ :  $59.7^\circ \pm 6.5^\circ$  SD;

387 Figure 5, Table 2), which was not true for stream populations (average  $\theta$ :  $29.7^\circ \pm$   
388  $21.0^\circ$  SD). Using only feeding related traits, the freshwater  $p_{max}$  differs commonly  
389 from the marine one with relatively low angles  $\theta$  (lake vs. marine: average  $\theta =$   
390  $9.2^\circ \pm 3.3^\circ$  SD; stream vs. marine: average  $\theta = 7.8^\circ \pm 2.0^\circ$  SD)). In contrast, the  
391 freshwater  $p_{max}$  based on defense or body shape and swimming performance  
392 related traits differed less commonly from the marine  $p_{max}$  (Figure 5, Table 2). In  
393 all but one case (marine vs. lake populations using all traits combined:  $F_{1,4} = 15.9,$   
394  $p = 0.016$ ), the observed angle  $\theta$  between a freshwater derived  $p_{max}$  and the  
395 marine  $p_{max}$  were not statistically correlated with the relative age of each  
396 freshwater system (all  $p > 0.1$ , results not shown).

397

## 398 Discussion

399

400 The extent of parallel evolution of phenotypically similar ecotypes depends on  
401 the genetic constraints, the selective environment and the time for evolution to  
402 act (Schluter & Nagel 1995; Langerhans & DeWitt 2004; Nosil *et al.* 2009;  
403 Kaeuffer *et al.* 2012; Nosil 2012). Nonparallel phenotypic features may thus  
404 occur between independently evolved yet ecologically similar ecotypes. The  
405 extent of convergent evolution and hence the degree to which two independent  
406 populations become more similar may further depend on the dimensionality  
407 with which ecotypic divergence is being measured. Rapid evolution may be  
408 inferred to be parallel when only few traits are being measured (e.g. Schluter *et*  
409 *al.* 2004), whereas parallel divergence in the multivariate phenotype, leading to  
410 overall phenotypic convergence of parallel evolved ecotypes, may need much  
411 longer time (Young *et al.* 2009; Kolbe *et al.* 2011).

412

413 Comparing the phenotypic variance-covariance (**P**) matrices of different  
414 stickleback freshwater ecotypes and their marine ancestors, we find that  
415 phenotypic divergence can result in parallel lines of least resistance ( $p_{\max}$ ) both  
416 for the marine-freshwater transition (Figure 5, Table 2) and the subsequent  
417 ecotype formation within freshwater (Figure 4, Table 3). The extent and  
418 parallelism of parapatric ecotype formation within freshwaters seems to be  
419 driven by historical contingency, potential differences in the divergent selective  
420 regimes between lake and streams and the time available for evolution, where  
421 much of the phenotypic variation is explained by differences between Icelandic  
422 and Swiss sticklebacks (Figure 2, Table 1). Parallel ecotypic divergence may  
423 trump historical contingency only in the oldest lakes, where the divergent  
424 selective regimes may moreover be strongest, an increased dimensionality of  
425 ecotypic differentiation and a clustering of ecotypes despite being genetically  
426 very distinct (Figure 2 & 3).

427

#### 428 *The evolution of freshwater stickleback*

429 The evolutionary transition between the marine and freshwater environment  
430 has been repeatedly studied in stickleback (e.g. Kristjánsson 2005; Leinonen *et*  
431 *al.* 2006; Wund *et al.* 2008; Berner *et al.* 2010b; Leinonen *et al.* 2011; Jones *et al.*  
432 2012a; Voje *et al.* 2013). Colonizing freshwater habitats requires adaptation to  
433 new selective regimes, which may differ between distinct freshwater habitats  
434 (Gross 1978; Gross & Anderson 1984; Reimchen 1994; Berner *et al.* 2009; 2010b,  
435 Lucek *et al.* 2014). The degree of phenotypic divergence from an ancestral-like  
436 marine population may consequently differ between distinct habitats and among

437 traits due to differences in the selective regime and the colonization history. In  
438 concordance, we find that the degree of phenotypic and genetic differentiation  
439 differs among systems (Figure 2). In the genetic tree, the two oldest lakes Mývatn  
440 and Thingvallavatn form distinct genetic clusters with the longest branch  
441 lengths, whereas the Swiss populations, albeit being genetically distinct from  
442 each other, form a separate branch. Conversely, ecotype specific clusters occur  
443 for the old lakes in the phenotypic tree, whereas all populations of young ecotype  
444 pairs cluster in concordance with their genetic lineage. Lineage dependent  
445 phenotypic constraints may have consequently been retained in Switzerland as  
446 the Constance and Geneva systems were colonized about 140 years ago by  
447 genetically distinct freshwater lineages and the Bern system lies in a hybrid zone  
448 between different lineages (Lucek *et al.* 2010). In contrast, Icelandic freshwater  
449 populations likely derive from a common marine population, where in some  
450 cases gene flow from the ancestral marine population may still be possible  
451 (Ólafsdóttir *et al.* 2007c, Figure 2a).

452

453 Historical contingency or potential differences in current selective regimes for  
454 different trait categories are further indicated by the changes in the **P** matrices  
455 among the different marine-freshwater comparisons. Here, patterns for the  $p_{\max}$   
456 of freshwater stickleback vary generally among all comparisons (Table 2, Figure  
457 5). Whereas  $p_{\max}$  of freshwater populations differ commonly from the marine  
458  $p_{\max}$  when all phenotypic traits were combined, they are similar to the marine  
459 one in all comparisons for anti-predator related traits. Predator communities are  
460 thought to differ though, where marine and freshwater lake populations  
461 experience a predation regime dominated by gape limited predators such as

462 birds and piscivorous fish (Gross 1978; Reimchen 1992), which shifts to  
463 increased insect predation in freshwater streams (Reimchen 1994; Marchinko  
464 2009). Invertebrate predation may however be negligible in Iceland (Lucek et al.  
465 2012b) and empirical evidence for the role of invertebrate predators as a source  
466 of selection is mixed for Swiss populations (Zeller et al. 2012a,b).

467

468 For trophic morphology on the other hand, freshwater  $p_{\max}$  differ commonly  
469 from the marine one with small but significant angles  $\theta$ , suggesting that the  
470 marine-freshwater transition may be generally associated with a change in the **P**  
471 matrix (Figure 5). Habitat dependent ecotypic differentiation in stickleback is  
472 indeed thought to be coupled with a change in diet and trophic morphology,  
473 where marine and some freshwater lake populations forage commonly on  
474 zooplankton in contrast to stream fish and some lake populations that feed on  
475 benthic prey (Gross & Anderson 1984; Berner *et al.* 2009; Kaeuffer *et al.* 2012;  
476 Lucek *et al.* 2012a). The small but significant angles  $\theta$ , which we observe  
477 especially for lake populations are consistent with prior findings in Canadian  
478 stickleback, where freshwater lake populations have been shown to have a  $p_{\max}$   
479 that has diverged from the marine one, involving a shift in gill raker lengths  
480 (Berner *et al.* 2010b). Lastly, differences in the selective regimes between our  
481 studied systems and countries may account for the observed changes in  $p_{\max}$  for  
482 body shape and swimming related traits that are linked to different foraging  
483 strategies in lakes and streams (Hendry & Taylor 2004; Reid & Peichel 2010;  
484 Hendry *et al.* 2011). The wide range for  $\theta$  for both the overall system and stream  
485 populations may further reflect different selection regimes for each stream due

486 to environmental differences such as differences in flow regimes (Steppan *et al.*  
487 2002; Ravinet *et al.* 2013).

