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Predation drives local adaptation of phenotypic plasticity

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

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

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

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

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

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

Results

The D. pulex system

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

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

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

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

Local Adaptation I: Plasticity x Regime Interactions

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

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

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

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

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

Within each predation regime (e.g. n=4 populations/regime), we detected no size 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

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

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

Local Adaptation IV: Predator Regime Drives Divergent Selection

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

between environments. Plasticity could thus align with g_{max} and ultimately selection [11]. This combination of theory and data suggests that phenotypic plasticity might actually 'aid evolution'.

Local adaptation of phenotypic plasticity might even be interpreted as a positive feedback to local adaptation *per se* via this alignment mechanism. Such an idea must be considered in light of theory on the effects of adaptive/maladaptive plasticity on local adaptation [29]. Schmid and Guillaume's theory [29] (and see Hendry [30]) shows how undifferentiated and un-evolving plasticity can none-the-less have substantial effects on the interplay between gene-flow and selection. Plasticity can, for example, neutralize fitness difference of migrants leading to increased phenotypic divergence but low genetic divergence, while maladaptive plasticity can increase genetic differentiation by increasing strength of selection, but also increase the risk of population extinction. Our evidence that plasticity can itself be locally adapted, and align genetic variation with selection [11], adds a compelling dimension to their call to consider more thoroughly the role of both adaptive and maladaptive plasticity in local adaptation and the response of populations to environmental change.

Our results also strongly suggest that to fully understand the ecological and evolutionary implications of plasticity, we must employ a multi-trait and multivariate analysis of phenotypic plasticity. Our data strengthen the call for multivariate approaches to research on plasticity and local adaptation [11, 21, 26, 31-33]. First, although all five traits that we measured are considered theoretically important traits linked to survival and reproduction in the face of predation risk, not all of them show univariate signature of a regime by treatment interaction (Figure 1) or univariate divergence across regimes (Figure 4). Second, the multivariate phenotype shows always a greater signature of selection than

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

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

Methods

Study System

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

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

Vertebrate and/or invertebrate predators thus select against large and small sizes in *Daphnia* prey, requiring defensive adaptive traits that have been shown to be effective and heritable [1, 40-42]. We examined predator-induced plasticity in several life-history traits of *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 µl ml^{-1} *Chaoborus*
403 predator cue concentrate. The fish treatment received 0.5 µl 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 x 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 2^{nd} and 3^{rd} 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 µl buffer (made up of 10 mM Tris-Cl pH 8.2, 1 mM EDTA and 25 mM NaCl)
444 and 4 µl 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 µl PCR reactions,
450 containing approximately 10ng of lyophilised genomic DNA, 0.2 µM of each primer and 1
451 µl 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

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

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

Q_{ST}- F_{ST}

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

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Author Contributions

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

Figure Legends

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

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

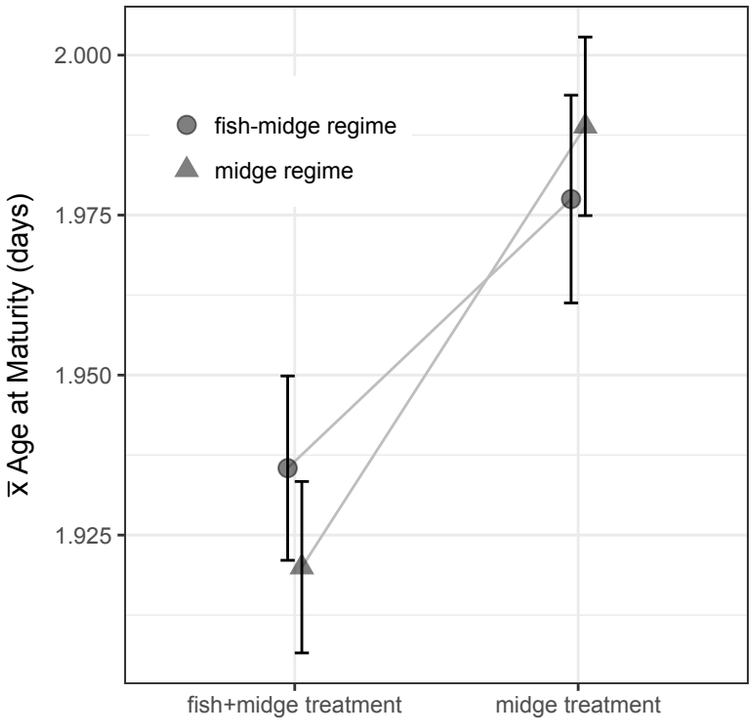
Figure 3. Genetic variance – covariance matrix visualisations for each regime within each treatment. The response to midge predation risk varies dramatically by regime, while there is little difference in response to fish predation risk between regimes. Size of the 3-D hull represents variance and the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

