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Predation drives local adaptation of phenotypic plasticity

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23 **Phenotypic plasticity is the ability of an individual genotype to alter aspects of its**
24 **phenotype depending on the current environment. It is central to the persistence,**
25 **resistance and resilience of populations facing variation in physical or biological**
26 **factors. Genetic variation in plasticity is pervasive which suggests its local**
27 **adaptation is plausible. Existing studies on adaptation of plasticity typically focus**
28 **on single traits and a few populations, while theory about interactions among genes**
29 **(e.g. pleiotropy) suggests that a multi-trait, landscape scale (e.g. multiple**
30 **populations) perspective is required. We present data from a landscape scale,**
31 **replicated, multi-trait experiment using a classic predator – prey system. We find**
32 **predator regime driven differences in genetic variation of multivariate plasticity.**
33 **These differences are associated with strong divergent selection linked to predation**
34 **regime. Our findings are evidence for local adaptation of plasticity, suggesting that**
35 **responses of populations to environmental variation depend on the conditions in**
36 **which they evolved in the past.**

37

38 All organisms face variability in their environment, which can make it difficult for
39 specialised phenotypes to survive and reproduce. An important outcome of this
40 environmental variability is that natural selection can favour flexibility in the form of
41 phenotypic plasticity [1]. Phenotypic plasticity, the ability of an individual genotype to alter
42 aspects of their phenotype depending on the current environment, is central to
43 understanding the persistence of populations facing variation in physical (e.g. weather) or
44 biological (e.g. predators and disease) factors [2]. Because phenotypic plasticity can
45 change the mean and variance of traits, and the alignment of genetic variation with the
46 targets of selection, it is also central to several recent theories about the pace of
47 evolutionary change, adaptive radiation and evolutionary responses to rapid and extreme
48 changes in climate [3-7].

49

50 But can phenotypic plasticity be locally adapted? For natural selection to drive the
51 evolution of phenotypic plasticity, there must be genetic variation in plasticity upon which
52 selection can act, the presence and impact of which has been established among plants
53 and animals and across aquatic and terrestrial habitats [2, 8, 9]. Additionally, individuals
54 that can modify how they develop in different environments must be those best equipped
55 to reproduce and survive. Quantitative genetic theory provides a framework to predict how
56 the patterns of variation in traits among environments can constrain or promote
57 evolutionary change and ultimately diversification [4, 5, 10, 11]. In this context of data and
58 theory, local adaptation of plasticity is predicted.

59

60 However, there remains little empirical evidence for local adaptation of plasticity. Where
61 gathering data has been attempted, studies have typically focused on the plasticity of
62 single traits and how they are related to environmental heterogeneity [8] However, genetic
63 pleiotropy among traits appears commonplace, which implies that effective evaluation of
64 local adaptation of phenotypic plasticity requires investigating how multiple traits evolve
65 together in response to environmental variation [12].

66

67 Here, we present evidence of local adaptation of multivariate plasticity using the
68 freshwater crustacean *Daphnia pulex* as a model system in a replicated experiment over a
69 landscape scale. Based on four tests of local adaptation, we show that there is a genetic
70 basis for the evolution of plasticity in multivariate trait space among *D. pulex* populations
71 associated with divergent selection tied to size-selective predation regimes (midge vs. fish-
72 midge). These conclusions emerge from multivariate analysis of five traits that include life
73 history and morphology, traits evaluated because of their significance in theory about
74 adaptation to size selective predation [13-15]. Evolutionary history shapes the ability of

75 individuals to respond to future variation in predation risk. Phenotypic plasticity can be
76 locally adapted and selection can act on it.

77

78 **Results**

79 *The D. pulex system*

80 We collected and analysed data from eight populations of the water-flea *D. pulex* and their
81 invertebrate midge larvae (*Chaoborus flavicans*) and vertebrate fish (*Gasterosteus*
82 *aculeatus*) predators (Supplementary Fig 1; Supplementary Table 1). Predator induced
83 phenotypic plasticity in morphological, life historical and behavioural traits of water fleas,
84 responding to chemical cues from invertebrate and fish predators, is an iconic example of
85 adaptive phenotypic plasticity [14, 16-18].

86

87 We evaluated whether phenotypic plasticity in five traits depends on the predator regime
88 they experience. Four traits are commonly evaluated alone in predation risk research and
89 are strongly linked to survival and reproduction: 1) induced morphological defense
90 (neckteeth); 2) age at maturity; 3) size at maturity; 4) somatic growth rate. Neckteeth are
91 known to increase survival by up to 50% in the face of small size selective predation by the
92 larvae of *Chaoborus* and are only produced when midge larvae are present [16, 19]. Late
93 maturation and large size at maturity are induced by small size selective midge predators
94 as part of investing in growth over early reproduction. In contrast, early reproduction and
95 small size at maturity is induced by large size selective fish predators as part of investing
96 in early reproduction over growth [11, 13]. We also included 5) population growth rate
97 (PGR), estimated from life table data using the Euler equation.

98

99 We performed a common garden experiment and carried out four statistical tests of local
100 adaptation of phenotypic plasticity. We used 70 genotypes from eight natural populations

101 in the UK, four of which experienced predation only by midge larvae, while the other four
102 experience a combination of fish and midge predation (Supplementary Table 1). This
103 classification of the ponds defines the predation *regime*. All genotypes were then reared
104 within the laboratory in either invertebrate midge or a combination of vertebrate fish +
105 invertebrate midge predator chemical cues. These two treatments (midge, fish + midge)
106 induce adaptive plastic changes in morphology and life history [11, 20] and are referred to
107 as the *treatments* between which we estimate plasticity. All analyses focus on testing
108 whether the predator induced plasticity defined between treatments depends on predator
109 regime. Because all populations experience midge predation in nature, a complementary
110 interpretation of our experimental design is that it is evaluating how evolution in the
111 presence or absence of fish constrains how individuals respond to pervasive midge
112 predation risk.

113

114 *Local Adaptation I: Plasticity x Regime Interactions*

115 We first evaluated local adaptation of plasticity via univariate tests of whether the effect of
116 predation risk (treatment) varies by the predation regime in which the *Daphnia* evolved -
117 an interaction between phenotypic plasticity and predator regime. Using linear mixed
118 models (see Online Methods), accounting for clones nested within ponds, we found that
119 the effect of predation risk on Size, Somatic Growth and Induction (neckteeth), varies by
120 the predator regime, while the effect of predation risk on Age and PGR does not vary by
121 regime (Figure 1).

122

123 *Local Adaptation II: Multivariate genetic variation in plasticity varies by regime*

124 We next performed a multivariate test of whether the effect of predation risk (treatment) on
125 the multivariate phenotype (multivariate plasticity), depends on the predation regime in
126 which the *Daphnia* evolved.

127

128 This multi-trait assessment of genetic variation in plasticity is evaluated by comparing
129 statistically the volume, shape and orientation of G-matrices between treatments, and
130 whether this pattern differs by regime [10, 21; a multivariate character-state evaluation of
131 genetic variation in plasticity]. We estimated, for each of the four combinations of *regime*
132 and *treatment*, the pattern of genetic variation and covariance (the G-matrix) among the
133 five traits using Bayesian MCMC mixed models (see Online Methods).

134

135 Genetic variation in multivariate plasticity can manifest via changes in the volume, shape
136 and orientation of the G-matrix. The volume and shape of the G-matrix capture the clonal
137 genetic variance (V_G) available to selection. Differences in volume and shape reflect
138 environment specific differences in the potential magnitude of the response to selection
139 [22]. Differences in the shape specifically reveal whether variation shifts between being
140 biased to a small number of traits or distributed evenly among all traits. We report on total
141 clonal variance to capture information on the volume and on the magnitude of this total
142 clonal variance associated with the major axis (g_{max}) to make inference about shape [21].
143 Differences in the orientation of the G-matrix reflect environment specific differences in the
144 identity and number of traits that comprise g_{max} in each treatment. Orientation differences
145 are a multivariate perspective on whether reaction norms cross and reveal how phenotypic
146 plasticity can change the set of traits associated with substantial genetic variation. We
147 evaluate two aspects of the G-matrix orientation [21]. The first is the identity of traits that
148 correlate most strongly with g_{max} . The second is the angle between the g_{max} in each
149 treatment.

150

151 Within each predation regime (e.g. n=4 populations/regime), we detected no size
152 differences between the G-matrix expressed in each treatment (Table 1; Figure 2; no

153 difference in either estimate of total variance or the variance of g_{\max}). In contrast, we
154 detected significant variation in the identity of the traits associated with g_{\max} , and in its
155 orientation between treatments in each regime. This result, centred on the covariation
156 among traits (see [21]), suggests that genetic variation in multivariate plasticity is locally
157 adapted.

158

159 Specifically, we detected in both regimes, a significant predator induced rotation of the
160 major axis of genetic variation towards somatic growth rate in the fish treatment (Table 1:
161 Angle Between g_{\max} ; Figure 2: The major axis of blue hulls is not aligned with the major
162 axis of the red hulls). Furthermore, in the midge treatment, the identity of the traits
163 comprising g_{\max} differed markedly depending upon the regime from which the *D. pulex*
164 originated (e.g. midge treatment loadings on the red hull major axes are different, Figure
165 2). Age is strongly positively correlated and size, somatic growth, and population growth
166 rate strongly negatively correlated with the major axis in the fish-midge regime, while the
167 opposite is true in the midge regime (Figure 2). The traits along which selection can act
168 most rapidly under the midge treatment are different in each of the predation regimes. The
169 phenotype starts, and rotates through trait space differently, depending on the predation
170 regime the populations have experienced.

171

172 *Local Adaptation III: Regimes Drive Different Response to Same Predation Cue*

173 With these same G-matrices, we also ask the complementary question of whether the
174 response to a specific predator treatment is constrained by the predator regime. Formally
175 this is testing whether the variance and co-variance among traits, in a predation treatment,
176 differs by the predator regime, again defined by differences in size, shape and orientation
177 of the G-matrix. Results in *Local Adaptation II* foreshadow significant differences between
178 regimes in the midge cue treatment where the major axis loadings differ, but not in the

179 fish+midge cue treatments, as the rotation in this treatment is consistently towards somatic
180 growth (see above and Figure 2). In line with this expectation, we detected a significant
181 rotation of the major axis between regimes in the midge cue, but not in the fish cue
182 treatment (Table 1: Angle Between g_{\max}), a difference that is clearly visible in Figure 3.

183

184 These three assessments provide strong support for local adaptation of plasticity.

185 Furthermore, the results from both multivariate analyses highlight that local adaptation is
186 manifest via the covariance among traits, not the variance – we detected no differences in
187 patterns of variance between environments (*Local Adaptation II*) or between regimes in
188 either environment (*Local Adaptation III*). While theory and empirical work routinely
189 highlight how plasticity alters variation (reviewed in [7]), our multivariate assessment shifts
190 attention to covariation among traits.