488

489 *Contingency, selection and parallelism during lake-stream divergence*

490 Both the occurrence and the extent of parapatric divergence depend mainly on  
491 the underlying environmental and selective gradient and the time for evolution  
492 to act (Endler 1977; Doebeli & Dieckmann 2003; Nosil *et al.* 2009). Parallel  
493 parapatric divergence is consequently only expected under comparable selective  
494 regimes (Kaeuffer *et al.* 2012) where selection is acting on a similar gene pool  
495 (Barrett & Schluter 2008). The repeated formation of parapatric lake-stream  
496 freshwater stickleback systems has been proposed to provide such a case  
497 (Reimchen *et al.* 1985; Thompson *et al.* 1997; Hendry & Taylor 2004; Berner *et*  
498 *al.* 2009; Lucek *et al.* 2013). However, recent studies find non-parallelisms in the  
499 realized divergence that occur both on smaller geographical scales (Kaeuffer *et*  
500 *al.* 2012; Hendry *et al.* 2013; Ravinet *et al.* 2013; Lucek *et al.* 2013) as well as  
501 between continents (Berner *et al.* 2010a). In the latter case, the authors  
502 suggested that genomic constraints could be responsible for the observed lower  
503 degree of divergence among Swiss populations and the evolutionary younger  
504 Atlantic stickleback lineage in general, where only the Constance system showed  
505 a level of divergence that is comparable to older Canadian systems (Berner *et al.*  
506 2010a; Ravinet *et al.* 2013, but see Lucek *et al.* 2013). However, the respective **P**  
507 matrices have not been compared.

508

509 Our results suggest that the evolution of parapatric lake-stream populations in  
510 stickleback can result in common and hence predictable  $p_{\max}$  independent of the

511 age of a system as it is indicated by the non-significant angles between the  
512 overall  $p_{\max}$  of different lake-stream systems for defense and feeding related  
513 traits (Table 3). Thus ecotype formation along parallel axes may start quickly.  
514 However, although parapatric lake-stream systems share similar  $p_{\max}$ , only a  
515 relatively small fraction of the overall phenotypic variation can be attributed to  
516 parallel habitat dependent differentiation (Table 1), where the directionality of  
517 parapatric divergence often differs between ecotype pairs in Switzerland and  
518 Iceland and sometimes also between pairs from different lake-stream systems  
519 within each country (Figure 3). In contrast, a much larger fraction is explained by  
520 the system and habitat interaction and thus the combined effect of system-  
521 related historical contingency and/or system-related selection with ecotypic  
522 divergence (Langerhans & DeWitt 2004; Eroukhmanoff *et al.* 2009; Kaeuffer *et al.*  
523 2012). The increased dimensionality of parapatric differentiation in the two  
524 oldest lake systems, Mývatn and Thingvallavatn (Figure 3), may either reflect  
525 stronger habitat dependent divergent selection or that longer time is needed for  
526 a parallel evolutionary response to similar divergent selection during ecotype  
527 formation to trump historical contingency (Nosil *et al.* 2009; Young *et al.* 2009;  
528 Nosil 2012). The increase in dimensionality further suggests that parallel  
529 phenotypic divergence is associated with increased phenotypic integration  
530 (Figure 3), which is consistent with studies on older adaptive radiations that  
531 show increased convergence in multivariate trait dimensions in comparison to  
532 younger radiations (Young *et al.* 2009; Kolbe *et al.* 2011).

533

534 In contrast to the observed phenotypic divergence and convergence, the degree  
535 of neutral parapatric genetic differentiation is correlated with the parapatric

536 environmental gradient rather than the evolutionary age of the system.  
537 Altitudinal gradients have similarly been found to explain the degree of  
538 parapatric genetic divergence in other freshwater systems (Caldera & Bolnick  
539 2008; Ravinet *et al.* 2013) as well as during the marine and freshwater transition  
540 (Deagle *et al.* 2013, Lucek *et al. unpublished data*) and may be linked to physical  
541 barriers restricting the potential for gene flow.

542

#### 543 *Rapid evolution versus plasticity*

544 Although phenotypic divergence was greatest in the oldest lakes, the observed  
545 differentiation in  $p_{\max}$  was not associated with our studied temporal gradient.  
546 Hence, plasticity could have initially promoted the colonization of freshwater  
547 habitats (Smith & Skúlason 1996) by rapidly shifting  $p_{\max}$  (Lande 2009; Draghi &  
548 Whitlock 2012). Marine stickleback are known to be phenotypically plastic,  
549 allowing them to respond to different diets readily when colonizing new  
550 freshwater environments (Wund *et al.* 2008). Plasticity can furthermore evolve  
551 in freshwater to initially promote a generalist life style where divergent selection  
552 may then lead to canalization and a reduction in plasticity (Svanbäck & Schluter  
553 2012), matching theoretical predictions (Lande 2009; Thibert-Plante & Hendry  
554 2011). In theory, however, phenotypic plasticity and thus  $p_{\max}$  may evolve quite  
555 fast, i.e. over fewer generations than those separating the lake and stream  
556 populations in our youngest system (Lande 2009; Draghi & Whitlock 2012).  $p_{\max}$   
557 based on phenotypically plastic traits may thus align if populations experience a  
558 comparable selective regime as we observe for defense and feeding related traits.  
559



560 Phenotypic shifts during the marine-freshwater transition as well as between  
561 distinct freshwater habitats in stickleback have similarly been suggested to occur  
562 through recurrent selection on standing genetic variation in the marine  
563 population (Deagle *et al.* 2012; Jones *et al.* 2012b). This is especially true for anti-  
564 predator related phenotypic shifts, where selection drives phenotypic  
565 divergence over only a few generations (Bell *et al.* 2004; Barrett *et al.* 2008;  
566 Schluter & Conte 2009) and may similarly account for phenotypic divergence in  
567 other genetically determined traits such as gill rakers (Hermida *et al.* 2002). Our  
568 observed parapatric divergence as well as the changes in the **P** matrix for these  
569 traits may therefore be a combined result of both plasticity and adaptation from  
570 standing genetic variation (Wund *et al.* 2008; Eroukhmanoff & Svensson 2011,  
571 Lucek *et al.* 2014). Indeed, empirical evidence suggests that although  $p_{\max}$  and  
572  $g_{\max}$  are correlated in stickleback,  $p_{\max}$  can only approximate  $g_{\max}$  and hence the  
573 underlying evolutionary constraints (Leinonen *et al.* 2011).