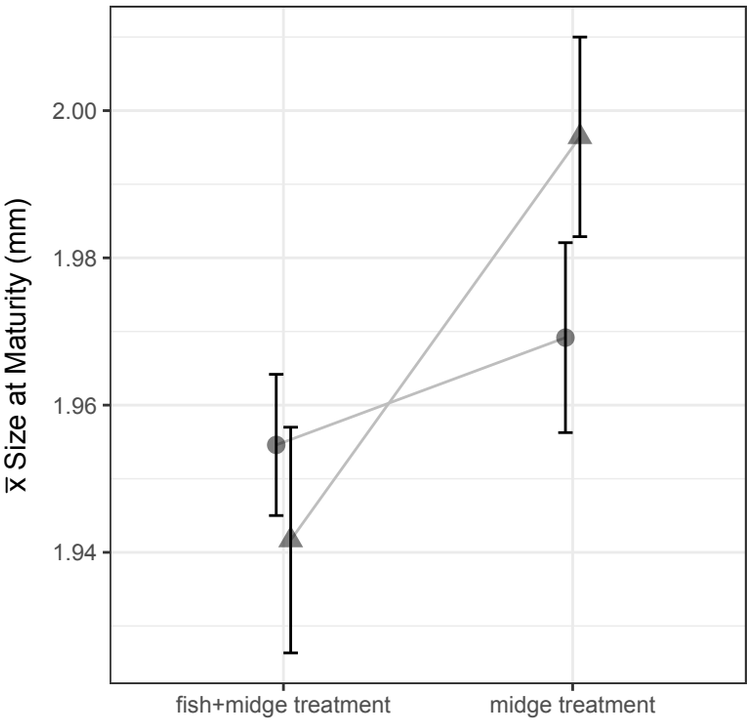
Fig 4. Multivariate Q_{ST}-F_{ST} analyses, following [23, 25, 26], showing evidence of strong divergent selection among all eight populations, estimated in each predation risk treatment; this is associated with predation regime (see text for detail). Each panel represents an environment (e.g. midge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

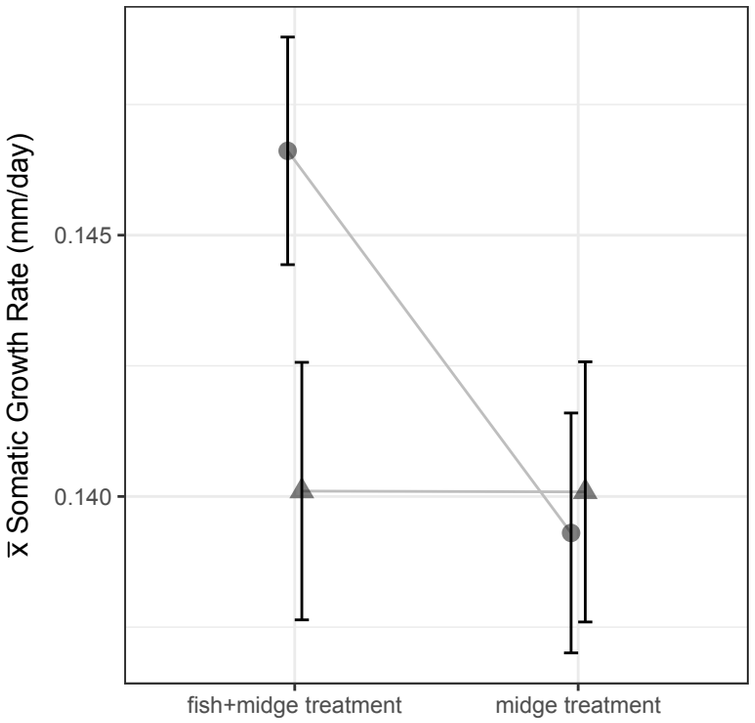
691 **Table 1**. Matrix comparison statistics for plasticity and local adaptation. Four metrics are
692 reported with their mode and 95% credible interval. VarGmax Diff estimates the change in
693 additive/clonal genetic variation between two matrices; Angle Between Gmax estimates
694 the angle of rotation between the two major axes of a G-matrix [21]; prob-VolDiff and sum-
695 VolDiff 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-VolDiff and sum-
697 VolDiff, 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