191

192 *Local Adaptation IV: Predator Regime Drives Divergent Selection*

193 In addition to evaluating local adaptation of phenotypic plasticity through pattern in the G-
194 matrix, we also explore patterns of selection on the multivariate phenotype in the context
195 of plasticity, using Q_{ST} - F_{ST} analyses. Comparing selection patterns within treatments but
196 between regimes (*i.e. as in Local Adaptation III*), we specifically ask whether there is
197 evidence of divergent or convergent selection among the eight populations within each
198 treatment (predator cue), whether the strength of selection depends on the treatment, and
199 whether evidence of divergence or convergence, if present, can be tied to predator
200 regime. Our data indicate that divergent selection, linked to predator regime, has acted at
201 an equal magnitude under predation risk from each predator to shape how individuals
202 respond to predation risk.

203

204 We reach this conclusion via univariate and multivariate Q_{ST} - F_{ST} analyses following
205 multivariate Bayesian MCMC methods developed by Ovaskainen et al and Karhunen et al
206 [23-26] that overcome several challenges associated with more traditional Q_{ST} - F_{ST}
207 analyses. We used these tools to estimate F_{ST} , gene flow and the signature of selection
208 among populations on all single trait, 2-trait, 3-trait, 4-trait and 5-trait combinations (Figure
209 4). Our primary objective was to estimate selection on the 5-trait phenotype, but we follow
210 Karhunen et al [25] in exploring how a univariate vs. multivariate approach to Q_{ST} - F_{ST}
211 influences inference.

212

213 We first estimated a co-ancestry matrix via an admixture F-model (AFM, [24]) deriving
214 units of drift separating the populations, as well as a MCMC based estimate of F_{ST} and
215 estimates of gene flow. We estimate an F_{ST} of 0.37 (95% Credible Interval 0.32-0.43) and
216 negligible gene flow (0.00001 – 0.0005; see Supplementary Table 2). In the absence of
217 gene flow and given the large distances separating many populations, a high F_{ST} of 0.2-
218 0.4 is not unexpected [25, 27].

219

220 We then used the co-ancestry matrix as the template on which to make strong inference
221 about any evidence of deviation from a formal model of drift [24, 26]. We present the S-
222 statistic of deviation from drift and a credible interval derived from the joint posterior of the
223 MCMC models. S can range between 0-1, where values of ~0.5 indicate drift, 0 - 0.2
224 stabilising selection, and 0.8 - 1 divergent selection among the populations [22].

225

226 We derive four major conclusions from this analysis. First, there is evidence of strong
227 divergent selection in each treatment and among populations when considering all five
228 traits ($S_{\text{midge}} = 0.85 (0.54-0.99)$; $S_{\text{fish}} = 0.88 (0.66-0.99)$; Fig 4). Overall, under a null

229 expectation of drift, we would only expect this signature of selection in 12-15% of the
230 cases (*probabilities evaluated from joint posterior distribution*).

231

232 Second, the signature of divergent selection increases monotonically, but with variation, as
233 the number of traits defining the phenotype increases (Fig 4; see [25]). A whole-organism,
234 multi-trait perspective on how phenotypic plasticity mediates organismal response to
235 environmental variation is therefore both influential and vital. Third, the strongest
236 univariate estimates of selection are on age at maturity, PGR and size at maturity under
237 the fish treatment but age at maturity, PGR and induced morphology under the midge
238 treatment. However, univariate estimates of selection are uniformly lower than multi-trait
239 estimates.

240

241 Fourth, the strongest signature of selection is detected on combinations of traits that do
242 include the traits associated with strong selection on their own, with 'surprising' omissions
243 and additions (Fig 4). As discussed above, and by Karhunen et al [25], what we are likely
244 witnessing is the effect of trait covariation which can only manifest under a multivariate
245 analysis (see Supplementary Figure 4 for more detail on covariance linked to divergence).

246

247 More specifically, under fish predation risk, where age at maturity, PGR and size at
248 maturity are the top univariate traits, the strongest signature of selection is associated with
249 a phenotype comprised of size at maturity - PGR or size at maturity - somatic growth rate -
250 PGR; while age at maturity is a 'surprising' omission from the multivariate phenotype under
251 strong selection (e.g. despite its strong univariate signature). In contrast, under midge
252 predation risk, where age at maturity, PGR and induced morphology are the top univariate
253 traits, the strongest signature of selection is associated with size at maturity-PGR-induced
254 morphology, age at maturity-size at maturity-somatic growth rate-induced morphology and

255 size at maturity-somatic growth rate-PGR-induced morphology; in this case, somatic
256 growth and age are 'surprising' additions to the multivariate phenotype under selection
257 (e.g. despite their weak univariate signatures).

258

259 We also found that the divergence is strongly linked to the predator regime. We applied
260 the H-test of Karhunen et al [25] to test whether the divergent selection was linked to the
261 predation regime across the landscape spanning ~540km. Controlling for how a shared
262 phylogenetic history may arise among populations in similar habitats, H estimates the
263 similarity between the distribution of quantitative traits and the distribution of environmental
264 conditions. A value of H close to one indicates a strong association, suggesting that the
265 distribution of trait means among the populations are more strongly linked to
266 environmental covariates than would be expected under a model of drift.

267

268 We ran two H-tests. First, we specified the environment solely by predation regime. This
269 resulted in $H = 0.86$ under the midge cue treatment and $H = 0.87$ under the fish+midge
270 cue treatment, suggesting a strong association of divergent selection with predator regime
271 across the landscape. Second, we generated three independent covariates of additional
272 environmental variables using PCA applied to the pond variables latitude, longitude, the
273 index of midge density, pH and temperature (see Supplementary Table 1; Supplementary
274 Fig 3). We used the first three principle components (90% variation) and predator regime
275 as the covariates in the second H-test.

276

277 Revealing the strong role of predation regime, this second H-test indicates that the
278 additional environmental variables contribute very little to our inference about the drivers of
279 divergence (H -midge = 0.89, H -fish = 0.88). We conclude that in both predation risk
280 treatments, divergent selection is strongly driven by predator regime.

281

282 **Discussion**

283 Genetic variation in phenotypic plasticity is found in nearly every assessment of reaction
284 norms, across taxa and habitat types [2, 8, 9], a source of variation on which selection can
285 act. In a landscape scale, replicated, natural experiment, we show that divergent natural
286 selection linked to predation regime shapes the inducible, plastic responses of *D. pulex* life
287 history and morphology to predation risk. We believe this to be the first demonstration that
288 multiple populations of the same species can differ consistently in their ability to respond to
289 variation in their environment that is tied to common conditions they have previously
290 experienced. Our data suggest that genetic variation in plasticity is locally adapted and
291 that evolution by natural selection, here associated with predator regime, can differentiate
292 genetic variation in plasticity among populations.

293

294 Predator induced, plastic changes in *D. pulex* morphology and life history is one of the
295 most well studied examples of phenotypic plasticity. Decades of work have consistently
296 shown that induced changes in traits caused by predator chemical cues can generate
297 patterns in morphology and life history that match those predicted by evolutionary theory
298 about small and large size selection [1, 11, 13, 14, 28]. This alignment between plastic
299 responses and the expectations of evolutionary theory generates the strong hypothesis
300 that phenotypic plasticity is indeed a trait on which selection acts.

301

302 These historical data are augmented by recent theory [5] and empirical work [11]
303 highlighting that plastic changes in traits may align the phenotype along the major axis of
304 genetic variation (g_{\max}) and the direction of selection. Draghi and Whitlock [5] proposed
305 that phenotypic plasticity may predispose the developmental machinery and increase the
306 genetic variance, covariance and mutational variance in the direction of most divergence

307 between environments. Plasticity could thus align with g_{\max} and ultimately selection [11].
308 This combination of theory and data suggests that phenotypic plasticity might actually 'aid
309 evolution'.
310
311 Local adaptation of phenotypic plasticity might even be interpreted as a positive feedback
312 to local adaptation *per se* via this alignment mechanism. Such an idea must be considered
313 in light of theory on the effects of adaptive/maladaptive plasticity on local adaptation [29].
314 Schmid and Guillaume's theory [29] (and see Hendry [30]) shows how undifferentiated and
315 un-evolving plasticity can none-the-less have substantial effects on the interplay between
316 gene-flow and selection. Plasticity can, for example, neutralize fitness difference of
317 migrants leading to increased phenotypic divergence but low genetic divergence, while
318 maladaptive plasticity can increase genetic differentiation by increasing strength of
319 selection, but also increase the risk of population extinction. Our evidence that plasticity
320 can itself be locally adapted, and align genetic variation with selection [11], adds a
321 compelling dimension to their call to consider more thoroughly the role of both adaptive
322 and maladaptive plasticity in local adaptation and the response of populations to
323 environmental change.
324
325 Our results also strongly suggest that to fully understand the ecological and evolutionary
326 implications of plasticity, we must employ a multi-trait and multivariate analysis of
327 phenotypic plasticity. Our data strengthen the call for multivariate approaches to research
328 on plasticity and local adaptation [11, 21, 26, 31-33]. First, although all five traits that we
329 measured are considered theoretically important traits linked to survival and reproduction
330 in the face of predation risk, not all of them show univariate signature of a regime by
331 treatment interaction (Figure 1) or univariate divergence across regimes (Figure 4).
332 Second, the multivariate phenotype shows always a greater signature of selection than

333 any univariate measure of divergence; univariate divergence measures may
334 underestimate or even fail to detect population divergence [25]. Finally, findings from
335 univariate divergence of traits do not necessarily hold when considering the multivariate
336 phenotype. We found that traits indicated to be important for univariate divergence might
337 not contribute to divergence of the multivariate phenotype, while traits considered
338 unimportant for univariate divergence can contribute to important aspects of the
339 divergence of the multivariate phenotype. Failing to accommodate the genetic covariance
340 among multiple traits can thus result in misleading conclusions.

341

342 The role of plasticity in how populations respond to variation in their environment, from
343 predation and disease risk to climate change, continue to be crystalized [4, 34]. In fact,
344 several recent bodies of theory provide compelling ideas that phenotypic plasticity may be
345 central to adapting to both steady and extreme events linked to climate change [4, 35].
346 Such hypotheses are deeply rooted in evolutionary theory about how plasticity can alter
347 the mean and variance of traits, the alignment of genetic variation with the targets of
348 selection, and its capacity to influence the pace of evolutionary change, adaptive radiation
349 and evolutionary responses to rapid and extreme changes in climate [3-6]. Our results,
350 drawn from four assessments of local adaptation, and focusing on variance and
351 covariance among five traits, provide a robust conclusion that such phenotypic plasticity is
352 locally adapted. Importantly, our evidence is drawn from replicate, natural populations of
353 each of two predation regimes and aligns with theoretical expectations that natural
354 selection linked to contrasting size selective predation regimes drive constraints on how
355 predator induced phenotypic plasticity evolves. Multivariate phenotypic plasticity can
356 evolve in response to strong selection pressures that operate at large scales and this
357 shapes future environmental responses.