574

### 575 *Conclusions*

576 Our results suggest that parapatric ecotype formation can result in parallel and  
577 hence predictable  $p_{\max}$  for some trait combinations, i.e. trophic morphology, but  
578 that the directionality of change may differ for others due to historical  
579 contingency or environmental effects. Whereas changes in the **P** matrix during  
580 the marine-freshwater transition seem to evolve independently of our studied  
581 temporal axis, both the extent and the dimensionality of parapatric ecotype  
582 formation depend on the available time for evolution to occur. Thus evolutionary  
583 changes towards novel adaptive peaks may occur readily during ecotype

584 formation and may be aided by phenotypic plasticity, yet convergent phenotypic  
585 evolution needs time to overcome contingency.

586

587 **Acknowledgment**

588 This research was supported by an European Science Foundation Exchange  
589 Grant in the FroSpects program (grant number 2912) to KL and a Swiss National  
590 Science Foundation Grant 31003A-118293 to OS. We thank Mélissa Lemoine,  
591 Fabrice Eroukhmanoff and Pamela Woods for constructive feedback and  
592 suggestions. Andrew Hendry, Catherine E. Wagner, Julia Schwarzer and one  
593 anonymous reviewer provided valuable comments on earlier versions of the  
594 manuscript.

595 **References**

- 596 Antao, T., A. Lopes, R. J. Lopes, A. Beja-Pereira, & Luikart G. 2008. LOSITAN: a  
597 workbench to detect molecular adaptation based on a Fst-outlier method. *BMC*  
598 *Bioinf* **9**:323.
- 599 Arnold, S. J. & Phillips P. C. 1999. Hierarchical comparison of genetic variance-  
600 covariance matrices. II. Coastal-inland divergence in the garter snake,  
601 *Thamnophis elegans*. *Evolution* **53**:1516–1527.
- 602 Arnold, S. J., Bürger R., Hohenlohe P. A., Ajie B. C. & Jones A. G. 2008.  
603 Understanding the evolution and stability of the G-matrix. *Evolution* **62**:2451–  
604 2461.
- 605 Bacigalupe, L. D. 2009. Biological invasions and phenotypic evolution: a  
606 quantitative genetic perspective. *Biol Inv* **11**:2243–2250.
- 607 Barrett, R. D. H. & Schluter D. 2008. Adaptation from standing genetic variation.  
608 *Trends Ecol Evol* **23**:38–44.
- 609 Barrett, R. D. H., Rogers S. M. & Schluter D. 2008. Natural selection on a major  
610 armor gene in threespine stickleback. *Science* **322**:255–257.
- 611 Baumgartner, J. V. 1995. Phenotypic, genetic, and environmental integration of  
612 morphology in a stream population of the threespine stickleback, *Gasterosteus*  
613 *aculeatus*. *Can J Fish Aquat Sci* **52**:1307–1317.
- 614 Bell, M. A. & Foster S. A. 1994. The Evolutionary Biology of the Threespine  
615 Stickleback. Oxford University Press, Oxford, UK.

- 616 Bell, M. A., Aguirre W. & Buck N. 2004. Twelve years of contemporary armor  
617 evolution in a threespine stickleback population. *Evolution* **58**:814–824.
- 618 Berner, D. 2009. Correction of a bootstrap approach to testing for evolution along  
619 lines of least resistance. *J Evol Biol* **22**:2563–2565.
- 620 Berner, D., Grandchamp A.-C. & Hendry A. P. 2009. Variable progress toward  
621 ecological speciation in parapatry: stickleback across eight lake-stream  
622 transitions. *Evolution* **63**:1740–1753.
- 623 Berner, D., Adams D. C., Grandchamp A.-C. & Hendry A. P. 2008. Natural selection  
624 drives patterns of lake-stream divergence in stickleback foraging morphology. *J*  
625 *Evol Biol* **21**:1653–1665.
- 626 Berner, D., Roesti M., Hendry A. P. & Salzburger W. 2010a. Constraints on  
627 speciation suggested by comparing lake-stream stickleback divergence across  
628 two continents. *Mol Ecol* **19**:4963–4978.
- 629 Berner, D., Stutz W. E. & Bolnick D. I. 2010b. Foraging trait (co)variances in  
630 stickleback evolve deterministically and do not predict trajectories of adaptive  
631 diversification. *Evolution* **64**:2265–2277.
- 632 Caldera, E. J. & Bolnick D. I. 2008. Effects of colonization history and landscape  
633 structure on genetic variation within and among threespine stickleback  
634 (*Gasterosteus aculeatus*) populations in a single watershed. *Evol Ecol Res* **10**:575-  
635 598.
- 636 Chapuis, E., Martin G., Goudet J. 2008. Effects of selection and drift on G matrix  
637 evolution in a heterogeneous environment: a multivariate Qst-Fst Test with the

- 638 freshwater snail *Galba truncatula*. *Genetics* **180**:2151–2161.
- 639 Cheverud, J. M. 1988. A comparison of genetic and phenotypic correlations.  
640 *Evolution* **42**:958–968.
- 641 Deagle, B. E., Jones F. C., Absher D. M., Kingsley D. M. & Reimchen T. E. 2013.  
642 Phylogeography and adaptation genetics of stickleback from the Haida Gwaii  
643 archipelago revealed using genome-wide single nucleotide polymorphism  
644 genotyping. *Mol Ecol* **22**:1917–1932.
- 645 Deagle, B. E., Jones F. C., Chan Y. F., Absher D. M., Kingsley D. M. & Reimchen T. E.  
646 2012. Population genomics of parallel phenotypic evolution in stickleback across  
647 stream-lake ecological transitions. *P R Soc B* **279**:1277–1286.
- 648 Doebeli, M. & Dieckmann U. 2003. Speciation along environmental gradients.  
649 *Nature* **421**:259–264.
- 650 Draghi, J. A. & Whitlock M. C. 2012. Phenotypic plasticity facilitates mutational  
651 variance, genetic variance, and evolvability along the major axis of  
652 environmental variation. *Evolution* **66**:2891–2902.
- 653 Einarsson, A., Stefansdottir G., Johannesson H., Olafsson J., Gislason G., Wakana I.,  
654 Gudbergsson G. & Gardarsson A. 2004. The ecology of Lake Myvatn and the River  
655 Laxa: Variation in space and time. *Aquat Ecol* **38**:317–348.
- 656 Endler, J. A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton  
657 University Press, Princeton, NY, USA.
- 658 Eroukhmanoff, F., Hargeby A., Arnberg N. N., Hellgren O., Bensch S. & Svensson E.

