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Experimental evolution of local adaptation under unidimensional and multidimensional selection

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Summary

Local adaptation is a fundamental evolutionary process generating biological diversity and potentially enabling ecological speciation. Divergent selection underlies the evolution of local adaptation in spatially structured populations by driving their adaptation towards local optima. Environments rarely differ along just one environmental axis, and so divergent selection may often be multidimensional. How the dimensionality of divergent selection affects local adaptation is unclear: Evolutionary theory predicts that increasing dimensionality will increase local adaptation when associated with stronger overall selection but may have less predictable effects if selection strengths are equal. Experiments are required that allow the effect of the dimensionality of selection on local adaptation to be tested independently of the total strength of selection. We experimentally evolved 32 pairs of monogonont rotifer populations under either unidimensional divergent selection (a single pair of stressors) or multidimensional divergent selection (three pairs of stressors), keeping the total strength of selection equal between treatments. At regular intervals, we assayed fitness in home and away environments to assess local adaptation. We observed an initial increase and subsequent decline of local adaptation in populations exposed to multidimensional selection, compared to a slower but eventually stronger increase in local adaptation in populations exposed to unidimensional selection. Our results contrast with existing predictions such as the 'weak multifarious' and 'stronger selection' hypotheses. Instead, we hypothesise that adaptation to multidimensional divergent selection may favour generalist genotypes and only produce transient local adaptation.

Keywords

Divergent selection, Dimensionality, Fitness, Generalists, Specialists, Divergence

Introduction

When faced with selection pressures that vary spatially, populations may adapt to local conditions[1]. Such local adaptation produces a pattern in which populations have higher fitness in their home environment than they would if transplanted to a different environment[2]. Local adaptation is an important component of within-species diversity and an increase in local adaptation is also a vital step towards ecological speciation, providing an extrinsic form of reproductive isolation[3,4]. The strength of local adaptation, and the speed at which it increases, depends on environmental factors such as the strength of selection and gene flow[5,6], and genomic conditions such as the amount and distribution of standing genetic variation[7,8] and the genomic architecture[9,10].

One variable that has received far less attention, however, is the number of simultaneous divergent selection pressures experienced by a population, i.e. the dimensionality of divergent selection[11–13]. Local adaptation can be driven by between-population heterogeneity in a single selection pressure (unidimensional divergent selection) or by multiple selection pressures (multidimensional divergent selection). Multidimensional divergent selection is generally predicted to drive the evolution of reproductive barriers, including local adaptation, more effectively than unidimensional selection and so to promote ecological speciation[11–14]. However, to date, no study has tested how local adaptation builds under different dimensionalities of selection[15].

It is important to distinguish the dimensionality of divergent selection from the overall dimensionality of selection (sometimes referred to as 'niche dimensionality')[16–19]. Overall dimensionality reflects the total number of selection pressures impacting a population, many of which will act via stabilising selection[18–21]. In any environment selection is likely to be imposed by multiple environmental variables and to act on multiple traits that are determined by many genes. The dimensionality of selection then depends on the extent to which environmental variables, and organismal traits are independent. For example, extensive pleiotropy reduces the effective dimensionality because generalist genotypes can increase fitness in response to multiple selection pressures simultaneously[22,23].

However, the dimensionality of divergent selection considers only selection pressures which vary between environments. Using a framework such as Fisher's Geometric Model[24], any two environments are separated by a single vector in multidimensional space. We can, therefore, define the dimensionality of divergent selection as the number of orthogonal environmental variables that are correlated with this vector. The dimensionality of divergent selection is also likely to be related to the number of traits and number of loci that must evolve to achieve local adaptation, although these ways of defining the available space do not map directly, for example because genetic correlations (G-

matrix covariances) mean that fewer genetic axes may underlie responses to multiple environmental variables or traits[25–27].