358

359 **Methods**

360 *Study System*

361 Our data come from eight populations of *D. pulex* along a 540km N-S gradient in the UK
362 (Supplementary Fig 1 and Supplementary Table 1). Four of the populations are classified
363 as midge only and the other four as fish+midge. As detailed in the text, this designation
364 defines our regime, or evolutionary background. Several other features of the ponds,
365 including a categorical index of midge predation density are provided in Supplementary
366 Table 1.

367

368 *D. pulex* inhabit either ephemeral, seasonal, ponds with predominately invertebrate
369 predators, or permanent lakes that also harbour vertebrate predators. Midge larvae,
370 *Chaoborus* spp., are gape- and size-limited predators, selectively feeding on small
371 cladocerans, whilst fish are active visual hunters and typically select large daphnids .
372 When exposed to kairomone from small-size selective *Chaoborus* during early
373 development, daphnids have a longer developmental time and mature at a larger size and
374 later age [16]. *D. pulex* also respond to cues released from *Chaoborus* by producing a
375 morphological defence, termed neckteeth, which are discrete, small protuberances on top
376 of a transformed neck region. These structures are directly linked to increases in body size
377 and survival [36, 37]. Under large-size selective predation, such as from juvenile fish,
378 daphnids have a shorter developmental time and mature at a smaller size and younger
379 age, without expressing the morphological defence during development [38, 39].

380

381 Vertebrate and/or invertebrate predators thus select against large and small sizes in
382 *Daphnia* prey, requiring defensive adaptive traits that have been shown to be effective and
383 heritable [1, 40-42]. We examined predator-induced plasticity in several life-history traits of
384 *D. pulex* in response to two major predators: phantom midge larvae (*Chaoborus flavicans*),

385 active in the early summer, and juvenile fish, three-spined stickleback (*Gasterosteus*
386 *aculeatus*), active in spring [37]. These opposing selection pressures, and the seasonal
387 heterogeneity of predator type and abundance, make the *Daphnia*-midge-fish system a
388 perfect candidate for studying genotype-environment interactions in plastic traits.

389

390 *Phenotype Data*

391 The phenotype data were collected from 70 genotypes collected from among the eight
392 populations (range 6-10/population; Supplementary Table 1) in a common-garden
393 experiment defined by the midge versus fish cue treatments. As detailed in the text, the
394 cue treatments define our environments for estimating predator induced plasticity.

395

396 We generated the treatment cues for midge and fish kairomone following an established
397 protocol [11, 14, 19, 20, 37, 43] that involves several steps of coarse filtration followed by
398 solid phase extraction on a C18 column to recover a concentrate containing the active
399 compounds that generate strong responses in the daphnids equivalent to exposure to
400 natural predators [37].

401

402 Cue treatments were as follows. The midge treatment received $0.5 \mu\text{l ml}^{-1}$ *Chaoborus*
403 predator cue concentrate. The fish treatment received $0.5 \mu\text{l ml}^{-1}$ *Chaoborus* predator cue
404 (midge treatment) and 5 ml fish kairomone conditioned water. This mix of cues for the fish
405 treatment was required to generate expression of the morphological defence, specific to
406 the midge cue treatment, but conspicuously absent under fish cue only treatments. We
407 thus required such a mix of cues to allow all five traits to be measured in two treatments.

408

409 Ten third-generation mothers of at least the third brood from each of the 70 genotypes
410 holding black-eyed embryos (12 hours prior to parturition) were placed in individual jars

411 containing 50 ml hard artificial pond water, algae (2×10^5 cells ml⁻¹ *Chlorella vulgaris*), 100
412 µl 30% marinure (liquid seaweed extract, Wilfrid Smith Limited) and either the *Chaoborus*
413 predator cue (midge treatment), or midge + fish cue (fish treatment).

414

415 After parturition, three neonates were randomly selected from each of the five mothers per
416 treatment, a total of 15 embryos per treatment for each genotype. They were placed
417 individually in 50 ml glass vials containing the same medium as their mothers experienced
418 with either midge or fish conditioned water, generating the two predator cue treatments.
419 Each animal was photographed daily (Canon DS126071) and transferred to a new glass
420 vial containing fresh media and predator cue until sexual maturity was reached, indicated
421 by the first appearance of eggs in the brood pouch.

422

423 In both treatments, we measured five traits. Three of them are life history traits: (i) body
424 size at maturity (the linear distance from the top of the head capsule through the eye to the
425 base of the tail), measured using the image analysis software ImageJ 1.37v; (ii) age at
426 maturity (number of days from birth to sexual maturity); and (iii) clutch size (number of
427 eggs in the brood pouch at maturity). Recording these life history traits allowed us to
428 calculate somatic growth rate (log difference in size at maturity and size at birth divided by
429 age at maturity), as well as intrinsic rate of population increase, r , estimated using the
430 stable-age (Euler's) equation combining a clone's age at maturity in days and number of
431 eggs [42, 44]

432

433 The classic induced morphological defence was measured at 2nd and 3rd instar following
434 [20, 37, 43, 44]. As the maximum induction varies with clone and age, we chose the
435 maximum of each of these measures as our estimate of induced morphology.

436

437 All variables included in this study are continuously varying quantitative traits. Before
438 analysis, we standardized all traits using Z-score scaling, resulting in all variables in the
439 data set having means centred at zero and a standard deviation of one.

440

441 *Genotyping*

442 Genomic DNA was extracted from whole adults by crushing iso-females in a 1.5 ml flip-top
443 tube with 50 μ l buffer (made up of 10 mM Tris-Cl pH 8.2, 1 mM EDTA and 25 mM NaCl)
444 and 4 μ l proteinase K (10mg/ml), followed by an incubation period of one hour at 55°C and
445 finally three minutes at 80°C to denature the proteinase K. We used 11 polymorphic
446 microsatellite markers to characterize our genotypes. The following sets of loci were taken
447 from Cristescu et al. [45] and developed by Reger et al. [46]: (i) Dp802; Dp1236, Dp1290;
448 (ii) Dpu122, Dp1079, Dp675; and (iii) Dp1123, Dp45, Dp460, Dp43. Following standard
449 protocols outlined in Kenta *et al.* [47], genotyping was performed in 2 μ l PCR reactions,
450 containing approximately 10ng of lyophilised genomic DNA, 0.2 μ M of each primer and 1
451 μ l QIAGEN multiplex PCR mix . We used a touchdown PCR to lower nonspecific
452 amplification [45]. Amplified products were genotyped in an ABI 3730 48-well capillary
453 DNA Analyser (Applied Biosystems) and allele sizes were scored using GENEMAPPER
454 v3.7 software (Applied Biosystems). For samples where the extraction did not yield
455 sufficient amounts of genomic DNA, the extraction process was repeated and samples that
456 failed to amplify at all loci were re-amplified and re-scored.

457

458 *Univariate Plasticity*

459 We estimated univariate plasticity and tested for an interaction with regime using linear
460 mixed effects models. Models were fit with lme4 using R 3.3.1 [48] and specified a fixed
461 effects interaction of treatment x regime and nested random effects structure of pond (n=8)
462 /clone (n = 66).

463

464 *Multivariate Plasticity*

465 We implemented the workflow and tools developed for comparison of G-matrices by
466 Robinson and Beckerman [21]. We first estimated the genetic variance-covariance matrix
467 for five traits in each treatment from each regime (four models): 1) induced morphological
468 defence (neckteeth); 2) age at maturity; 3) size at maturity; 4) somatic growth rate; 5)
469 population growth rate. In contrast to above, because we are fitting models to populations
470 within regimes, we fit clone ID as a random effect to capture the estimate of genetic
471 variation (broad sense; clonal variance) and pond (n=4 for each model) as a fixed effect.
472 We used a Bayesian multivariate mixed model (MCMCglmm in R [49]) to recover the joint
473 posterior distribution of trait variances and covariances, and define the genetic variance-
474 covariance matrix (G-matrix).

475

476 All models were fit with parameter expanded priors and run multiple times for 1 million
477 iterations and sampled 1000 times after a burn-in of at least 500000. All models were
478 checked for lack of autocorrelation and several diagnostics to ensure proper mixing.

479

480 The tools developed in Robinson and Beckerman [21] to evaluate plasticity draw on
481 several established metrics for comparing two G-matrices estimated from each treatment.
482 Their approach to characterizing plasticity emerges directly from the character-state
483 representation of plasticity. Via and Lande [10] showed that it is straightforward to estimate
484 plasticity by treating the same trait in each two environments as two traits. In contrast to
485 other approaches, estimating the G-matrices with Bayesian MCMC methods allows one to
486 estimate features of plasticity with strong inference using several metrics of change in
487 variance and covariance. They show that it is straightforward to compare total genetic
488 variation, variance allocated to the major axis of variation, and an estimate of the number

489 of major axes. They also show, extending theory from Ovaskainen et al [50], how to
490 estimate with strong inference whether the rotation of the major axis, if present, is
491 significant.

492

493 Their tools (see Robinson and Beckerman [21]; [www.github.com/andbeck/mcmc-plus-](http://www.github.com/andbeck/mcmc-plus-tensor)
494 [tensor](http://www.github.com/andbeck/mcmc-plus-tensor)) provide a) a table of plasticity metrics and their 95% Credible intervals from the
495 comparisons; b) a graphical representation of the comparison and c) a definition of the
496 major and two additional minor axes of variation (e.g. loadings associated with the
497 ordination of the G-matrix).

498

499 Q_{ST} - F_{ST}

500 We made univariate and multivariate Q_{ST} - F_{ST} analyses using the methods of Ovaskainen
501 et al and Karhunen et al [23-26] and the packages RAFM and driftsel modified to handle
502 clonal organisms (Karhunen, *personal communication*). The methods implement Bayesian
503 MCMC algorithms to a) reconstruct the ancestral phenotype, b) estimate the change in
504 that phenotype that has arisen due to genetic drift (F_{ST}) and then c) an estimate, S, of
505 whether there is any evidence of directional (S<0.1; only 10% of the time would
506 populations be closer under a null model drift) or divergent selection (S>0.9; only 10% of
507 the time would populations be further apart under a null model of drift). Their methods
508 also include an additional test (H) that estimates whether the selection intensity estimates
509 (S) are correlated with some description of the environment. We used this “H-test” to
510 examine whether the patterns of selection were linked to the predation regime, controlling
511 for geographic distance (isolation by distance) and evaluating multivariate patterns of
512 divergence or convergence, relative to expectations of drift.