- 659 I. 2009. Parallelism and historical contingency during rapid ecotype divergence  
660 in an isopod. *J Evol Biol* **22**:1098–1110.
- 661 Eroukhmanoff, F. & Svensson E. I. 2011. Evolution and stability of the G-matrix  
662 during the colonization of a novel environment. *J Evol Biol* **24**:1363–1373.
- 663 Falconer, D. S. 1989. Introduction to quantitative genetics. 3rd ed. John Wiley &  
664 Sons, New York, NY, USA.
- 665 Gross, H. P. 1978. Natural selection by predators on defensive apparatus of the  
666 three-spined stickleback, *Gasterosteus aculeatus* L. *Can J Zool* **56**:398–413.
- 667 Gross, H. P. & Anderson J. M. 1984. Geographic variation in the gillrakers and the  
668 diet of European threespine stickleback, *Gasterosteus aculeatus*. *Copeia* **1**:87–97.
- 669 Hendry, A. P., Hendry A. S. & Hendry C. A. 2013. Hendry vineyard stickleback:  
670 testing for contemporary lake-stream divergence. *Evol Ecol Res* **15**:1–32.
- 671 Hendry, A. P. & Taylor E. B. 2004. How much of the variation in adaptive  
672 divergence can be explained by gene flow? An evaluation using lake-stream  
673 stickleback pairs. *Evolution* **58**:2319–2331.
- 674 Hendry, A. P., Hudson K., Walker J. A., Räsänen K. & Chapman L. J. 2011. Genetic  
675 divergence in morphology-performance mapping between Misty Lake and inlet  
676 stickleback. *J Evol Biol* **24**:23–35.
- 677 Hermida, M., Fernandez C., Amaro R. & San Miguel E. 2002. Heritability and  
678 “evolvability” of meristic characters in a natural population of *Gasterosteus*  
679 *aculeatus*. *Can J Zool* **80**:532–541.

- 680 Jones, A. G. A., Arnold S. J. S. & Bürger R. 2004. Evolution and stability of the G-  
681 matrix on a landscape with a moving optimum. *Evolution* **58**:1639–1654.
- 682 Jones, F. C., Chan Y. F., Schmutz J., Grimwood J., Brady S. D., Southwick A. M. *et al.*  
683 2012a. A genome-wide SNP genotyping array reveals patterns of global and  
684 repeated species-pair divergence in sticklebacks. *Curr Biol* **22**:83–90.
- 685 Jones, F. C., Grabherr M. G., Chan Y. F., Russell P., Mauceli E., Johnson R. *et al.*  
686 2012b. The genomic basis of adaptive evolution in threespine sticklebacks.  
687 *Nature* **484**:55–61.
- 688 Kaeuffer, R., Peichel C. L., Bolnick D. I. & Hendry A. P. 2012. Parallel and  
689 nonparallel aspects of ecological, phenotypic, and genetic divergence across  
690 replicate population pairs of lake and stream stickleback. *Evolution* **66**:402–418.
- 691 Kolbe, J. J., Revell L. J., Szekely B., Brodie E. D., Losos J. B. 2011. Convergent  
692 evolution of phenotypic integration and its alignment with morphological  
693 diversification in Caribbean Anolis ecomorphs. *Evolution* **65**:3608–3624.
- 694 Kristjánsson, B. K. 2005. Rapid morphological changes in threespine stickleback,  
695 *Gasterosteus aculeatus*, in freshwater. *Environ Biol Fish* **74**:357–363.
- 696 Kristjánsson, B. K., Skúlason S. & Noakes D. 2002a. Morphological segregation of  
697 Icelandic threespine stickleback (*Gasterosteus aculeatus* L.). *Biol J Linn Soc*  
698 **76**:247–257.
- 699 Kristjánsson, B. K., Skúlason S. & Noakes D. 2002b. Rapid divergence in a recently  
700 isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). *Evol*  
701 *Ecol Res* **4**:659–672.

- 702 Lande, R. 2009. Adaptation to an extraordinary environment by evolution of  
703 phenotypic plasticity and genetic assimilation. *J Evol Biol* **22**:1435–1446.
- 704 Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to  
705 brain - body size allometry. *Evolution* **33**:402–416.
- 706 Lande, R. & Arnold S. J. 1983. The measurement of selection on correlated  
707 characters. *Evolution* **37**:1210–1226.
- 708 Lande, R. & Shannon S. 1996. The role of genetic variation in adaptation and  
709 population persistence in a changing environment. *Evolution* **50**:434–437.
- 710 Langerhans, R. B. & DeWitt T. J. 2004. Shared and unique features of evolutionary  
711 diversification. *Am Nat* **164**:335–349.
- 712 Leinonen, T., Cano J. M. & Merilä J. 2011. Genetics of body shape and armour  
713 variation in threespine sticklebacks. *J Evol Biol* **24**:206–218.
- 714 Leinonen, T., Cano J. M., Mäkinen H. S., Merilä J. 2006. Contrasting patterns of  
715 body shape and neutral genetic divergence in marine and lake populations of  
716 threespine sticklebacks. *J Evol Biol* **19**:1803–1812.
- 717 Lucek, K., Roy D., Bezault E., Sivasundar A., Seehausen O. 2010. Hybridization  
718 between distant lineages increases adaptive variation during a biological  
719 invasion: stickleback in Switzerland. *Mol Ecol* **19**:3995–4011.
- 720 Lucek, K., Sivasundar A. & Seehausen O. 2012a. Evidence of adaptive  
721 evolutionary divergence during biological invasion. *PLoS ONE* **7**:e49377.
- 722 Lucek, K., Haesler M. P. & Sivasundar A. 2012b. When phenotypes do not match



- 723 genotypes - unexpected phenotypic diversity and potential environmental  
724 constraints in Icelandic stickleback. *J Hered* **103**:579–584.
- 725 Lucek, K., Sivasundar A., Roy D., Seehausen O. 2013. Repeated and predictable  
726 patterns of ecotypic differentiation during a biological invasion: lake-stream  
727 divergence in parapatric Swiss stickleback. *J Evol Biol* **26**:2691–2709.
- 728 Lucek, K., Sivasundar A., Seehausen O. 2014. Disentangling the role of phenotypic  
729 plasticity and genetic divergence in contemporary ecotype formation during a  
730 biological invasion. *Evolution in press*.
- 731 Marchinko, K. B. 2009. Predation's role in repeated phenotypic and genetic  
732 divergence of armor in threespine stickleback. *Evolution* **63**:127–138.
- 733 Marroig, G. & Cheverud J. M. 2005. Size as a line of least evolutionary resistance:  
734 diet and adaptive morphological radiation in New World monkeys. *Evolution*  
735 **59**:1128–1142.
- 736 Mäkinen, H. S., Cano J. M., Merilä J. 2006. Genetic relationships among marine and  
737 freshwater populations of the European three-spined stickleback (*Gasterosteus*  
738 *aculeatus*) revealed by microsatellites. *Mol Ecol* **15**:1519–1534.
- 739 McKinnon, J. S. & Rundle H. 2002. Speciation in nature: the threespine stickleback  
740 model systems. *Trends Ecol Evol* **17**:480–488.
- 741 Meirmans, P. G. & Van Tienderen P. 2004. GENOTYPE and GENODIVE: two  
742 programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol*  
743 *Notes* **4**:792–794.