Predictions of how dimensionality might impact local adaptation and speciation, such as Nosil *et al*.'s[11] 'stronger selection' vs 'weak multifarious' hypotheses, describe how multidimensionality might distribute divergent selection over more traits. In reality, there are several different ways in which the dimensionality of divergent selection might impact these processes, though these ideas currently lack empirical support. Firstly, increasing the dimensionality of divergent selection could drive the evolution of local adaptation in a variety of ways[25]. For instance, multidimensionality might be associated with an increase in the overall strength of divergent selection, driving local adaptation via reduced fitness of migrants and hybrids, thus decreasing effective gene flow[12,27]. Moreover, more selection pressures might produce divergence in more orthogonal traits[26,28]. This may speed local adaptation as a greater number of orthogonal traits implies more genes, and hence more standing additive genetic variation, enabling more rapid increase of local adaptation[7,29].

Alternatively, increasing the dimensionality of divergent selection might plausibly slow, or even prevent, local adaptation if the total strength of divergent selection does not increase with dimensionality[25]. In this scenario, if increased dimensionality leads to more loci contributing to genetic variation, but the overall strength of selection does not increase, selection may become diluted across many loci, leading to weaker per-locus selection coefficients. Here, outcomes are harder to predict[30]. Greater total genetic variance, due to increased mutational input, may allow faster and stronger local adaptation[30,31]. Alternatively, per-locus selection may be too low to overcome the homogenising force of gene flow, preventing local adaptation, whereas unidimensional selection concentrated onto few loci may provide a stronger response[11]. Furthermore, local adaptation may take longer, as a greater number of new, locally-adaptive alleles must be brought together by recombination arising during sexual reproduction (overcoming Hill-Robertson interference[32]) to produce locally-adapted genotypes.

Attempting to quantify the dimensionality of divergent selection is difficult in natural populations. Studies that determine the relationship between local adaptation and environmental heterogeneity tend to focus on single environmental differences between populations[33–36]. This may be because it is considerably easier to identify adaptation along a single consistent environmental gradient, although there are excellent examples of studies identifying multidimensional divergent selection[37–39]. Comparison of these studies indicates that multidimensionality is associated with stronger local adaptation. A meta-analysis of 35 reciprocal transplant studies [27] identified that the dimensionality of environmental divergence (maximum likelihood estimate from available data, as defined in

93 Hohenlohe and Arnold[40]) accounted for a larger proportion of variance in local adaptation (20%) than
94 environmental variables alone (4%)[41].

95 Laboratory experiments have been proposed as one of the most effective methods available to assess
96 the impact of dimensionality on local adaptation[1]. Using experimental evolution, environmental
97 selection pressures and levels of ongoing gene flow can be manipulated and controlled[42]. However, a
98 review identified no single experimental evolution study that has varied the dimensionality of
99 selection[15]. Although comparisons between experiments have been made[12,13], only 5 studies have
100 imposed divergent selection along more than one axis[43–47]. Although three of these produced strong
101 reproductive isolation, it has been argued that this was due to selection on multiple-effect traits (traits
102 that underlie more than one component of reproductive isolation[48]) rather than multidimensional
103 divergent selection[25,42].

104 In this study, we used experimental evolution to test how the dimensionality of divergent
105 environmental selection affected the speed and magnitude of evolution of local adaptation. We
106 exposed populations of the monogonont rotifer, *Brachionus plicatilis*, to unidimensional and
107 multidimensional divergent selection pressures (Table 1) whilst keeping total selection equal (Figure
108 1). Populations were paired and connected via gene flow to form metapopulations with 'home' and
109 'away' environments for each subpopulation. We tested the strength of local adaptation in each
110 metapopulation at regular intervals over the course of the experiment, defined by differences in
111 fitness when exposed to home vs. away environments[1,49]. Our planned analyses sought to answer
112 three questions: 1) Do the speed and magnitude of local adaptation vary by dimensionality, 2) if so,
113 are these patterns specific to dimensionality, or could they be explained by the individual stressors
114 used, and 3) what underlying patterns of fitness in the home and away environments are responsible
115 for changes in local adaptation?