513

514

515 **References**

- 516 1. Tollrian, R. and C.D. Harvell, *The ecology and evolution of inducible defenses*.
517 1999: Princeton University Press.
- 518 2. Miner, B.G., et al., *Ecological consequences of phenotypic plasticity*. Trends in
519 Ecology & Evolution. **20**(12): p. 685-692.
- 520 3. Pfennig, D.W., et al., *Phenotypic plasticity's impacts on diversification and*
521 *speciation*. Trends in Ecology & Evolution, 2010. **25**(8): p. 459-467.
- 522 4. Chevin, L.-M., R. Lande, and G.M. Mace, *Adaptation, Plasticity, and Extinction in a*
523 *Changing Environment: Towards a Predictive Theory*. PLoS Biol, 2010. **8**(4): p.
524 e1000357.
- 525 5. Draghi, J.A. and M.C. Whitlock, *Phenotypic plasticity facilitates mutational variance,*
526 *genetic variance, and evolvability along the major axis of environmental variation*.
527 Evolution, 2012. **66**(9): p. 2891-2902.
- 528 6. Ghalambor, C.K., et al., *Non-adaptive plasticity potentiates rapid adaptive evolution*
529 *of gene expression in nature*. Nature, 2015. **525**(7569): p. 372-375.
- 530 7. Ghalambor, C.K., et al., *Adaptive versus non-adaptive phenotypic plasticity and the*
531 *potential for contemporary adaptation in new environments*. Functional Ecology,
532 2007. **21**(3): p. 394-407.
- 533 8. Hendry, A.P., *Key Questions on the Role of Phenotypic Plasticity in Eco-*
534 *Evolutionary Dynamics*. Journal of Heredity, 2016. **107**(1): p. 25-41.
- 535 9. Pigliucci, M., *Evolution of phenotypic plasticity: where are we going now?* Trends in
536 Ecology & Evolution, 2005. **20**(9): p. 481-486.
- 537 10. Via, S. and R. Lande, *Genotype-environment interaction and the evolution of*
538 *phenotypic plasticity*. Evolution, 1985. **39**(3): p. 505-522.
- 539 11. Lind, M.I., et al., *The alignment between phenotypic plasticity, the major axis of*
540 *genetic variation and the response to selection*. Proceedings of the Royal Society of
541 London B: Biological Sciences, 2015. **282**(1816).
- 542 12. Merilä, J. and A.P. Hendry, *Climate change, adaptation, and phenotypic plasticity:*
543 *the problem and the evidence*. Evolutionary Applications, 2014. **7**(1): p. 1-14.
- 544 13. Taylor, B.E. and W. Gabriel, *To Grow or Not to Grow - Optimal Resource-Allocation*
545 *for Daphnia*. American Naturalist, 1992. **139**(2): p. 248-266.
- 546 14. Tollrian, R., *Predator-Induced Morphological Defenses - Costs, Life-History Shifts,*
547 *and Maternal Effects in Daphnia pulex*. Ecology, 1995. **76**(6): p. 1691-1705.
- 548 15. Reznick, D. and J.A. Endler, *The impact of predation on life-history evolution in*
549 *Trinidadian guppies (Poecilia reticulata)*. Evolution, 1982. **36**(1): p. 160-177.
- 550 16. Riessen, H., *Predator-induced life history shifts in Daphnia: a synthesis of studies*
551 *using meta-analysis*. Can J Fisheries Aquat Sci, 1999. **56**.
- 552 17. De Meester, L. and L.J. Weider, *Depth selection behavior, fish kairomones, and the*
553 *life histories of Daphnia hyalina X galeata hybrid clones*. Limnology and
554 Oceanography, 1999. **44**(5): p. 1248-1258.
- 555 18. Tollrian, R. and S.I. Dodson, *Inducible defenses in Cladocera: constraints, costs*
556 *and multipredator environments*, in *The Ecology and Evolution of Inducible*
557 *Defenses*, R. Tollrian and C.D. Harvell, Editors. 1999, Princeton University Press:
558 Princeton, NJ. p. 177-202.
- 559 19. Tollrian, R., *Daphnia pulex as an Example of Continuous Phenotypic Plasticity -*
560 *Morphological Effects of Chaoborus Kairomone Concentration and Their*
561 *Quantification*. Journal of Plankton Research, 1993. **15**(11): p. 1309-1318.
- 562 20. Dennis, S.R., et al., *Phenotypic convergence along a gradient of predation risk*.
563 Proceedings of the Royal Society B-Biological Sciences, 2011. **278**(1712): p. 1687-
564 1969.

- 565 21. Robinson, M.R. and A.P. Beckerman, *Quantifying multivariate plasticity: genetic*
566 *variation in resource acquisition drives plasticity in resource allocation to*
567 *components of life history*. Ecology Letters, 2013: p. 281-290.
- 568 22. Calsbeek, B. and C.J. Goodnight, *Empirical comparison of g matrix test statistics:*
569 *Finding biologically relevant change*. Evolution, 2009. **63**(10): p. 2627-2635.
- 570 23. Karhunen, M., et al., *driftsel: an R package for detecting signals of natural selection*
571 *in quantitative traits*. Molecular Ecology Resources, 2013. **13**(4): p. 746-754.
- 572 24. Karhunen, M. and O. Ovaskainen, *Estimating Population-Level Coancestry*
573 *Coefficients by an Admixture F Model*. Genetics, 2012. **192**(2): p. 609-617.
- 574 25. Karhunen, M., et al., *Bringing Habitat Information Into Statistical Tests Of Local*
575 *Adaptation In Quantitative Traits: A Case Study Of Nine-Spined Sticklebacks*.
576 Evolution, 2014. **68**(2): p. 559-568.
- 577 26. Ovaskainen, O., et al., *A New Method to Uncover Signatures of Divergent and*
578 *Stabilizing Selection in Quantitative Traits*. Genetics, 2011. **189**(2): p. 621-632.
- 579 27. Lynch, M., et al., *The quantitative and molecular genetic architecture of a*
580 *subdivided species*. Evolution, 1999. **53**(1): p. 100-110.
- 581 28. Reznick, D., *The Impact of Predation On Life-History Evolution in Trinidadian*
582 *Guppies - Genetic-Basis of Observed Life-History Patterns*. Evolution, 1982. **36**(6):
583 p. 1236-1250.
- 584 29. Schmid, M. and F. Guillaume, *The role of phenotypic plasticity on population*
585 *differentiation*. Heredity, 2017. **119**(4): p. 214-225.
- 586 30. Hendry, A.P., T. Day, and E.B. Taylor, *Population mixing and the adaptive*
587 *divergence of quantitative traits in discrete populations: A theoretical framework for*
588 *empirical tests*. Evolution, 2001. **55**(3): p. 459-466.
- 589 31. Hine, E., et al., *Characterizing the evolution of genetic variance using genetic*
590 *covariance tensors*. Philosophical Transactions of the Royal Society B-Biological
591 Sciences, 2009. **364**(1523): p. 1567-1578.
- 592 32. Aguirre, J.D., et al., *Comparing G: multivariate analysis of genetic variation in*
593 *multiple populations*. Heredity, 2014. **112**(1): p. 21-29.
- 594 33. Delahaie, B., et al., *Conserved G-matrices of morphological and life-history traits*
595 *among continental and island blue tit populations*. Heredity, 2017.
- 596 34. Gienapp, P., et al., *Predicting demographically sustainable rates of adaptation: can*
597 *great tit breeding time keep pace with climate change?* Philosophical Transactions
598 of the Royal Society B: Biological Sciences, 2013. **368**(1610).
- 599 35. Chevin, L.M. and R. Lande, *When do adaptive plasticity and genetic evolution*
600 *prevent extinction of a density-regulated population?* Evolution, 2010. **64**(4): p.
601 1143-1150.
- 602 36. Tollrian, R. and S. Dodson, *Inducible defences in cladocera: constraints, costs, and*
603 *multipredator environments*. The ecology and evolution of inducible defenses.
604 Princeton University Press, Princeton, NJ, 1999: p. 177-202.
- 605 37. Hammill, E., A. Rogers, and A.P. Beckerman, *Costs, benefits and the evolution of*
606 *inducible defences: a case study with Daphnia pulex*. Journal of Evolutionary
607 Biology, 2008. **21**(3): p. 705-715.
- 608 38. Stibor, H., *Predator Induced Life-History Shifts in a Freshwater Cladoceran*.
609 Oecologia, 1992. **92**(2): p. 162-165.
- 610 39. Weider, L. and J. Pijanowska, *Plasticity of Daphnia life histories in response to*
611 *chemical cues from predators*. Oikos, 1993. **67**.
- 612 40. Parejko, K. and S.I. Dodson, *The Evolutionary Ecology of an Antipredator Reaction*
613 *Norm: Daphnia pulex and Chaoborus americanus*. Evolution, 1991. **45**(7): p. 1665-
614 1674.

- 615 41. Spitze, K., *Chaoborus* Predation and Life-History Evolution in *Daphnia-Pulex* -
616 Temporal Pattern of Population Diversity, Fitness, and Mean-Life History. *Evolution*,
617 1991. **45**(1): p. 82-92.
- 618 42. Spitze, K., *Predator-mediated plasticity of prey life history and morphology:*
619 *Chaoborus americanus* predation on *Daphnia pulex*. *American Naturalist*, 1992.
620 **139**(2): p. 229-247.
- 621 43. Beckerman, A.P., G.M. Rodgers, and S.R. Dennis, *The reaction norm of size and*
622 *age at maturity under multiple predator risk*. *Journal Of Animal Ecology*, 2010. **79**:
623 p. 1069-1076.
- 624 44. Tollrian, R., *Chaoborus Crystallinus* Predation on *Daphnia pulex* - Can Induced
625 Morphological-Changes Balance Effects of Body-Size on Vulnerability. *Oecologia*,
626 1995. **101**(2): p. 151-155.
- 627 45. Cristescu, M.E.A., et al., *A microsatellite-based genetic linkage map of the*
628 *waterflea, Daphnia pulex: On the prospect of crustacean genomics*. *Genomics*,
629 2006. **88**(4): p. 415-430.
- 630 46. Reger, J., *The quantitative genetic basis of inducible defences and life-history*
631 *plasticity in Daphnia pulex*, in *Department of Animal and Plant Sciences*. 2013,
632 University of Sheffield: Sheffield.
- 633 47. Kenta, T., et al., *Multiplex SNP-SCALE: a cost-effective medium-throughput single*
634 *nucleotide polymorphism genotyping method*. *Molecular Ecology Resources*, 2008.
635 **8**(6): p. 1230-1238.
- 636 48. Development Core Team, R., *R: A Language and Environment for Statistical*
637 *Computing*. 2016, R Foundation for Statistical Computing: Vienna, Austria.
- 638 49. Hadfield, J.D., *MCMC Methods for Multi-Response Generalized Linear Mixed*
639 *Models: The MCMCglmm R Package*. *Journal of Statistcal Software*, 2010. **33**(2):
640 p. 1-22.
- 641 50. Ovaskainen, O., J.M. Cano, and J. Merila, *A Bayesian framework for comparative*
642 *quantitative genetics*. *Proceedings of the Royal Society B-Biological Sciences*,
643 2008. **275**(1635): p. 669-678.
- 644

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651

652 **Author Contributions**

653 JR and APB designed the research. JR and MIL collected data. APB and MRR developed
654 methods. JR, APB, MRR and MIL analysed data and wrote the MS.

655

656 **Figure Legends**

657 **Figure 1.** Univariate plasticity in the five traits. Each panel shows the change in trait mean
658 (y) between the two environments (x), and how these responses vary by predator regime
659 [fish(midge) vs midge]. The inset table presents a test of whether plasticity (slopes) differ
660 between each regime (regime x treatment interaction). The effect of the environment on
661 Age at Maturity and Population Growth Rate (PGR) does not depend on regime, while the
662 effect of the environment on Size at Maturity, Somatic Growth Rate and Induction
663 (neckteeth) does depend on regime. Data are mean \pm 95% confidence interval.