- 744 Mori, S. & Takamura N. 2004. Changes in morphological characteristics of an  
745 introduced population of the threespine stickleback *Gasterosteus aculeatus* in  
746 Lake Towada, northern Japan. *Ichthyol Res* **51**:295–300.
- 747 Moser, D., Roesti M. & Berner D. 2012. Repeated lake-stream divergence in  
748 stickleback life history within a Central European lake basin. *PLoS ONE*  
749 **7**:e50620.
- 750 Nosil, P. 2012. *Ecological Speciation*. Oxford University Press, Oxford, UK.
- 751 Nosil, P., Harmon L. J. & Seehausen O. 2009. Ecological explanations for  
752 (incomplete) speciation. *Trends Ecol Evol* **24**:145–156.
- 753 Ólafsdóttir, G. A., Snorrason S. S. & Ritchie M. G. 2007a. Postglacial intra-  
754 lacustrine divergence of Icelandic threespine stickleback morphs in three  
755 neovolcanic lakes. *J Evol Biol* **20**:1870–1881.
- 756 Ólafsdóttir, G. A., Snorrason S. S. & Ritchie M. G. 2007b. Parallel evolution?  
757 Microsatellite variation of recently isolated marine and freshwater three-spined  
758 stickleback. *J Fish Biol* **70**:125–131.
- 759 Ólafsdóttir, G. A., Snorrason S. S. & Ritchie M. G. 2007c. Morphological and genetic  
760 divergence of intralacustrine stickleback morphs in Iceland: a case for selective  
761 differentiation? *J Evol Biol* **20**:603–616.
- 762 Peichel, C. L., Nereng K. S., Ohgi K. A., Cole B. L., Colosimo P. F., Buerkle C. A., *et al.*  
763 2001. The genetic architecture of divergence between threespine stickleback  
764 species. *Nature* **414**:901–905.

- 765 Pigliucci, M., Cammell K. & Schmitt J. 1999. Evolution of phenotypic plasticity a  
766 comparative approach in the phylogenetic neighbourhood of *Arabidopsis*  
767 *thaliana*. *J Evol Biol* **12**:779–791.
- 768 Raeymaekers, J. A. M., Van Houdt J. K. J., Larmuseau M. H. D., Geldof S. & Volckaert  
769 F. A. M. 2007. Divergent selection as revealed by P(ST) and QTL-based F(ST) in  
770 three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-  
771 inland gradient. *Mol Ecol* **16**:891–905.
- 772 Ravinet, M., Prodöhl P. A. & Harrod C. 2013. Parallel and nonparallel ecological,  
773 morphological and genetic divergence in lake-stream stickleback from a single  
774 catchment. *J Evol Biol* **26**:186–204.
- 775 Reid, D. T. & Peichel C. L. 2010. Perspectives on the genetic architecture of  
776 divergence in body shape in sticklebacks. *Integr Comp Biol* **50**:1057–1066.
- 777 Reimchen, T. E. 1992. Injuries on stickleback from attacks by a toothed predator  
778 (*Oncorhynchus*) and implications for the evolution of lateral plates. *Evolution*  
779 **46**:1224–1230.
- 780 Reimchen, T. E. 1994. Predators and morphological evolution in threespine  
781 stickleback. In: *The evolutionary biology of the threespine stickleback*. Oxford  
782 University Press, Oxford, UK. 240–276.
- 783 Reimchen, T. E., Stinson E. M. & Nelson J. S. 1985. Multivariate differentiation of  
784 parapatric and allopatric populations of threespine stickleback in the Sangan  
785 river watershed, Queen-Charlotte-Islands. *Can J Zool* **63**:2944–2951.
- 786 Saemundsson, K. 1992. Geology of the Thingvallavatn area. *Oikos* **64**:40–68.

- 787 Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance.  
788 *Evolution* **50**:1766–1774.
- 789 Schluter, D. & Conte G. L. 2009. Genetics and ecological speciation. *P Natl Acad Sci*  
790 *Usa* **106 Suppl 1**:9955–9962.
- 791 Schluter, D. & Nagel L. M. 1995. Parallel speciation by natural selection. *Am Nat*  
792 **146**:292–301.
- 793 Schluter, D., Clifford E. A., Nemethy M. & McKinnon J. S. 2004. Parallel evolution  
794 and inheritance of quantitative traits. *Am Nat* **163**:809–822.
- 795 Smith, T. B., S. Skúlason. 1996. Evolutionary significance of resource  
796 polymorphisms in fishes, amphibians, and birds. *Annu Rev Ecol Evol S* **27**: 111–  
797 133.
- 798 Spitze, K. 1993. Population structure in *Daphnia obtusa* - quantitative genetic and  
799 allozymic variation. *Genetics* **135**:367–374.
- 800 Stepan, S., Phillips P. C. & Houle D. 2002. Comparative quantitative genetics:  
801 evolution of the G matrix. *Trends Ecol Evol* **17**:320–327.
- 802 Svanbäck, R. & Schluter D. 2012. Niche specialization influences adaptive  
803 phenotypic plasticity in the threespine stickleback. *Am Nat* **180**:50–59.
- 804 Thibert-Plante, X. & Hendry A. P. 2011. The consequences of phenotypic  
805 plasticity for ecological speciation. *J Evol Biol* **24**:326–342.
- 806 Thompson, C., Taylor E. B. & McPhail J. D. 1997. Parallel evolution of lake-stream  
807 pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA

808 variation. *Evolution* **51**:1955–1965.

809 Voje, K. L., Mazzearella A. B., Hansen T. F., Ostbye K., Klepaker T. O., Bass A., *et al.*  
810 2013. Adaptation and constraint in a stickleback radiation. *J Evol Biol* **26**: 2396-  
811 2414.

812 Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection  
813 in evolution. *P 6<sup>th</sup> Int Cong Genet*: 356-366.

814 Wund, M. A., Baker J. A., Clancy B., Golub J. L. & Foster S. A. 2008. A test of the  
815 “Flexible stem” model of evolution: Ancestral plasticity, genetic accommodation,  
816 and morphological divergence in the threespine stickleback radiation. *Am Nat*  
817 **172**:449–462.

818 Young, K. A., Snoeks J. & Seehausen O. 2009. Morphological diversity and the  
819 roles of contingency, chance and determinism in African cichlid radiations. *PLoS*  
820 *ONE* **4**:e4740.

821 Zeller, M., Lucek K., Haesler M. P. & Seehausen O. 2012a. Little evidence for a  
822 selective advantage of armour-reduced threespined stickleback individuals in an  
823 invertebrate predation experiment. *Evol Ecol* **26**:1293–1309.

824 Zeller, M., Lucek K., Haesler M. P., Seehausen O. & Sivasundar A. 2012b. Signals of  
825 predation-induced directional and disruptive selection in the threespine  
826 stickleback. *Evol Ecol Res* **14**:193–205.