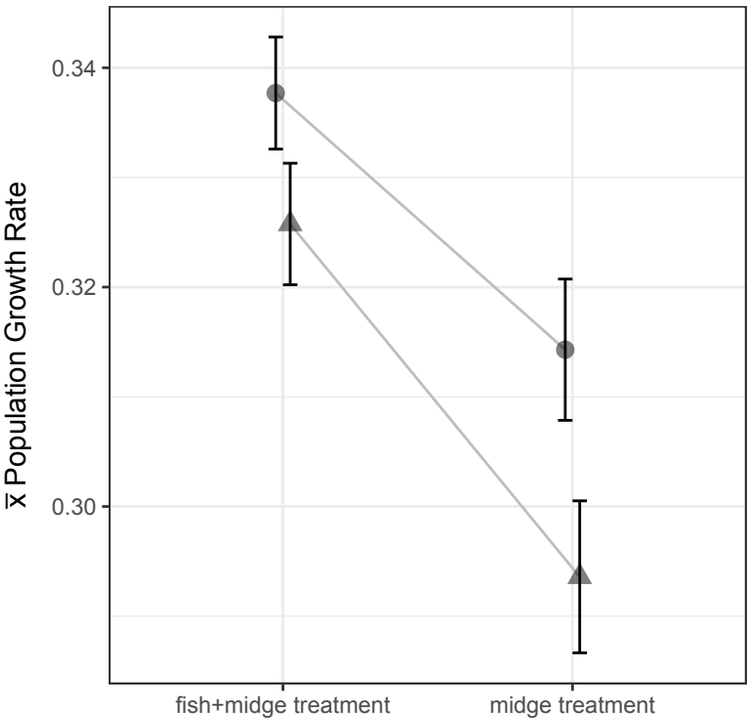
		Metric	mode	lower 95% CI	Upper 95% CI	Probability
Plasticity	Fish-Midge Regime	VarGmax Diff	0.049	-0.196	0.228	NA
		Angle Between Gmax	34.009	22.899	55.647	0.048
		prob-VolDiff	0.027	-0.012	0.075	NA
		sum-VolDiff	-0.001	-0.815	0.668	NA
	Midge Regime	VarGmax Diff	0.046	-0.1	0.312	NA
		Angle Between Gmax	39.063	20.908	61.426	0.08
		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
		Angle Between Gmax	32.096	18.233	49.11	0.03
		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