664

665 **Figure 2.** Genetic variance-covariance matrix visualisations for each treatment within each
666 regime. Size of the 3-D hull represents variance and the shape and rotation reflect
667 changes in covariance. Loadings (larger absolute values = stronger association) of traits
668 (see text for definitions) on each g_{\max} from the midge treatment are labeled indicating
669 differences in traits comprising the major axis of clonal variance in this system. See [21] for
670 methods.

671

672 **Figure 3.** Genetic variance – covariance matrix visualisations for each regime within each
673 treatment. The response to midge predation risk varies dramatically by regime, while there
674 is little difference in response to fish predation risk between regimes. Size of the 3-D hull
675 represents variance and the shape and rotation reflect changes in covariance. Loadings
676 (larger absolute values = stronger association) of traits (see text for definitions) on each
677 g_{\max} from the midge treatment are labeled indicating differences in traits comprising the
678 major axis of additive genetic (clonal) variance in this system. See [21] for methods.

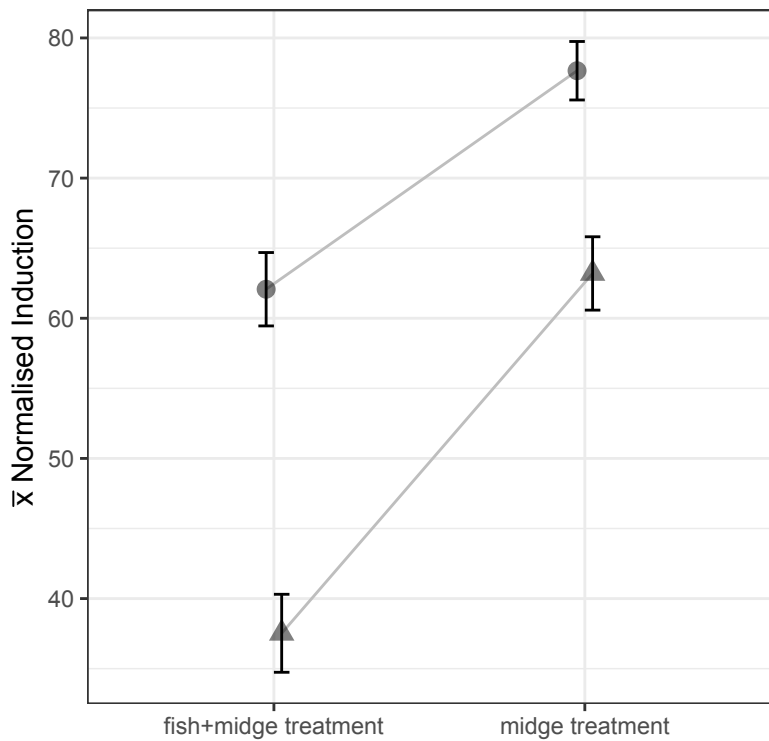
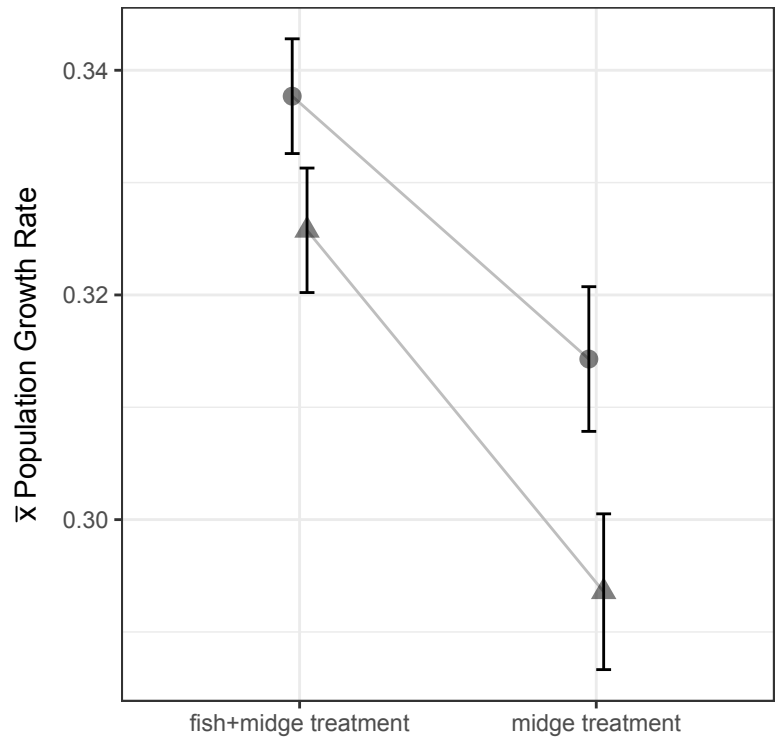
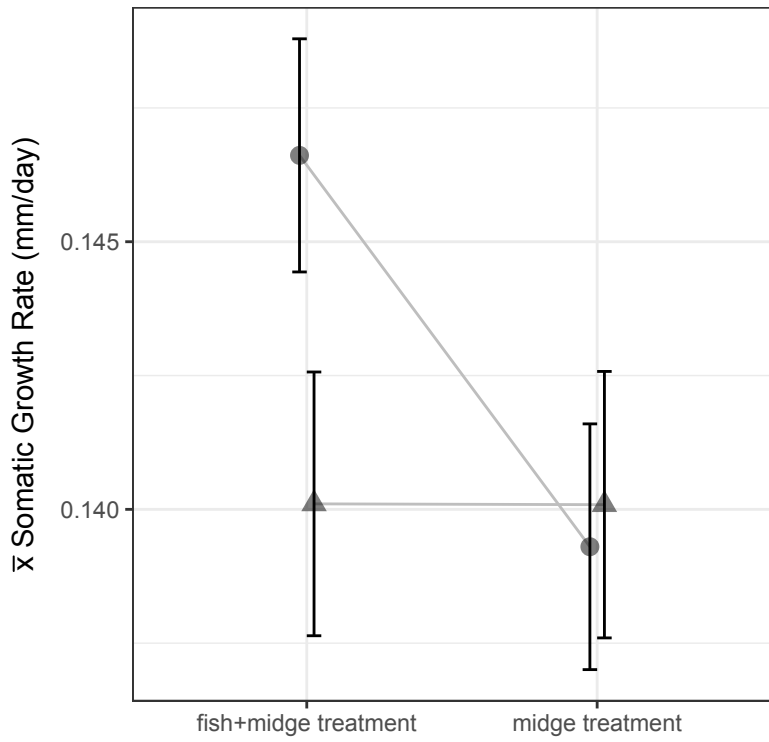
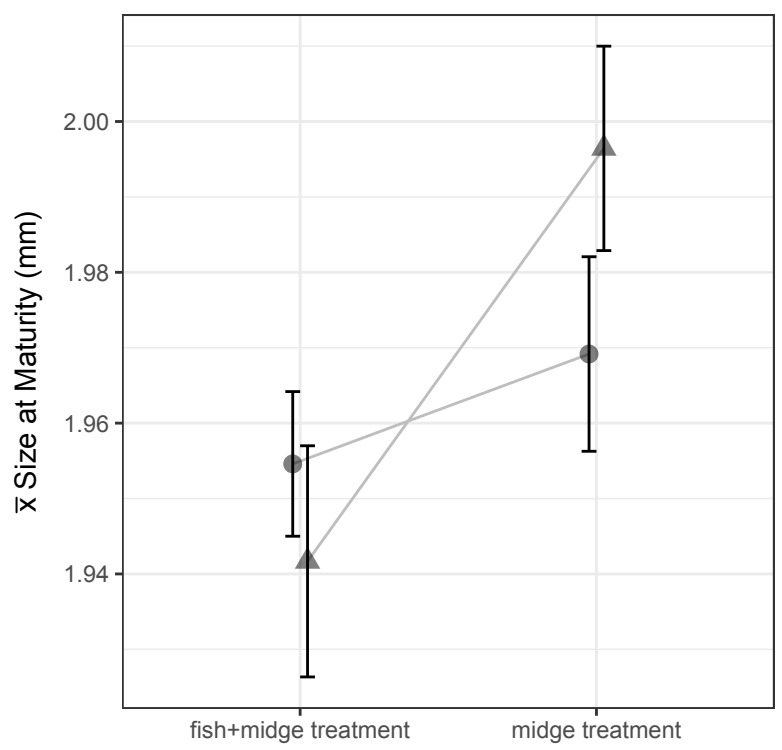
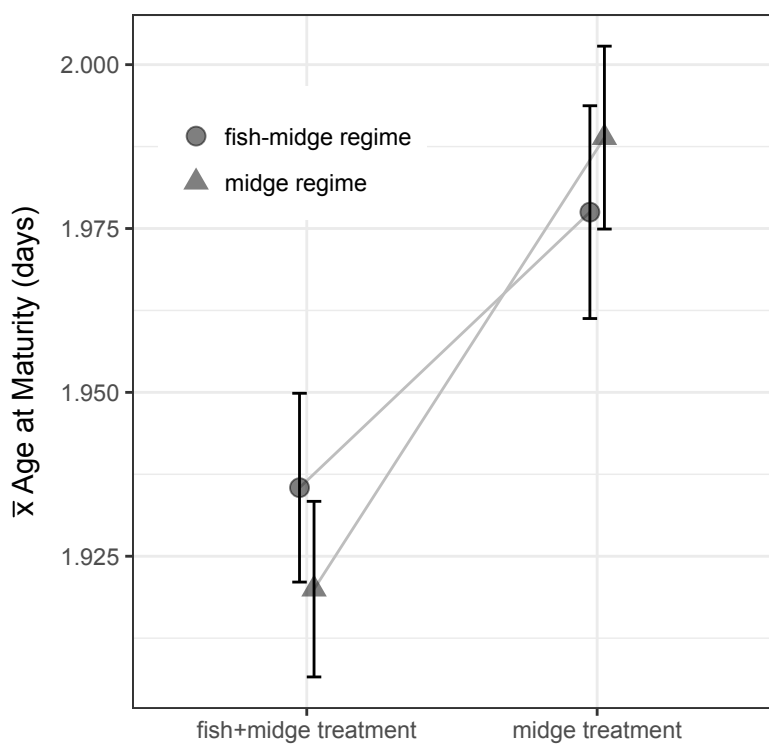
679

680

681 **Fig 4.** Multivariate Q_{ST} - F_{ST} analyses, following [23, 25, 26], showing evidence of strong
682 divergent selection among all eight populations, estimated in each predation risk
683 treatment; this is associated with predation regime (see text for detail). Each panel
684 represents an environment (e.g. midge or fish+midge predation risk) and presents the
685 signal of selection for univariate, 2-way, 3-way, 4-way and the 5-trait combination. S , which
686 can take values between 0 and 1, defines selection, where values of ~ 0.5 indicate drift, 0 -
687 0.2 stabilising selection, and 0.8 - 1 divergent selection among the populations [22].
688 (abbreviations: age = age at maturity, ind = morphological induction, pgr = population
689 growth rate, sGro = somatic growth rate, size = size at maturity).
690