827

828 Figure Legends:

829 Figure 1: Overview of the studied systems: a) Sampled lakes and corresponding  
830 sampling sites (squares: lake populations; diamonds: stream populations; circles:  
831 marine populations) for both Iceland (top) and Switzerland (bottom). b)  
832 Representative examples of each sex for the different stickleback ecotypes of  
833 each lake-stream system.

834

835 Figure 2: Genetic and phenotypic relationship among sampling sites. Shape of tip  
836 labels indicates habitat (square: lake; diamond: stream; circle: marine) and  
837 colors represent different lake-stream systems. a) Genetic differentiation among  
838 populations based on a neighbour-joining tree using Cavalli-Sforza distances  
839 amongst sampling sites included in this study (see Figure 1), calculated from  
840 allele frequencies at 10 microsatellite loci. The tree is midpoint rooted. Numbers  
841 beside nodes indicate percent bootstrap support based on 1000 resampling  
842 replicates. Bootstrap values below 50% are not shown. b) Dendrogram of  
843 phenotypic Mahalanobis distances among all sampling sites.

844

845 Figure 3: Pairwise phenotypic divergence between lake and stream ecotype ( $P_{ST}$   
846  $\pm$  95% CI) for each system, calculated for each trait separately, for all traits  
847 combined and for functionally distinct trait groups.  $P_{ST}$  for trait groups is based  
848 on scores of the first principal component axis for either all traits combined,  
849 defense related traits, feeding related traits, body shape related traits (see text  
850 for details). Asterisks indicate cases where the 95% confidence interval for  $P_{ST}$

851 exceeds 0. For the latter traits, triangles indicate the directionality of the  
852 pairwise divergence, where a trait is larger (pointing right) or smaller (pointing  
853 left) in lake fish in comparison to stream fish. For a description of each trait and  
854 its abbreviation see the main text and Figure S1.

855

856 Figure 4: Angles between the major axis of phenotypic variation ( $p_{\max}$ ) in  
857 parapatric lake versus stream populations. Angles were calculated including  
858 either all phenotypic traits (a) or a subset of defense (b), feeding (c) or body  
859 shape / swimming performance (d) related traits. Letters indicate the respective  
860 system: B – Bern (red), C – Constance (black), G – Geneva (green), H –  
861 Hraunsfjörður (blue), M – Mývatn (dark blue), T – Thingvallavatn (pink). Dashed  
862 lines denote lake-stream systems from Iceland, solid lines systems from  
863 Switzerland.

864

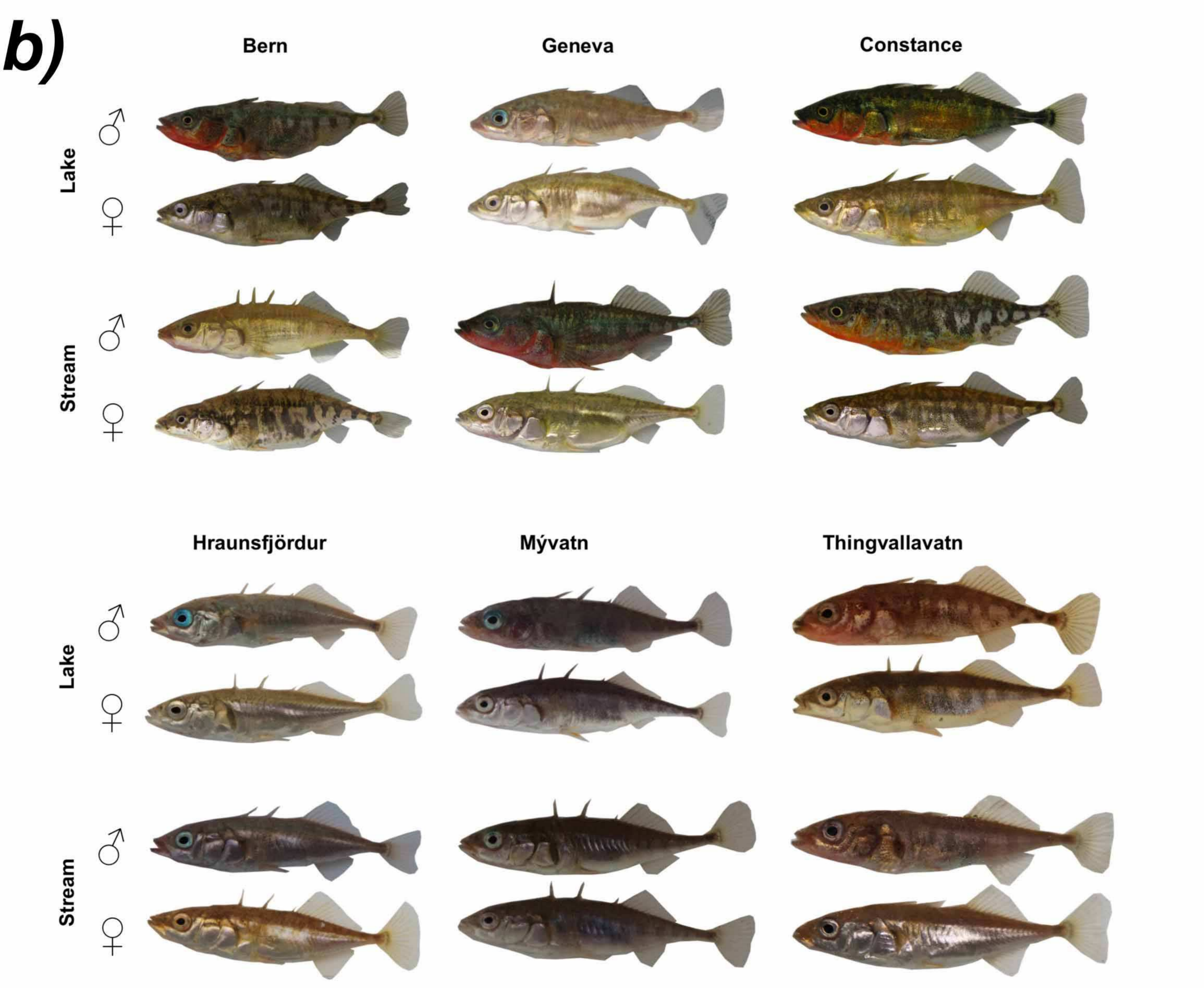
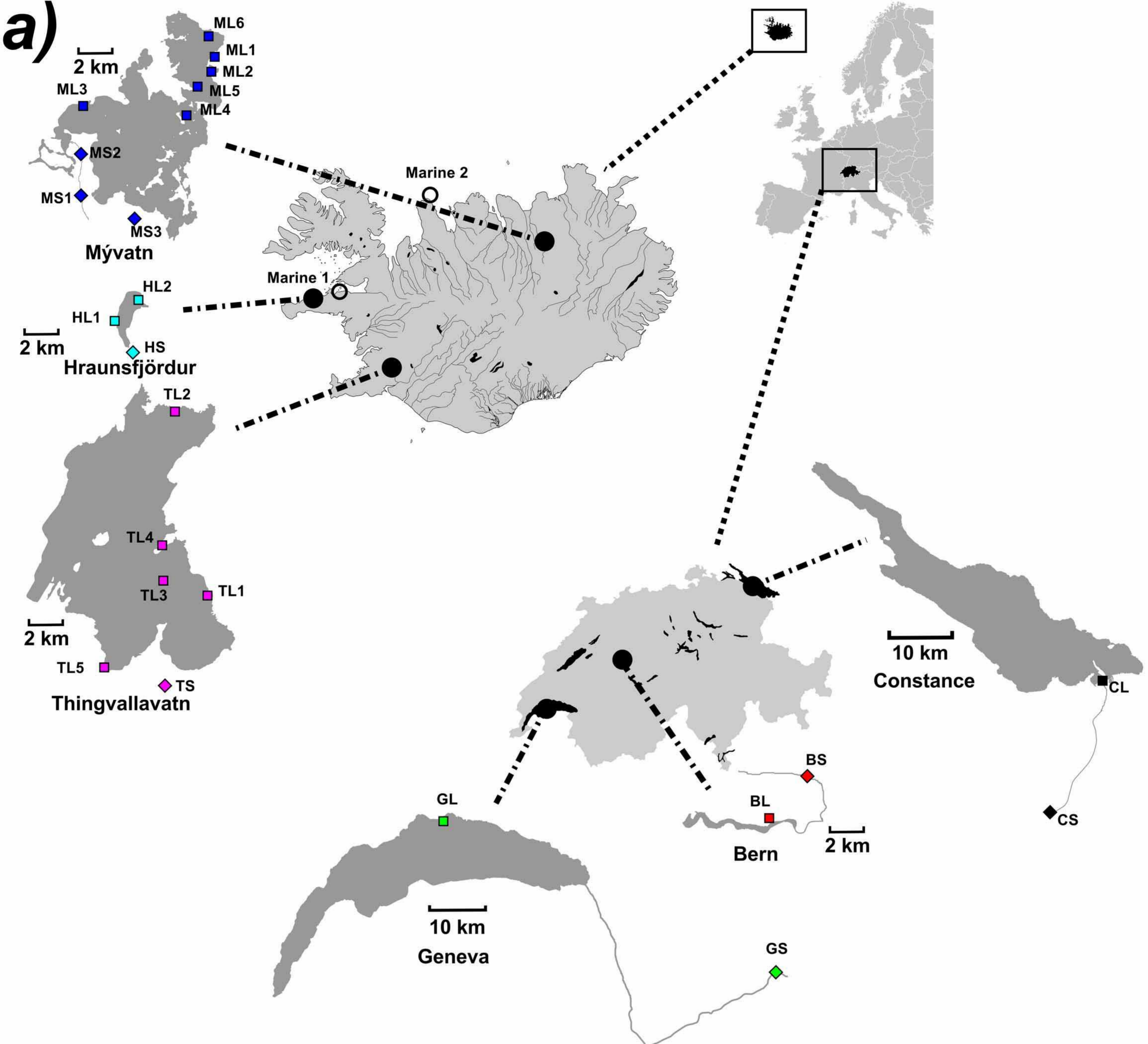
865 Figure 5: Angles between the major axis of phenotypic variation ( $p_{\max}$ ) between  
866 the marine population and either the freshwater stream populations or the lake  
867 populations. Angles were calculated using (from top to bottom): all phenotypic  
868 traits or a subset of defense, feeding or body shape / swimming performance  
869 related traits. For the overall divergence, indicated vectors are scaled according  
870 to the eigenvalue of the leading axis. Letters indicate the respective system: B –  
871 Bern (red), C – Constance (black), G – Geneva (green), H – Hraunsfjörður (blue),  
872 M – Mývatn (dark blue), T – Thingvallavatn (pink). Dashed lines denote lake-  
873 stream systems from Iceland, straight lines systems from Switzerland.