116 Because we kept the total strength of selection equal between dimensionality treatments, the
117 patterns of local adaptation were expected to depend primarily on the genetic basis of local
118 adaptation. We predicted that unidimensional divergent selection would result in stronger local
119 adaptation, at least initially, given the expectation that selection would be concentrated upon fewer
120 loci and so might overcome the opposing effect of gene flow more easily. We further predicted that,
121 under multidimensional divergent selection, the selection per locus may be too dilute to overcome
122 gene flow, with conflicting fitness effects among loci, and that this effect would dominate over the
123 greater availability of genetic variation, producing only weak or slow local adaptation. Our results
124 contradicted these predictions: local adaptation evolved more slowly under unidimensional divergent

selection but ultimately led to stronger local adaptation, whereas multidimensional selection resulted in initially stronger local adaptation that was transient and eventually declined to low levels.

Results

Laboratory adaptation

To assess the baseline of laboratory adaptation, we analysed population density of all demes throughout the experiment. We counted population density pre-shock at four-cycle intervals throughout the experiment whenever local adaptation was being assayed. Additionally, we counted population density post-shock each week for passaging purposes. We observed a significant increase in day 7 (post-shock) population density in all metapopulations at the start of the experiment which levelled off after approximately 10 cycles (Figure S2). During this laboratory adaptation period, shock survival was estimated at cycles 5 and 9 but did not significantly differ from the calibrated value in pilot experiments (1-sample t-test with expectation of 0.45; cycle 5 mean = 0.441, t = -0.343, df = 63, p = 0.737; cycle 9 mean = 0.406, t = -1.916, df = 63, p = 0.060). This indicated that the rise in density was due to increased asexual growth rather than increased shock survival.

Local adaptation

To determine the time-course and strength of local adaptation under each of the two dimensionality treatments, we used repeated reciprocal transplant assays. Using the SA contrast as a metric for local adaptation (see Methods), over 45 cycles of experimental evolution, we found that both unidimensional and multidimensional treatments led to local adaptation, but with contrasting temporal dynamics (Figure 2).

Firstly, our analysis using dimensionality treatment as a fixed effect, revealed a significant effect of dimensionality on the way local adaptation (SA) evolved (interaction between treatment and second-order polynomial effect of cycle: $F = 7.17$, $df = 2, 28.162$, $p = 3.04 \times 10^{-3}$; full ANOVA table presented in Table 2, model coefficients provided in Table S2). In populations exposed to multidimensional selection, local adaptation increased rapidly during the early stages of the experiment but subsequently declined to low levels. In contrast, local adaptation increased more slowly in populations exposed to unidimensional selection but ultimately reached higher levels by the end of the experiment (Figure 2).

Secondly, we tested whether the effect of the identity of the stressor pair on SA varied by cycle. This model identified no significant interaction between stressor pair and the second order polynomial of cycle ($F = 1.40$, $df = 14, 29.01$, $p = 0.217$), on local adaptation.

However, to test the *a priori* hypothesis that treatment (unidimensional vs. multidimensional divergent selection), would influence the evolution of local adaptation, regardless of the specific stressors involved, we performed planned contrasts of marginal means from this model. As detailed

above, we predicted differences between the two dimensionality treatments, with unidimensional divergent selection producing faster and stronger local adaptation. Although we did observe clear differences between treatments, they ran counter to this prediction. At the start of the experiment (cycle 5), there was no difference between dimensionalities ($t = -0.155$, $df = 23.97$, $p = 0.878$). However, by the midpoint (cycle 21), multidimensional stressor pairs had produced significantly greater local adaptation than unidimensional stressor pairs ($t = 3.24$, $df = 24.20$, $p = 3.40 \times 10^{-3}$), and by the end (cycle 41) this pattern had reversed so that unidimensional metapopulations now had produced significantly greater local adaptation than multidimensional metapopulations ($t = -2.65$, $df = 23.01$ $p = 0.0142$; Figure S3; Table S3).