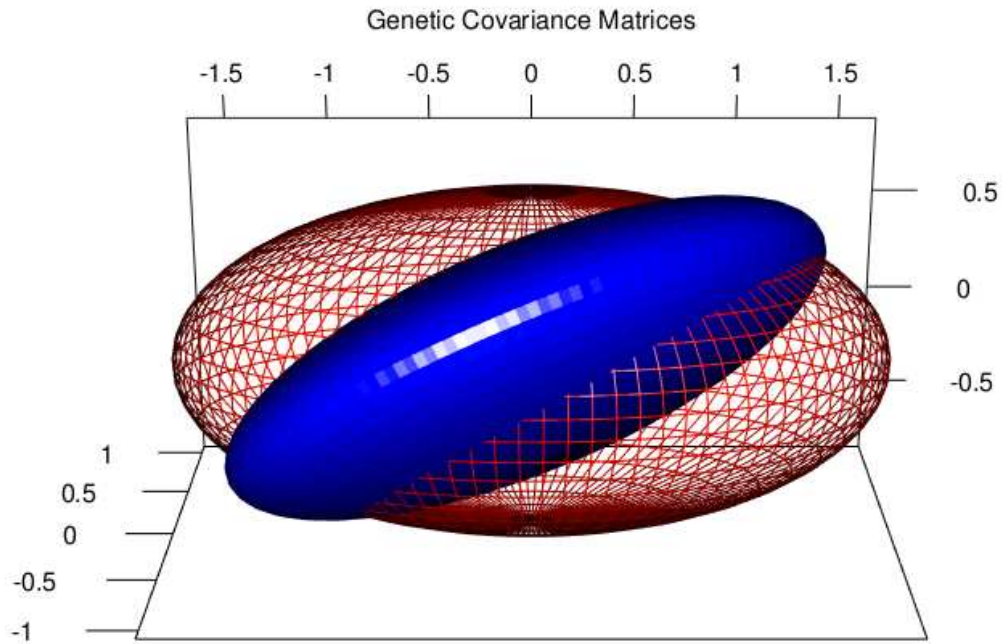
691 **Table 1.** Matrix comparison statistics for plasticity and local adaptation. Four metrics are
692 reported with their mode and 95% credible interval. VarGmax Diff estimates the change in
693 additive/clonal genetic variation between two matrices; Angle Between Gmax estimates
694 the angle of rotation between the two major axes of a G-matrix [21]; prob-VoIDiff and sum-
695 VoIDiff provide estimates of the change in total variance using two different methods for
696 estimating total variance of a G-matrix[21]. For VarGmax Diff, prob-VoIDiff and sum-
697 VoIDiff, significance is evaluated strictly by whether the 95% Credible Interval contains
698 zero. These metrics have NA (not applicable) placeholders in the Probability column. The
699 Angle Between g_{\max} is calculated by sampling from the posterior distribution of the
700 differences in angles within and between groups [21, 50]. With these samples, we can
701 calculate the probability that the between sample comparisons are larger than the within
702 sample comparisons. These are reported in the Probability column. Underlined rows
703 correspond to values discussed in the text (Local Adaptation II and III).
704

	Metric	mode	lower 95% CI	Upper 95% CI	Probability	
Plasticity	Fish-Midge Regime	VarGmax Diff	0.049	-0.196	0.228	NA
		<u>Angle Between Gmax</u>	<u>34.009</u>	<u>22.899</u>	<u>55.647</u>	<u>0.048</u>
		prob-VolDiff	0.027	-0.012	0.075	NA
		sum-VolDiff	-0.001	-0.815	0.668	NA
	Midge Regime	VarGmax Diff	0.046	-0.1	0.312	NA
		<u>Angle Between Gmax</u>	<u>39.063</u>	<u>20.908</u>	<u>61.426</u>	<u>0.08</u>
		prob-VolDiff	-0.002	-0.039	0.033	NA
		sum-VolDiff	0.063	-0.538	0.639	NA
Adaptation	Midge Environment	VarGmax Diff	0.008	-0.236	0.187	NA
		<u>Angle Between Gmax</u>	<u>32.096</u>	<u>18.233</u>	<u>49.11</u>	<u>0.03</u>
		prob-VolDiff	0.014	-0.021	0.07	NA
		sum-VolDiff	0.119	-0.5	0.885	NA
	Fish Environment	VarGmax Diff	0.045	-0.158	0.242	NA
		Angle Between Gmax	23.717	12.697	57.669	0.364
		prob-VolDiff	-0.005	-0.044	0.02	NA
		sum-VolDiff	0.137	-0.415	0.856	NA



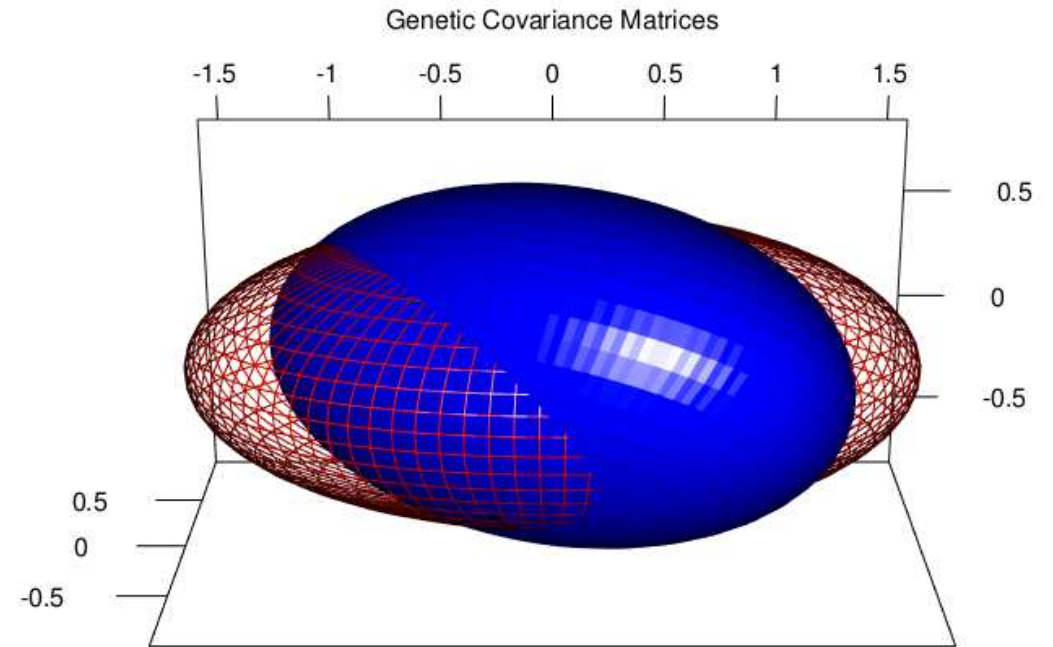
	Chisq	df	P
<i>Age</i>	0.008	1	0.93
<i>Size</i>	14.14	1	0.00017
<i>Somatic Growth</i>	16.397	1	5.14e-05
<i>Induction</i>	33.22	1	8e-09
<i>PGR</i>	0.052	1	0.8203

Fish-Midge Regime



induction : age : size : somatic growth : PGR
-0.02 : 0.39 : -0.54 : -0.62 : -0.42