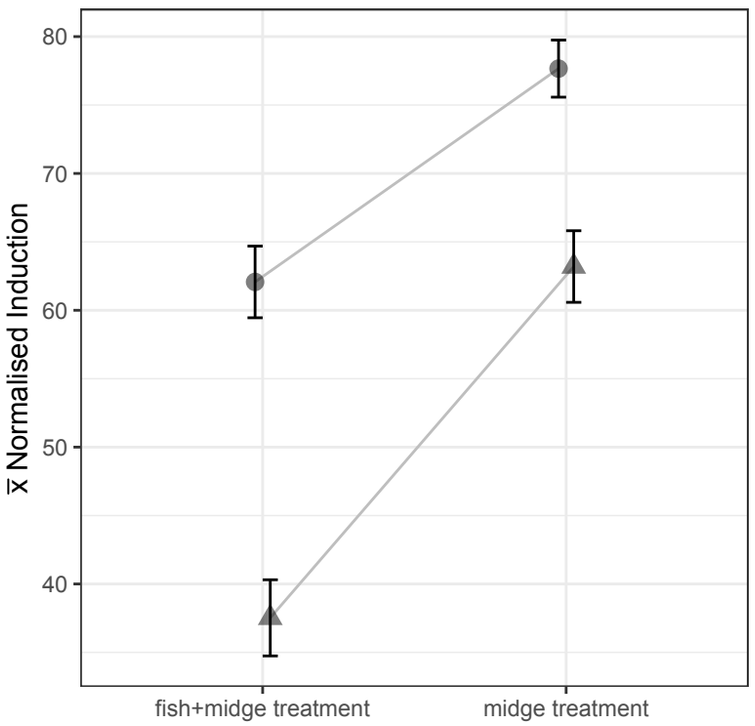
705





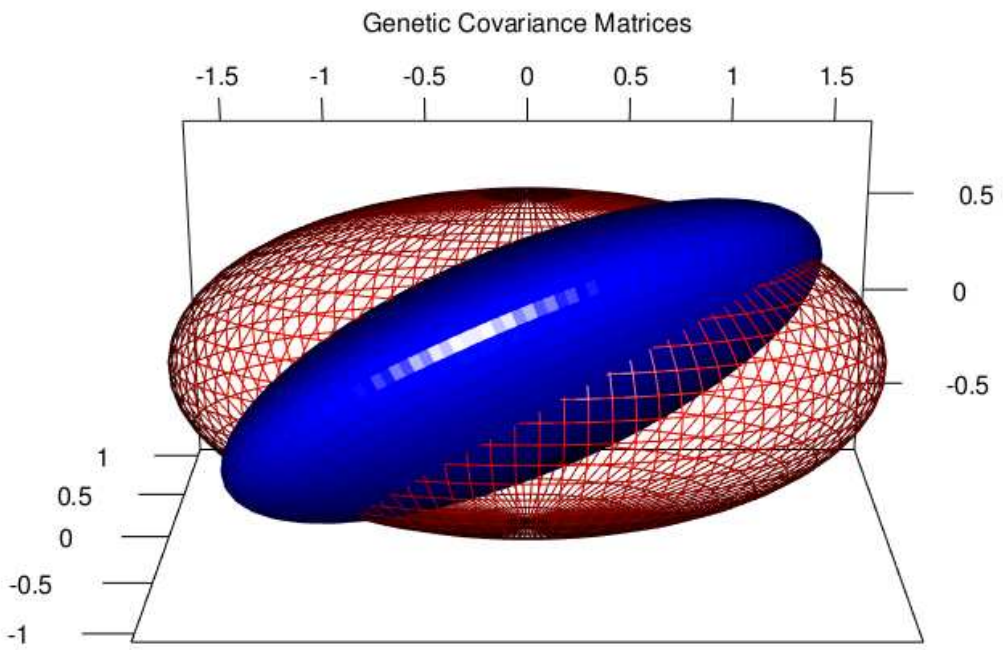






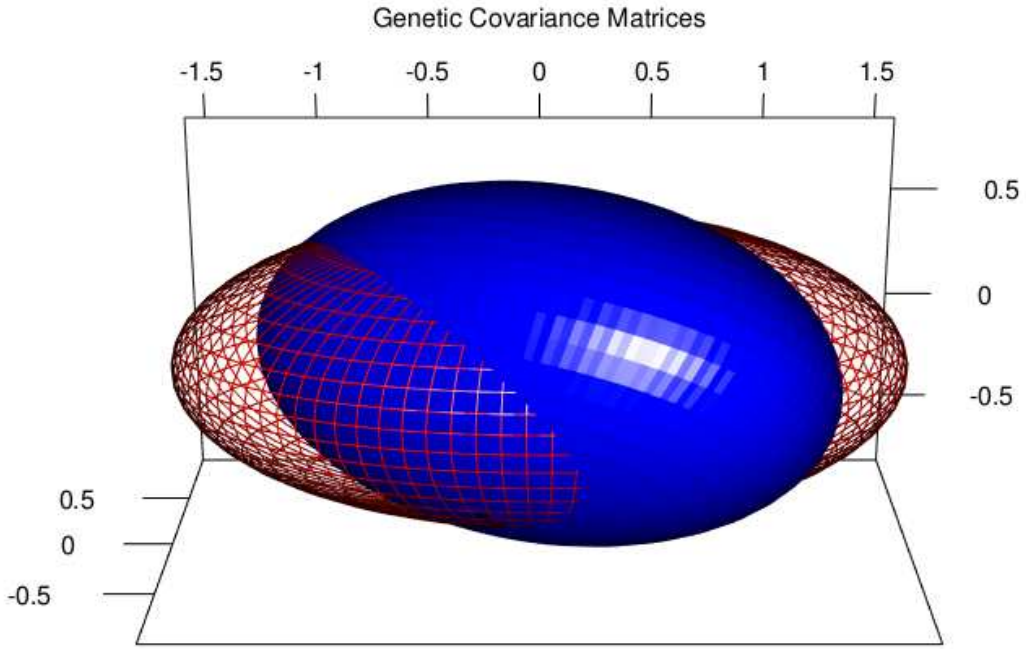
	Chisq	df	P
Age	0.008	1	0.93
Size	14.14	1	0.00017
Somatic Growth	16.397	1	5.14e-05
Induction	33.22	1	8e-09
PGR	0.052	1	0.8203