Repeating this analysis using the 'local-foreign' criterion for local adaptation instead of SA confirmed these results and replicated the pattern of trajectories (Figure S3; Figure S4; Table S4; Table S5. We also observed significantly higher local adaptation at the midpoint ($t = 3.14$, $df = 2.4.06$, $p = 4.37 \times 10^{-3}$) and lower local adaptation at the end ($t = -3.19$, $df = 23.93$, $p = 3.96 \times 10^{-3}$) in multidimensional lines compared to unidimensional lines when contrasting estimated means from a model using stressor pair, quadratic effect of time and their interaction (Figure S3; Table S5). Finally, although there was a significant quadratic effect of cycle on LF asymmetry ($F = 61.9$, $df = 2, 29.06$, $p = 5.76 \times 10^{-3}$), with local adaptation becoming initially less, then gradually more asymmetric over time, there was no effect of, nor interaction with, stressor pair or dimensionality (Figure S5).

Thirdly, we tested whether home fitness, away fitness, or some combination of both was responsible for the local adaptation. We found that home fitness increased linearly and at similar rates in response to all stressor pairs, and hence is unlikely to be responsible for the observed differences in local adaptation. Meanwhile, away adaptation displayed more idiosyncratic behaviour when comparing between stressor pairs and between dimensionality treatments and is, therefore, likely to be responsible for differences in local adaptation.

Fitness in home environments was found to increase linearly with time, without any rate variation with respect to stressor pair; there was a significant effect of cycle ($F = 14.94$, $df = 1, 30.53$, $p = 5.40 \times 10^{-4}$) and stressor pair identity ($F = 5.07$, $df = 7, 23.82$, $p = 1.25 \times 10^{-3}$), but with no interaction or quadratic effects (Figure 3). Repeating this analysis with dimensionality treatment rather than stressor pair yielded the same pattern of results; a significant effect of cycle ($F = 14.05$, $df = 1, 30.54$, $p = 7.44 \times 10^{-4}$) and dimensionality ($F = 9.11$, $df = 1, 29.92$, $p = 5.15 \times 10^{-3}$) but no interaction or quadratic effects. Fitness in the home environment was higher for unidimensional than for multidimensional treatments, throughout the experiment ($\Delta w = 0.066 \pm 0.022$).

The differences in evolution of local adaptation between treatments were driven mainly by fitness in the away environments. As for home fitness, there was a significant effect of stressor pair on fitness ($F = 5.01$, $df = 7, 23.72$, $p = 1.35 \times 10^{-3}$). Here, the interaction between stressor pair and a quadratic effect of cycle approached significance ($F = 2.03$, $df = 14, 29.02$, $p = 0.053$), suggesting variation in patterns of evolution of away fitness according to stressor pair. The four multidimensional stressor pairs showed consistent quadratic effects, an initial fall in away fitness followed by a rise. Together with a linear increase in home fitness, this explains the observed pattern of local adaptation: away fitness was lower than home fitness in the middle of the experiment, but not by the end (Figure 3). Unidimensional stressor pairs displayed a less consistent pattern, with two stressor pairs displaying negative quadratic effects and two stressor pairs displaying positive quadratic effects. The increase in local adaptation of unidimensional metapopulations towards the end of the experiment is therefore due to the linear increase in home fitness with little overall change in away fitness (Figure 3). Using dimensionality treatment rather than stressor pair yielded a significant effect of dimensionality ($F = 14.11$, $df = 1, 29.93$, $p = 7.44 \times 10^{-4}$), with unidimensional away fitness higher than multidimensional away fitness ($\Delta w = 0.093 \pm 0.024$) but no effect of, nor interaction with, cycle.

Discussion

In natural environments, divergent selection is frequently multidimensional, but how the dimensionality of selection affects the evolution of local adaptation remains unknown. Our experiment directly tests how the dimensionality of divergent selection affects the evolution of local adaptation whilst controlling for the overall strength of selection, identity of stressors, gene flow and recombination[15,25,42]. Our first prediction of faster evolution of local adaptation under unidimensional divergent selection, was not realised. Our second prediction of weak response to multidimensional selection was also inconsistent with the results. Metapopulations evolving under unidimensional divergent selection did not show local adaptation until late in the experiment. Meanwhile, metapopulations evolving under multidimensional divergent selection produced rapid, but ultimately transient local adaptation.