Midge Regime

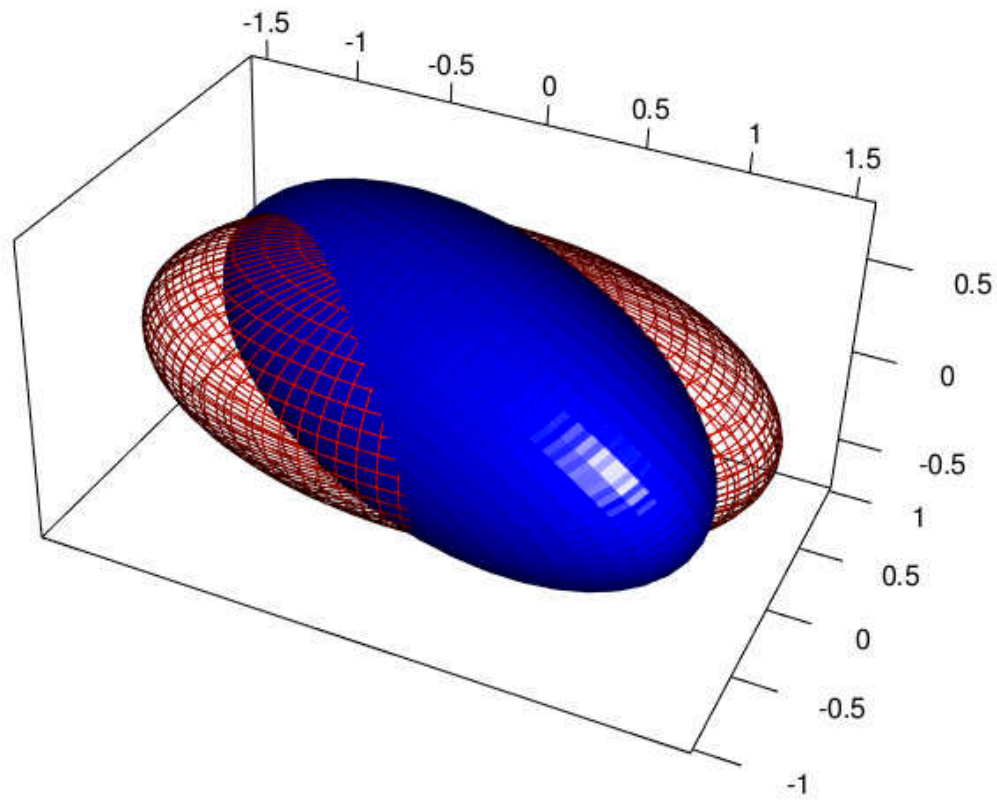


induction : age : size : somatic growth : PGR
-0.06 : -0.54 : 0.37 : 0.22 : 0.72

● Midge Treatment

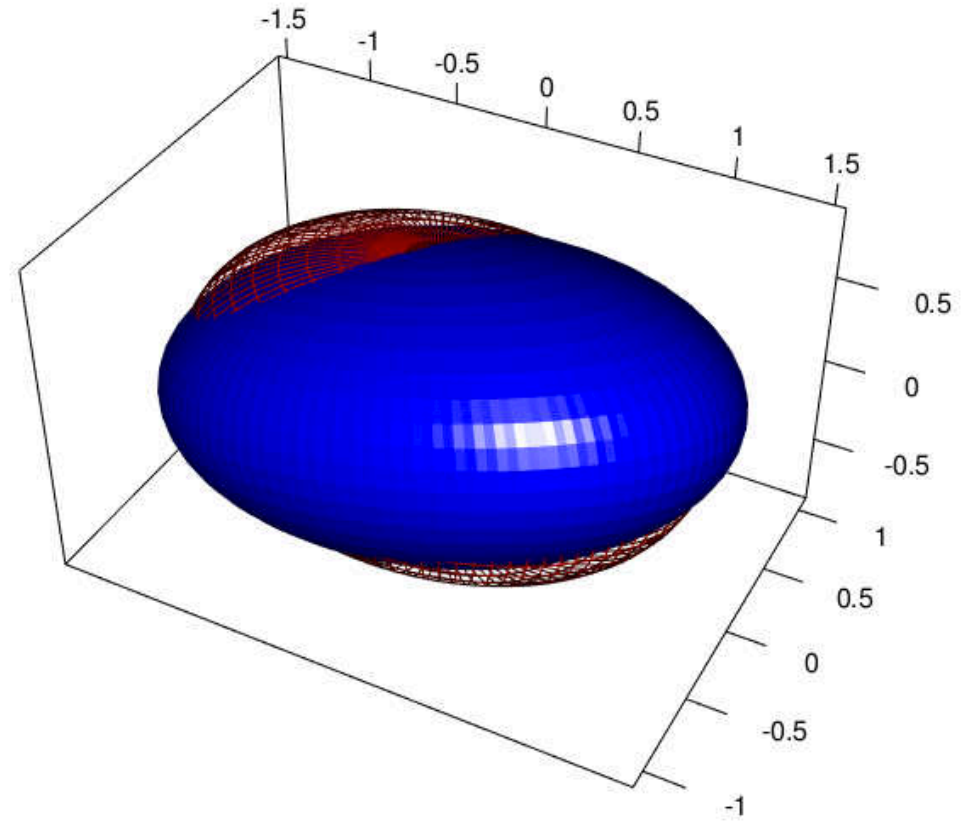
● Fish + Midge Treatment

Midge Treatment



induction : age : size : somatic growth : PGR
-0.06 : -0.54 : 0.37 : 0.22 : 0.72

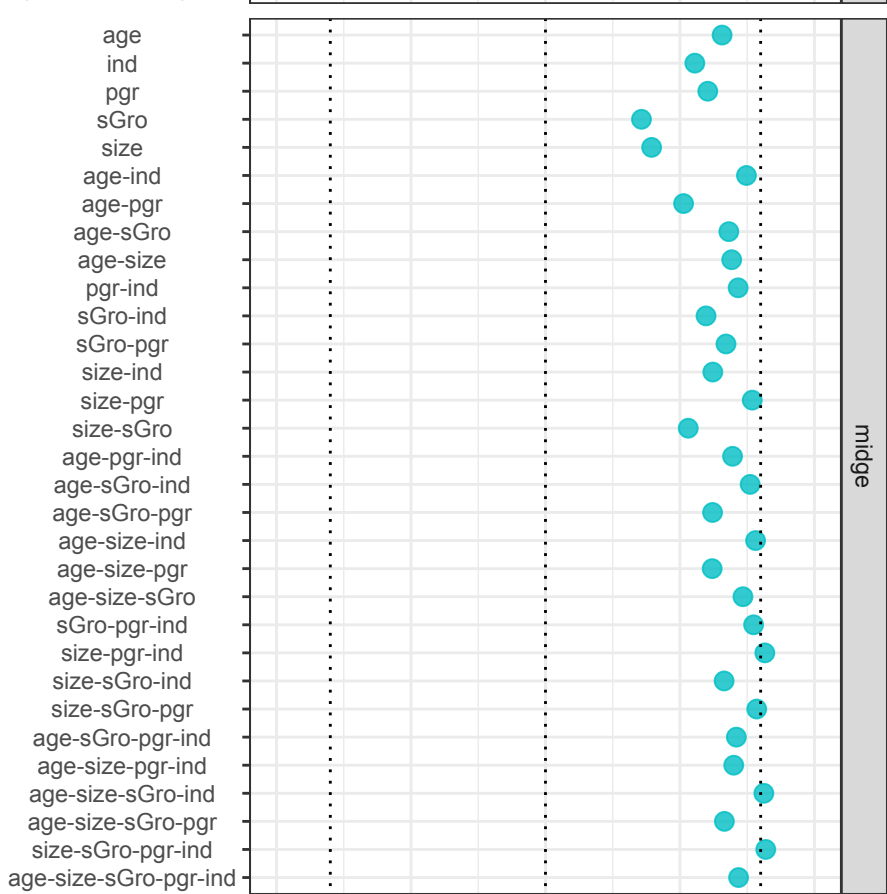
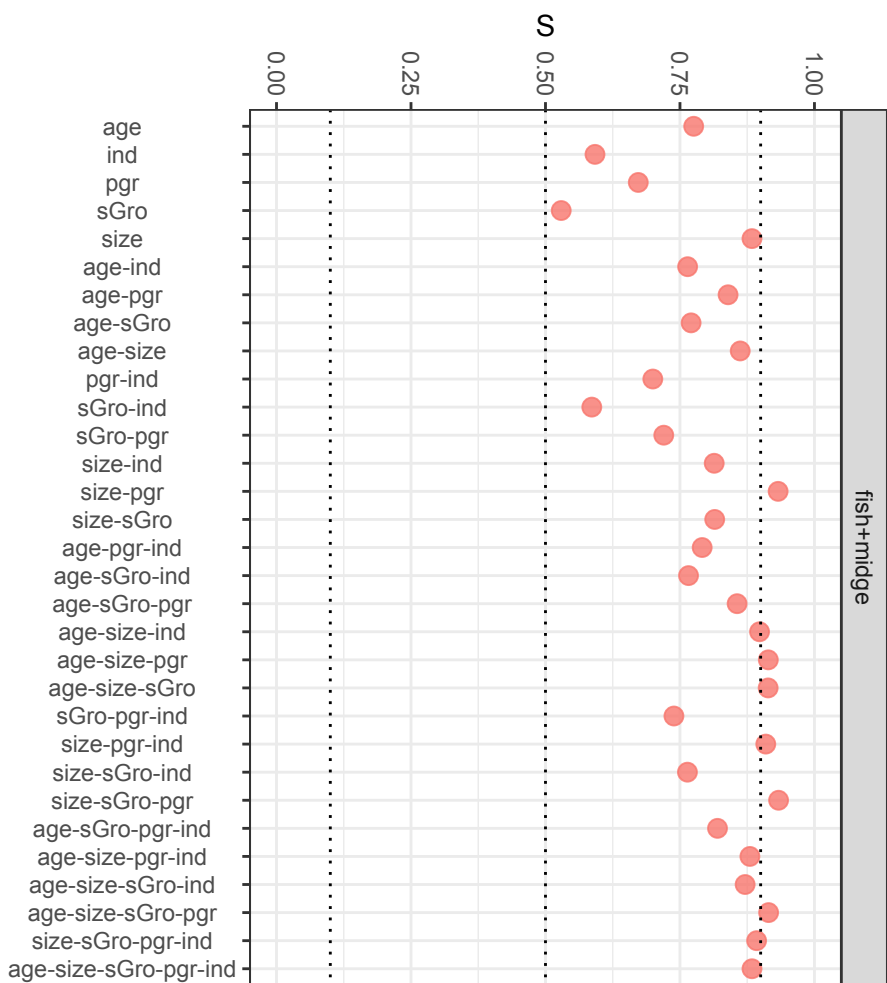
Fish+Midge Treatment



induction : age : size : somatic growth : PGR
0.01 : 0.46 : -0.16 : -0.68 : -0.54

● Midge Regime

● Fish-Midge Regime



Predation drives local adaptation of phenotypic plasticity

Supplementary Figures and Tables

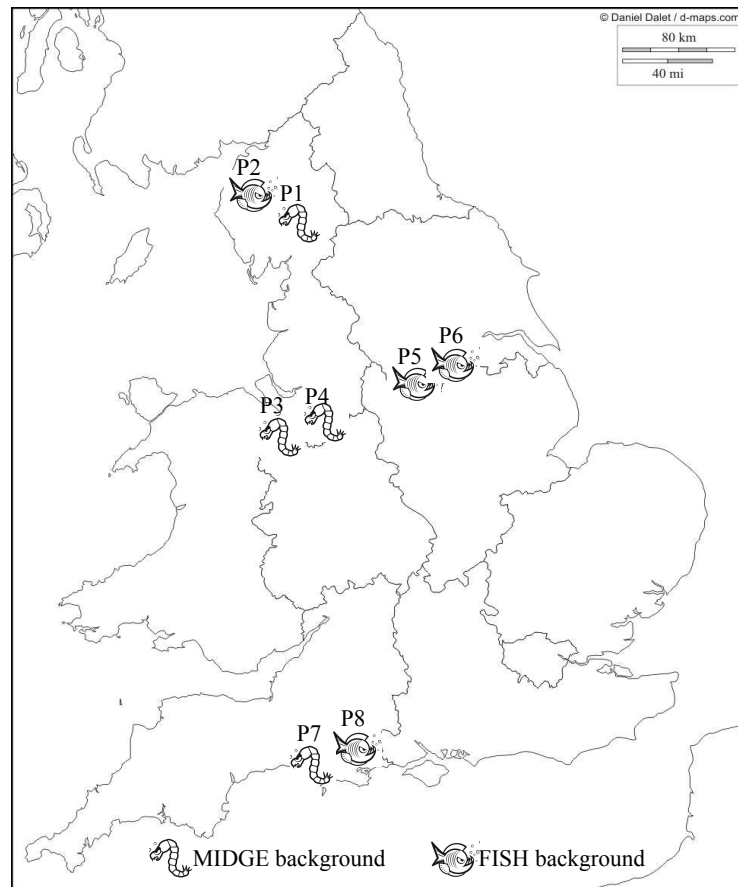
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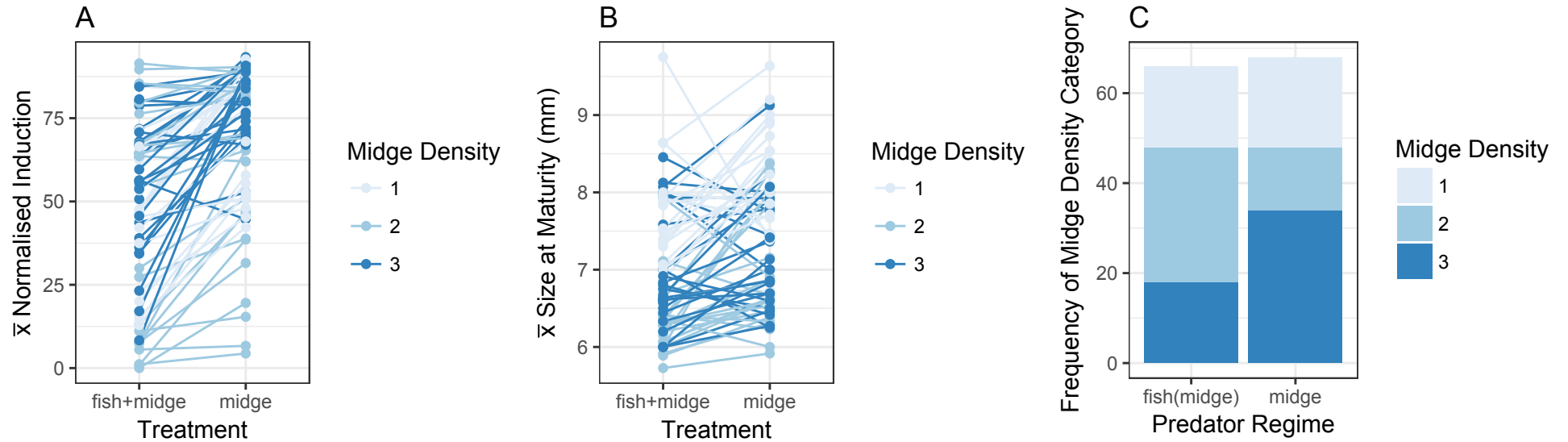
³Department of Computational Biology, University of Lausanne, Lausanne, Switzerland.