Fish-Midge Regime



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

Midge Regime

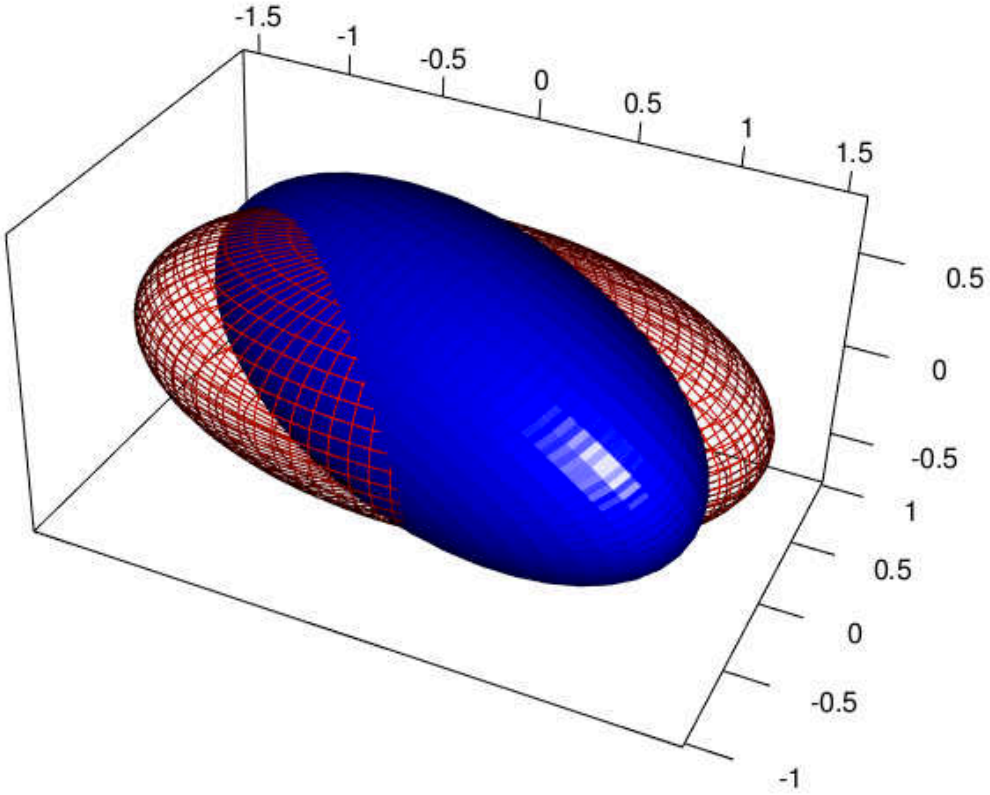


induction : age : size : somatic growth : PGR
-0.06 : -0.54: 0.37 : 0.22 : 0.72

Midge Treatment

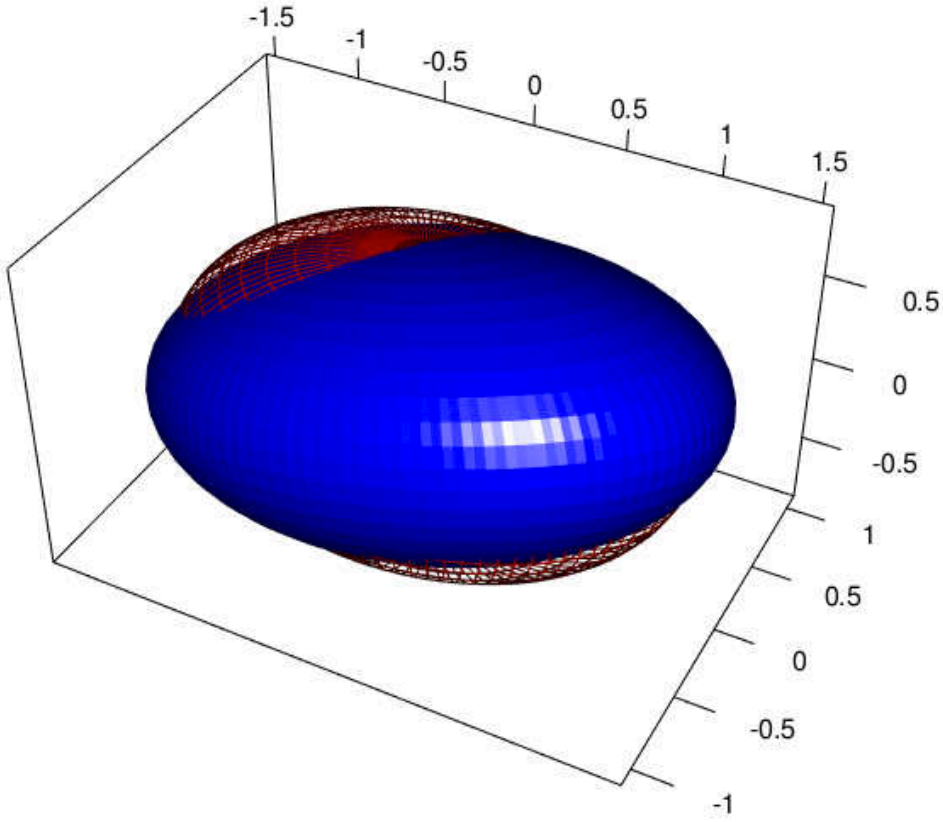
Fish + Midge Treatment

Midge Treatment



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