874 Figure S1: The evolution of the P matrix over time through gradual (a) or rapid  
875 changes (b). Shown is the ancestral P matrix with its line of least resistance or  
876  $p_{\max}$  (solid arrow) and the  $p_{\max}$  of diverged populations sampled along a temporal  
877 gradient (from yellow to violet, i.e.  $t_1$ - $t_4$ ) that evolve towards a novel adaptive  
878 optimum. The angle  $\theta$  indicates the degree of divergence over time. Assuming  
879 mainly heritable determined traits,  $p_{\max}$  may need to evolve and  $\theta$  may thus  
880 gradually change over time. On the other hand  $p_{\max}$  may align independently of  
881 time if the underlying traits are either mainly plastic or selection acted on  
882 adaptive standing genetic variation (see the main text for details)

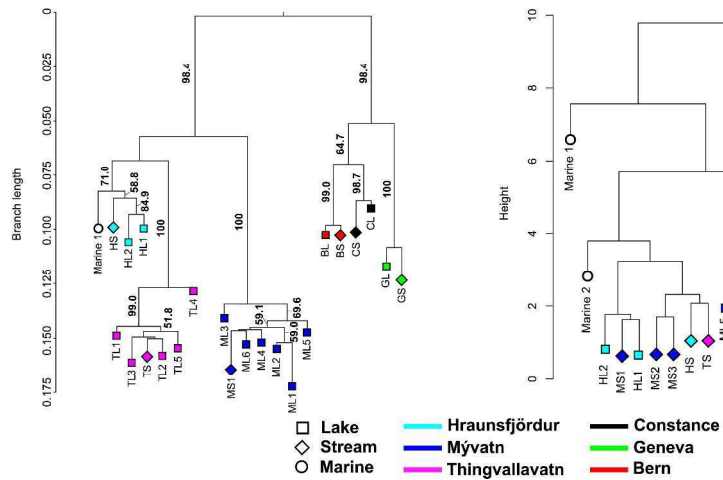
883

884 Figure S2: Summary of all linear measurements used in this study that were  
885 either obtained on the left side a), the gill arch b) or from the ventral side of each  
886 individuals. These measurements can be categorized to belong to either anti-  
887 predator defense (FSL - length of the first dorsal spine; DSL - length of the second  
888 dorsal spine; PSL - length of the pelvic spine; PGL - length of the pelvic girdle),  
889 feeding ecology (HL - head length; UJL - upper jaw length; SnL - snout length;  
890 SnW - snout width; ED - eye diameter) or being linked to general body shape and  
891 swimming performance (SL - standard length; PGW - width of the pelvic girdle;  
892 BD1 - body depth measured after the first dorsal spine; BD2 - body depth  
893 measured after the second dorsal spine; CPL - caudal peduncle length; BLA -  
894 basal length of the anal fin; BLD - basal length of the dorsal fin; TLP - total length  
895 of the pelvic fin; see main text for details). Two feeding related traits were  
896 measured on the gill arch: the length of the second gill raker (GRL2) and the  
897 length of the lower gill arch (AL).