Fitness was generally higher in unidimensional lines than multidimensional lines from the outset of the experiment (Figure 3). This could reflect rapid adaptation in unidimensional lines over the first four cycles (between the start of experimental evolution and the first assay), but to both environments, since local adaptation did not increase. Alternatively, it could be that, despite calibration, unidimensional selection was generally slightly weaker than multidimensional selection due to some experimental artefact such as the filtering process.

The patterns of home and away fitness in our data indicate that the factor which determined local adaptation was fitness of populations in the away environment since home fitness increased linearly, at similar rates, under both dimensionality treatments. The challenge is to understand the different patterns of fitness in away environments under unidimensional and multidimensional divergent selection. These patterns are inconsistent with the expectation that unidimensional divergent selection might concentrate selection strongly onto a few loci, leading to rapid local adaptation, and the opposite for multidimensional selection. What property of dimensionality limits fitness in away environments at an early stage for multidimensional divergent selection, but at a later stage for unidimensional divergent selection? Here, we propose an explanation based on the idea that locally-adaptive genotypes may be expected to have different fitness effects in away environments depending on the dimensionality of the environmental difference. This hypothesis is consistent with our results but will need to be verified by further experiments.

Specialists vs Generalists

Genotypes increased in frequency because they were advantageous under their home conditions, and indeed in both treatments we saw a gradual increase in home fitness across the experiment. Given

239 that the migration rate was low and hence gene flow from the other deme was not expected to be a
240 strong opposing force[6], we suggest that the main determinant of local adaptation was the fitness of
241 these genotypes in the away environment. With respect to their performance under away conditions,
242 three possible genotype classes might compete. A genotype class with positive fitness effects in an
243 away environment suggests a generalist genotype, whereas a genotype class with negative away
244 fitness effects suggests a specialist in the home environment with antagonistic pleiotropy. The third
245 genotype class represents conditional neutrality, where the genotype is a specialist in the home
246 environment but there are no observed costs in the away environment[50]. Interpreting patterns of local
247 adaptation in our experiment requires consideration of the away fitness of genotypes that were
248 available as standing variation at the start of the experiment and those that were generated by
249 mutation and recombination as the experiment progressed.

250 Our data suggest that the relative contribution of these genotypic classes to adaptation may have
251 varied by dimensionality and over the course of experimental evolution. Overall patterns in home vs
252 away fitness of metapopulations under unidimensional divergent selection suggest conditional
253 neutrality because home fitness increased with little change in away fitness. However, this varied
254 among stressor pairs, with some evidence for generalist effects early and antagonistic effects late in
255 the experiment for two stressor pairs (Sal and Misc). Meanwhile, patterns in metapopulations under
256 all four stressor pairs from the multidimensional divergent selection treatment were consistent with
257 early local adaptation driven by specialist genotypes with small (conditional neutrality) or negative
258 (antagonistic) effects on fitness in the away environment. Generalist genotypes that spread later could
259 explain reduced local adaptation without loss in home fitness. These genotypes could have been
260 created by recombination between conditionally-neutral genotypes that were exchanged between
261 demes.

262 The initial response to divergent selection is likely to depend on standing genetic variation, in this case
263 the diverse set of 500 clones used to initiate the experimental metapopulations[26]. Our results imply
264 that this included clones that increased fitness under all experimental treatments. They further imply
265 that clones with high fitness in response to one stressor also tended to have increased fitness in
266 response to at least one other stressor or to be neutral in the away environment and, hence, they
267 behaved as generalists in the context of the unidimensional treatments[22]. However, this generalist
268 behaviour appears to be limited: clones with positive fitness effects on average across the three
269 stressors used in a multidimensional treatment were neutral or antagonistic with respect to average
270 fitness across the three different stressors used in the away environment, i.e. they exhibited
271 conditional neutrality[50,51] or were specialist. This would explain the initial increase in home fitness

under both treatments, accompanied by local adaptation under multidimensional divergent selection but not under unidimensional selection.