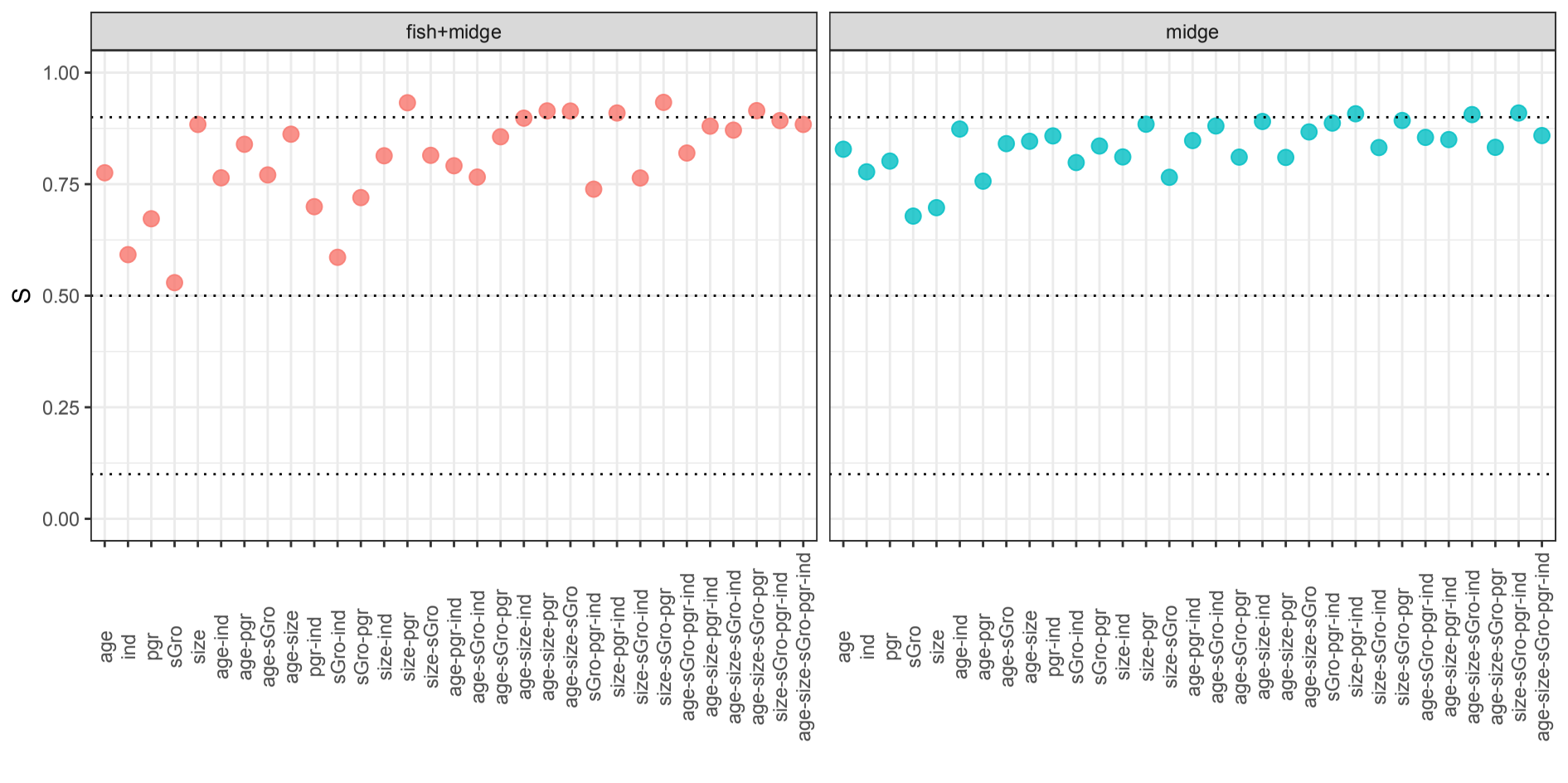
Fish+Midge Treatment



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

Midge Regime

Fish-Midge Regime


fish+midge
midge
S
1.00
0.75
0.50
0.25
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age
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Predation drives local adaptation of phenotypic plasticity