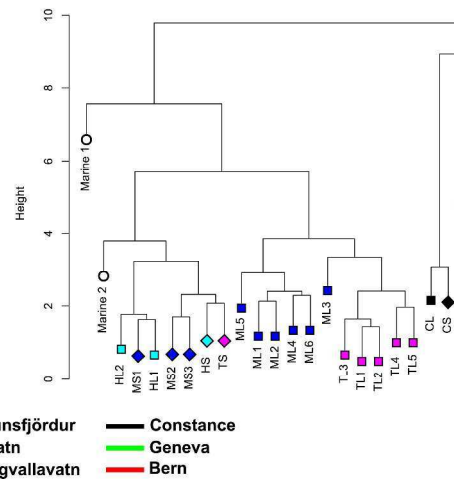




## a) Genetic differentiation

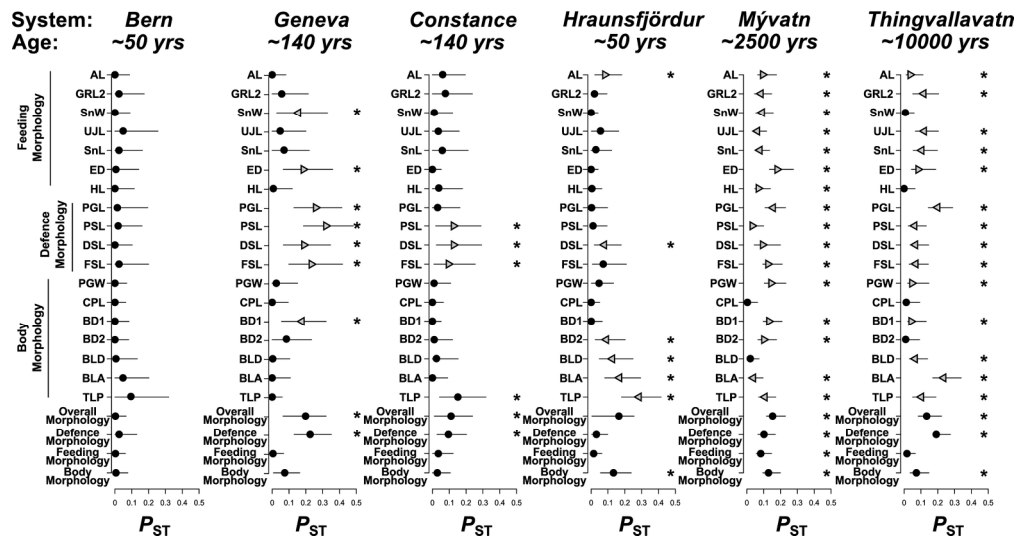


## b) Phenotypic differentiation



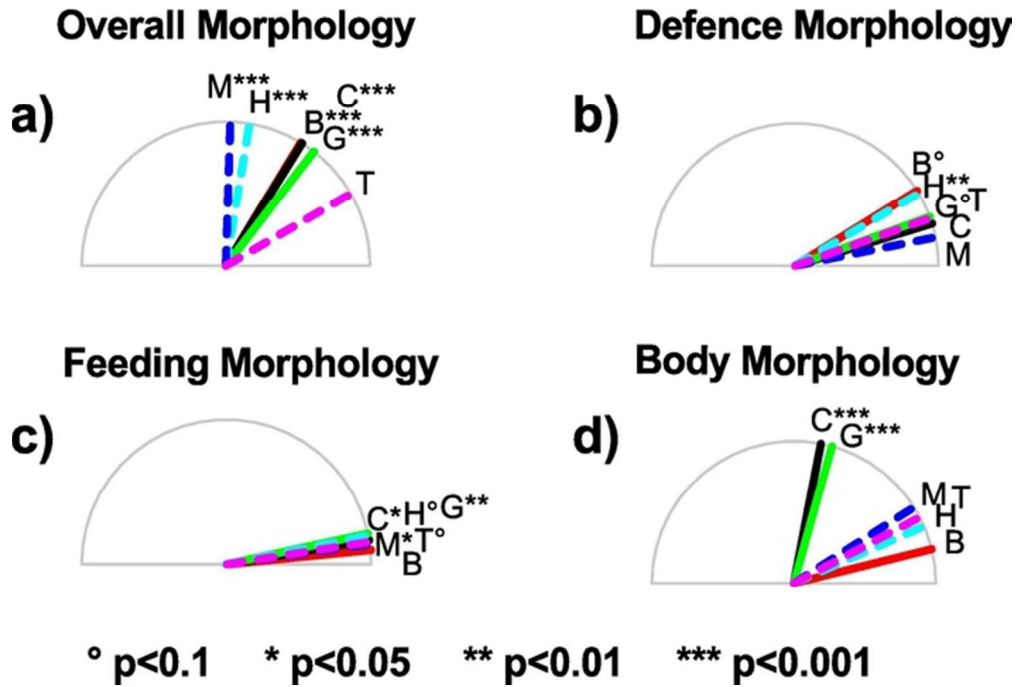
Genetic and phenotypic relationship among sampling sites. Shape of tip labels indicates habitat (square: lake; diamond: stream; circle: marine) and colors represent different lake-stream systems. a) Genetic differentiation among populations based on a neighbour-joining tree using Cavalli-Sforza distances amongst sampling sites included in this study (see Figure 1), calculated from allele frequencies at 10 microsatellite loci. The tree is midpoint rooted. Numbers beside nodes indicate percent bootstrap support based on 1000 resampling replicates. Bootstrap values below 50% are not shown. b) Dendrogram of phenotypic Mahalanobis distances among all sampling sites.

734x379mm (600 x 600 DPI)



Pairwise phenotypic divergence between lake and stream ecotype ( $P_{ST} \pm 95\%$  CI) for each system, calculated for each trait separately, for all traits combined and for functionally distinct trait groups.  $P_{ST}$  for trait groups is based on scores of the first principal component axis for either all traits combined, defence related traits, feeding related traits, body shape related traits (see text for details). Asterisks indicate cases where the 95% confidence interval for  $P_{ST}$  exceeds 0. For the latter traits, triangles indicate the directionality of the pairwise divergence, where a trait is larger (pointing right) or smaller (pointing left) in lake fish in comparison to stream fish. For a description of each trait and its abbreviation see the main text and Figure S1.

196x103mm (300 x 300 DPI)



Angles between the major axis of phenotypic variation (pmax) in parapatric lake versus stream populations. Angles were calculated including either all phenotypic traits (a) or a subset of defense (b), feeding (c) or body shape / swimming performance (d) related traits. Letters indicate the respective system: B – Bern (red), C – Constance (black), G – Geneva (green), H – Hraunfjörður (blue), M – Mývatn (dark blue), T – Thingvallavatn (pink). Dashed lines denote lake-stream systems from Iceland, solid lines systems from Switzerland.

59x40mm (300 x 300 DPI)