⁴Swiss Institute of Bioinformatics, Lausanne, Switzerland.



Supplementary Figure 1 Locations of study populations of *Daphnia pulex*, classified as either midge-dominated (midge regime), or fish-dominated ponds (fish-midge regime), along a 540km north-south axis in England, UK. See Supplementary Table 1 for further details on each population.

1
2

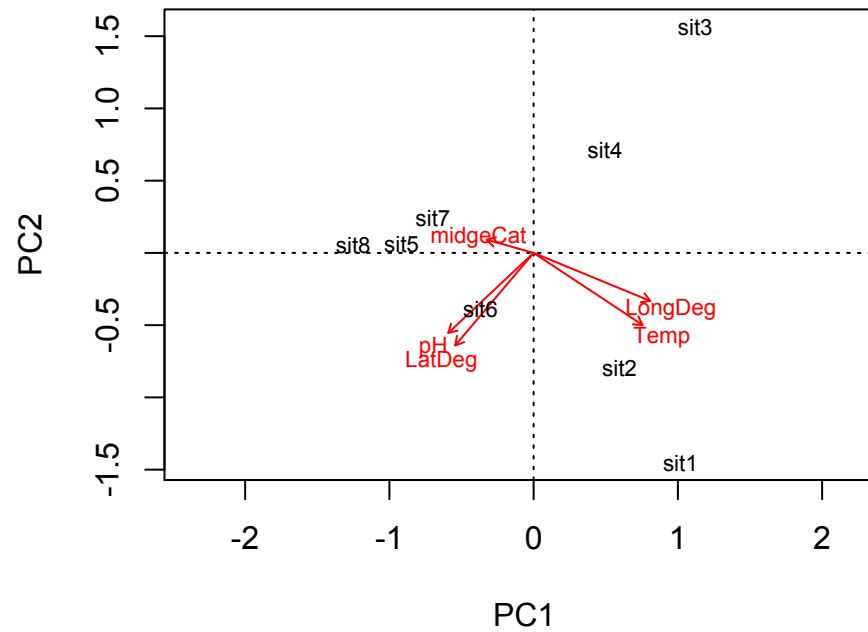


3

4 **Supplementary Figure 2.** Genetic variation in (a) morphological defense and (b) size at maturity plasticity is distributed across midge
5 densities. High midge density is more common in midge regimes (c). Each line in (A) and (B) connect a genotype mean trait value in
6 each treatment.

7

8

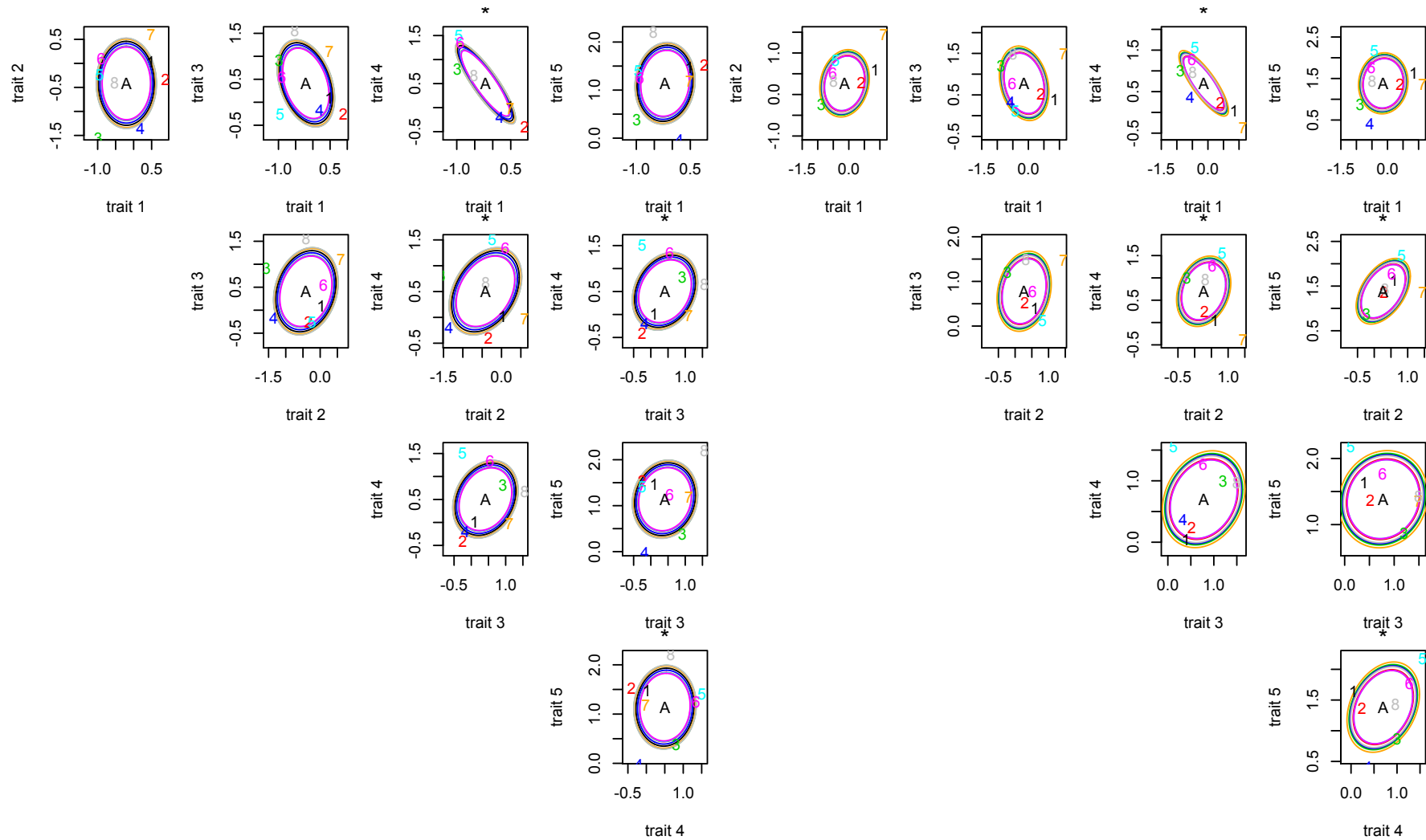


9

10 **Supplementary Figure 3.** A principle components analysis applied to five habitat variables measured for each population defined three
 11 major axes, capturing 90% of the variation. Longitude and Temperature are most closely associated with PC1, pH and Latitude with PC2
 12 and midge abundance most closely with PC3. None of the PC axes varied by predator regime (all $t < 1.6$, $p > 0.1$). sit1-8 = Pond 1-8.
 13 These PC variables were used in the H-test for association between divergent selection and predation regime.

14

15



16

17 **Supplementary Figure 4.** Distributions of mean additive genotypes and their expected divergences for all pairwise combinations of the
 18 five traits, revealing several strong patterns of covariance underpinning divergence patterns [23]. Mean phenotypes of populations are

19 denoted by number and the ellipses define the 50% probability sets for the range of random genetic drift for the respective populations.
20 When numbers are outside (inside) their lines, there is evidence for divergent (stabilising) selection. Trait 1 = age at maturity, Trait 2 =
21 size at maturity, Trait 3 = Somatic Growth Rate, Trait 4 = Population Growth Rate, Trait 5 = Morphological Induction.

22

Supplementary Table 1. Location details and categorization of the ponds. *Daphnia pulex* clones were collected from between May and September 2009. Sampling revealed two types of ponds: shallow small ponds with invertebrate (midge) predators and larger ponds that also host vertebrate (fish) predators. Ponds were thus classified as either midge (midge only background) or fish_midge (fish + midge background). Temperature and pH data are single values from mid-summer. Other predators include *Notonecta* and dragonfly larvae.

Pond	Location	Coordinates	Predation Regime	Hydroperiod	Temp (°C)	pH	Vegetation	Cover	Midge density	Other predators	No. genotypes
P1	Cumbria	54°20'39.8791"N	Midge	Temporary	13.1	8.5	Heavy	Light	High	No	10
		002°50'53.9422"W									
P2	Cumbria	54°20'51.8643"N	Fish/Midge	Permanent	17	8.46	Present	Light	Low	Yes	10
		002°53'07.1089"W									
P3	Cheshire	53°17'45.7623"N	Midge	Semi-permanent	12.1	8.63	None	Shaded	Low	No	10
		003°00'26.7868"W									
P4	Cheshire	53°18'17.7955"N	Midge	Temporary	12.1	8.88	None	Shaded	High	No	8
		003°01'05.3586"W									

P5	Yorkshire	53°20'06.0076"N	Fish/Midge	Permanent	19.4	8.45	Heavy	Light	Medium	No	9
001°27'09.3348"W											
P6	Yorkshire	53°24'18.4949"N	Fish/Midge	Permanent	21.7	8.62	Heavy	Light	Low	Yes	9
001°27'27.7570"W											
P7	Dorset	50°38'33.3445"N	Midge	Temporary	16.1	8.45	Present	Shaded	Medium	No	8
002°05'58.7449"W											
P8	Dorset	50°42'35.6367"N	Fish/Midge	Permanent	16.4	7.82	Heavy	Light	Low	Yes	6
002°12'26.7497"W											

Supplementary Table 2. The AFM model estimates F_{ST} and gene flow via population co-ancestry [24]. The QST-FST method we employ estimates a matrix of co-ancestry coefficients. The diagonals are the average co-ancestry within subpopulations and the off-diagonals are the average co-ancestry between subpopulations. F_{ST} is a function of all values, and gene-flow inferred from the off-diagonals, based on the coalescent definitions of F_{ST} (see [23, 24, 26]).

	1	2	3	4	5	6	7	8
1	0.41169							
2	0.00048	0.32503						
3	0.00002	0.00007	0.45599					
4	0.00001	0.00006	0.00024	0.34877				
5	0.00004	0.00007	0.0001	0.00002	0.29607			
6	0.00007	0.00004	0.00001	0.00003	0.00032	0.27084		
7	0.00006	0.00004	0.00008	0.00005	-0.00003	0.00049	0.49428	
8	0.00004	0.00005	0.00005	0.00003	0.00002	0.00009	0.00009	0.3378