Supplementary Figures and Tables

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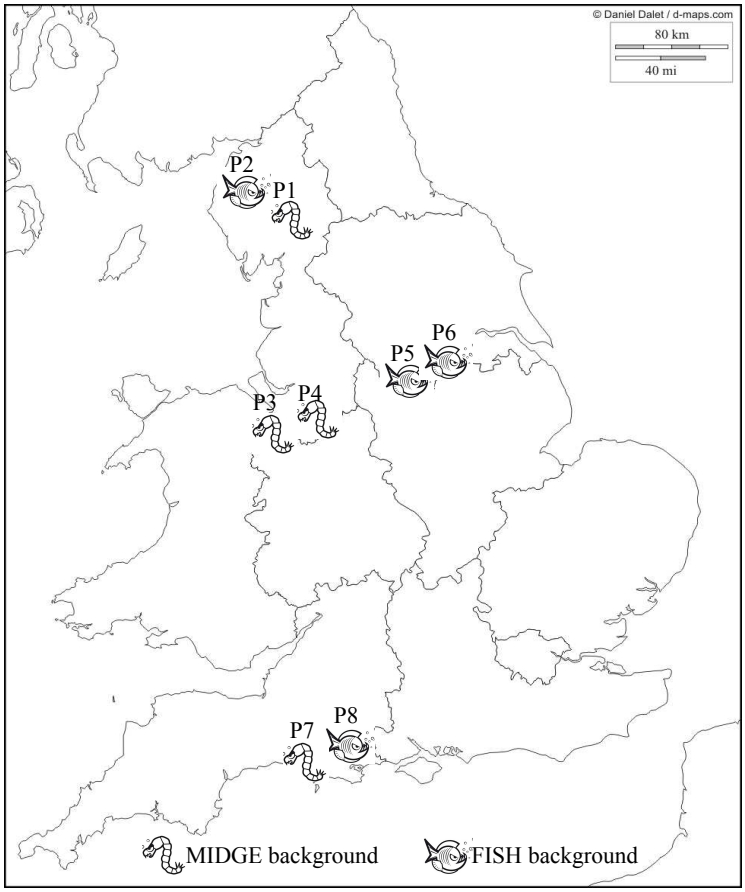
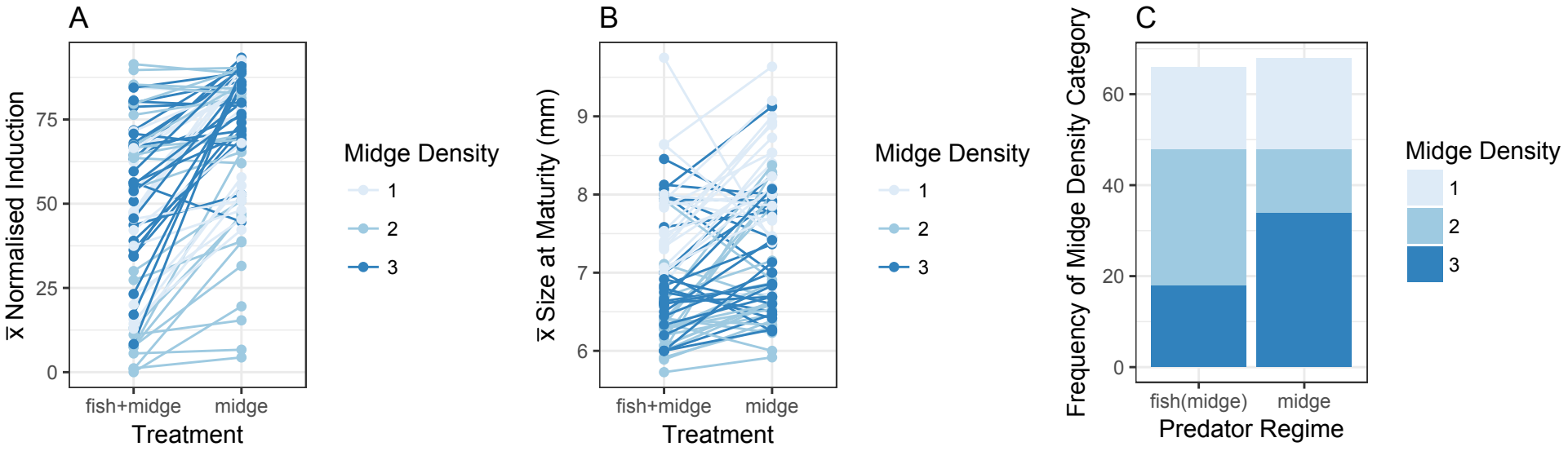
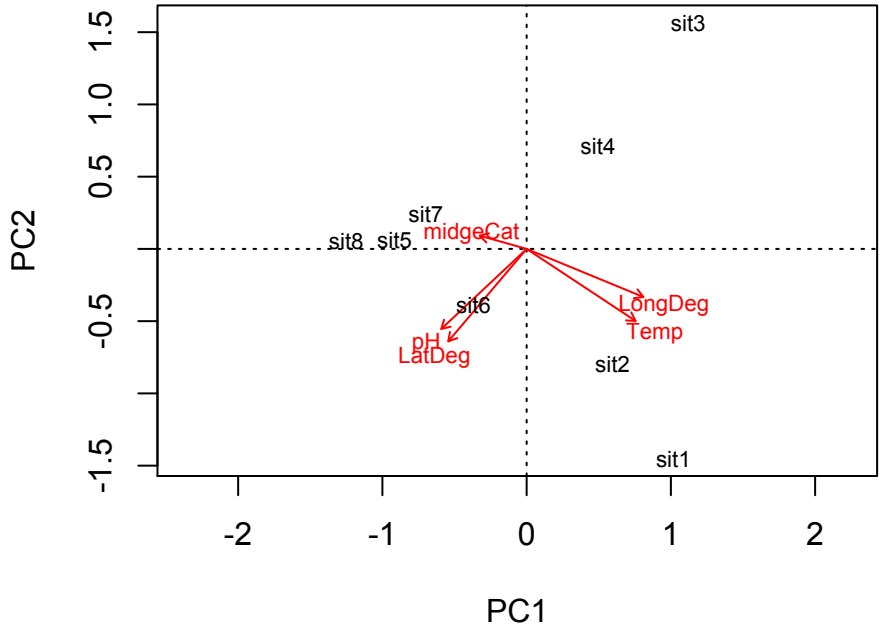