Later in experimental evolution, responses are likely to have changed due to increased frequency of initially rare clones, or generation of new clones by recombination or mutation[26,28]. For unidimensional metapopulations this resulted, on average, in a continued increase in fitness in the home environment but either little change or a decrease in fitness in the away environment. This implies that further evolution involved greater specialisation, i.e. that the clones with the highest fitness in one environment now tended to have antagonistic fitness effects in the other environment, unlike those clones initially available. Not surprisingly, this pattern was somewhat dependent on the specific stressors used (Figure 3). By contrast, under multidimensional divergent selection, conditionally neutral specialist genotypes might have flowed freely between demes, owing to the lack of negative effects in the away environment, resulting in a progressive loss of local adaptation without a reduction in home fitness[52,53]. It is conceivable that generalist clones might have been generated by recombination between specialist conditionally neutral genotypes which were adaptive in different environments. This pattern was consistent across stressor combinations (Figure 3).

Another way to picture this behaviour is to consider adaptive trajectories on a multidimensional adaptive landscape, using Fisher's Geometric Model[24]. As a result of calibration in pilot experiments, the starting populations were equally maladapted to each environment: the fitness reduction in response to each stressor or stressor combination was initially equal. Therefore the two demes had to adapt along trajectories of equal length, but the angle between these trajectories differed[54,55]. The divergence between these adaptive trajectories was shaped by the co-variance of environmental variables and the genetic covariance of adaptive traits at the point of divergence (as could be respectively described by an E-matrix or G-matrix[28,56,57]). The angle of divergence between adaptive trajectories is extremely challenging to predict *a priori* and may not be as wide for unidimensional divergent selection as one might assume[58]. For instance, high and low salinity shocks, as used in this study, might intuitively imply adaptive trajectories with a 180˚ angle of divergence (i.e. antiparallel trajectories *sensu* Bolnick *et al.*[55]). However, generalist 'osmotic shock' alleles could theoretically provide adaptation in both environments, producing adaptive trajectories with little divergence, and limited opportunity for local adaptation. For multidimensional divergent selection, the angle between trajectories is even more difficult to predict, although we argue that it is likely to be wider given a lower probability of 'one-size-fits-all' generalist genotypes, at least in the short-term. In the longer term, this would also depend on the nature of mutational input; do new alleles tend to have pleiotropic effects, and if so, in which direction[59]?

Specific features of our experiments might restrict the generality of the results. Firstly, *B. plicatilis* is a facultatively sexual organism. This is not an uncommon reproductive strategy[60] but it may influence the dynamics of local adaptation. Sex and recombination both create and decompose locally adapted genotypes[23,48,61]. However, a small amount of sex can be sufficient to gain most of its advantage and so we would not expect major differences in behaviour from obligate-sexual species[62].

Secondly, our means of delivering multidimensional selection mimics spatially and temporally heterogeneous selection within a single population, such that stressors are experienced separately and do not interact[23]. This was necessary for experimental tractability but reality is often likely to be more complex. Selection pressures often do not influence all individuals in all generations, but they are rarely independent and might often have interactive effects on fitness, whether experienced simultaneously or successively[63,64]. How this might influence the outcome of local adaptation is unknown but it is unlikely to change the broad distinction between low and high dimensionality of divergent selection that we sought to address.

Previously, unidimensional vs multidimensional divergent selection comparisons have focused on the effects of diluting divergent selection over few vs many traits or loci. For instance, Nosil *et al.*[11] present the 'strong selection' and 'weak multifarious' hypotheses in terms of distributing a fixed quantity of divergent selection over few vs many traits, and the consequences this may have for overcoming the homogenising force of gene flow.