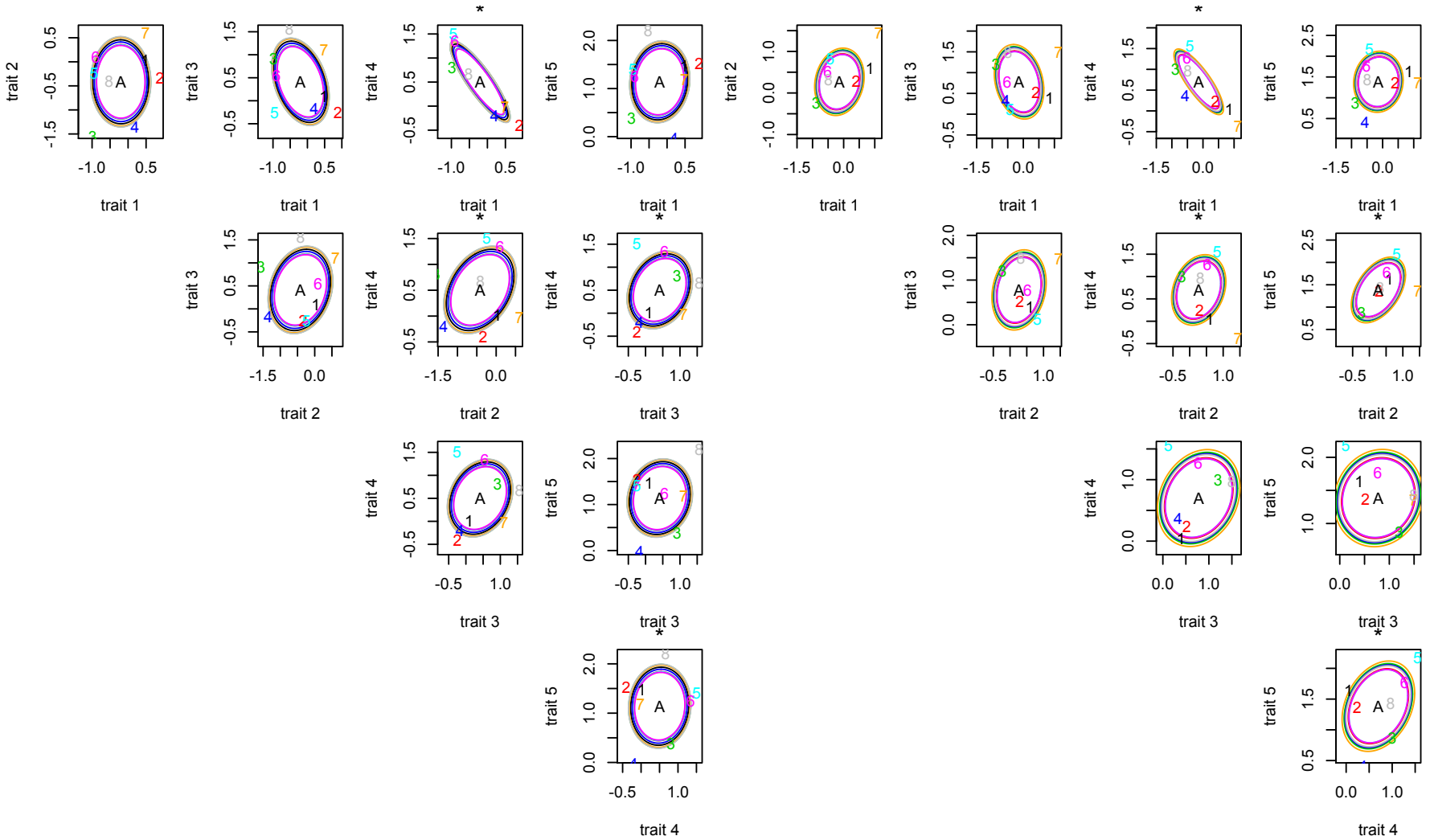
Supplementary Figure 1 Locations of study populations of *Daphnia pulex*, classified as either midge-dominated (midge regime), or fish-dominated ponds (fish-midge regime), along a 540km north-south axis in England, UK. See Supplementary Table 1 for further details on each population.



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



Supplementary Figure 3. A principle components analysis applied to five habitat variables measured for each population defined three major axes, capturing 90% of the variation. Longitude and Temperature are most closely associated with PC1, pH and Latitude with PC2 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. These PC variables were used in the H-test for association between divergent selection and predation regime.



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

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

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 Fst and gene flow via population co-ancenstry [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**