The interpretation of our study modifies this argument. In addition to the way selection is concentrated or diluted across the genome, it may be important to consider the performance of alleles when exposed to a range of environments. Models and perspectives have tended to focus on alleles with antagonistic effects[12]. However, outcomes are likely to be different if conditionally neutral and generalist alleles are available. The strength of the divergent component selection depends on the complex interaction between the environment and the available genetic variation, in ways that change as evolution proceeds. Unidimensional divergent selection may, in the short term, be less divergent than one might think due to the availability of generalist alleles. However, we suggest that on long evolutionary time-scales, this is likely to act only as a short-term barrier to local adaptation. Once generalist alleles fix, the G-matrix will have in effect been rotated to align with the E-matrix, allowing specialist alleles to drive local adaptation. We suggest that, where gene flow is ongoing, multidimensional divergent selection may be more likely to create an ecological generalist in the long term than locally adapted genotypes. However, in allopatry, multidimensional divergent selection remains likely to form locally adapted genotypes. Furthermore, by potentially impacting more loci, multidimensional divergent selection may access more standing or mutational genetic variation and

so drive long-term divergence and ecological speciation, through coupling with other barriers such as assortative mating[65]. Further studies that connect the dimensionality of novel selection pressures, the genetic basis of adaptation, and fitness in a range of environments are needed to test these ideas.

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Author contributions

RKB conceived the project. NJW and IE designed the experiment with contributions from all authors. IE established the starting populations. NJW performed the experiments. NJW and APB analysed the data. NJW wrote the manuscript with contributions from all authors.

Declaration of interests

The authors declare no conflicts of interest

Figure legends

Figure 1: Experimental design

Panel A depicts the treatment-replicate-metapopulation structure. Two demes formed a metapopulation, with divergent selection applied across the metapopulation. There were four unidimensional forms of divergent selection (four stressor pairs) and four multidimensional forms of divergent selection (four combinations of three stressor pairs). Each of these was replicated four times, yielding 16 metapopulations per dimensionality treatment. Panel B details the weekly cycle of experimental evolution. Panel C depicts a sympatric-allopatric (SA) local adaptation test in which the fitness of each deme of the metapopulation was assayed in each environment.

Figure 2: Local adaptation under experimental divergent selection with gene flow.

Points represent SA estimates for each metapopulation. Curved thick lines display model fit using the first and second order polynomial of cycle, dimensionality, and their interaction. Random effects not presented in this Figure. See also supplementary Figures S3-S5 and supplementary Tables S2-S5.

Figure 3: Home vs away fitness of metapopulations.

A: Loess fits to data grouped by dimensionality. Red/blue lines represent loess fits (span = 3) for each environment (without allowance for replicate effects). Data shown are the average fitnesses of the two demes per metapopulation in their home/away environments. Grey lines connect fitness through time for each metapopulation (see also supplementary Figure S2).

B: Data separated by stressor pair: unidimensional treatment on the top row, multidimensional treatment on the bottom row. Lines represent the best model fits for home and away fitnesses, separately.

Tables

Treatment	Stressor pair	Environment 'A'	Environment 'B'
Unidimensional	**pH**	**Alkali (Alk)** 0.0288 M NaOH solution	**Acid** 0.025 M HCl solution
	Salinity (Sal)	**High Salinity (HS)** 60 g/L salt solution	**Low Salinity (LS)** 0 g/L salt solution (pure water)
	Insecticide (Ins)	**Neurotoxin (Neuro)** 31.25 µg/L permethrin solution (Lignum)	**Digestive Inhibition (DI)** 6.6 g/L *Bacillus thuringiensis* subsp. *kurstaki* 54% w/w granule (DiPel DF)
	Miscellaneous (Misc)	**Hot** 40°C	**Ethanol (Eth)** 6% ethanol solution
Multidimensional	**Sal-Ins-Misc**	**HS-Neuro-Hot**	**LS-DI-Eth**
	pH-Ins-Misc	**Alk-Neuro-Hot**	**Acid-DI-Eth**
	pH-Sal-Misc	**Alk-HS-Hot**	**Acid-LS-Eth**
	pH-Sal-Ins	**Alk-HS-Neuro**	**Acid-LS-DI**

Table 1: Stressors used in this experiment.
Individual stressor pairs (unidimensional), and combinations of stressor pairs (multidimensional) given in bold. Each stressor, other than those on the salinity axis, is given as relating to a standard solution of 12 g/L saltwater. Any addition of, for instance, HCl to form an acidic shock media was sufficiently small as to not impact salinity to the degree that fitness would be affected.

Variable	F	DF	Residual DF	p-value
Poly(Cycle, 2)	3.54	2	27.86	0.043
Dimensionality	2.20	1	29.96	0.147
Poly(Cycle, 2) : Dimensionality	7.17	2	28.16	0.003

Table 2: Model statistics. Analysis of deviance table for a linear mixed effects model explaining variation in SA using the first and second order polynomial of cycle, dimensionality, and their interaction. P-values obtained through type II Wald F tests with Kenward-Roger degrees of freedom.

STAR Methods

Resource Availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Nathan White (nwhite3@sheffield.ac.uk)

Material availability

This study did not generate any new unique reagents

Data and code availability

- Local adaptation data has been deposited at DataDyrad and are publicly available as of the date of publication. DOIs will be listed in the key resources table
- All original code has been deposited at DataDyrad and is publicly available as of the date of publication. DOIs will be listed in the key resources table.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

Experimental Model and Subject Details

Brachionus plicatilis

Brachionus plicatilis is a facultatively sexual aquatic metazoan (Rotifera, Monogononta). It reproduces asexually at low population density, then switches to sexual reproduction at high population density[66]. Sexual reproduction in monogonont rotifers produces diapausing embryos (also known as 'resting eggs') that do not hatch under normal culture conditions, requiring a short period of dormancy and specific conditions to trigger hatching[67]. *B. plicatilis* rotifers used in this study were derived from diapausing embryos in sediment samples from two brackish ponds in the Juncar-Segura basin, Albacete province, Spain: Laguna del Salobrejo (38°54.765'N, 1°28.275'W, 0.36 km^2 surface area) and Hoy Yerba (38°46.7667'N, 1°26.1167'W, 0.03 km^2 surface area). Diapausing embryos were hatched in isolation under laboratory conditions and allowed to form clonal cultures. Due to the *B. plicatilis* species complex comprising 15 species[68], sequencing of mitochondrial cytochrome c oxidase subunit 1 (CO1) was performed to confirm species identity as *B. plicatilis sensu stricto*[69]. In total, 54 clonal cultures were selected for the experiment, 27 originating from each pond, on the basis that they grew well under laboratory conditions so limiting later laboratory adaptation. Further details of clones and collection methods are in García-Roger and Ortells[70]. These 54 cultures were combined in equal proportions and grown to high density to produce diapausing embryos via sexual reproduction. These embryos were hatched and cultured to form a genetically diverse set of 500 clonal cultures. These

cultures were pooled, and samples were taken from this pool over four successive weeks to form replicate experimental populations.

All cultures were maintained in 12 g/L artificial seawater (TetraMarine) at 25°C on a 12:12 light-dark cycle. Cultures were grown on a seven-day cycle. For each culture, after 7 days of population growth, a new culture was established through passaging individuals from the old high-density culture to establish a new culture at population density of 6 rotifers ml^{-1}. Total culture volume was made up to 400ml with fresh media (12g/L artificial seawater) containing 250µl/L *Nannochloropsis* paste as food (Seahorsebreeder). 50ml of fresh media containing concentrated *Nannochloropsis* paste (4ml/L) was added to each culture on day 3, increasing culture volume to 450ml.

Method Details

Pilot experiments to calibrate shock duration for each stressor

This study required the fitness effects of multiple forms of selection to be calibrated in order to deliver an equally strong shock, measured as 24-hour survival rate (see Main Text), and thus generate divergent selection of comparable strength across metapopulations and treatments. To calibrate these shocks, we performed pilot experiments using the same starting population as for experimental evolution. We aimed for a 45% survival rate. This rate was selected as preliminary experiments showed an approximately 90% survival rate when filtered and 'shocked' with standard growth media, hence 45% survival would represent a 50% mortality rate due to the shock media. To standardise each shock so that a 45% survival rate was achieved, we varied the incubation duration in the shock medium. The eight forms of selection are listed in Table 1.

Populations were grown on a weekly cycle over four weeks as described in the Main Text. At passage, rotifers from all populations were pooled together and passaged to establish a mixed population from which new populations were established. Shocks were delivered as described in the Main Text, but with variable incubation durations from 30-70 minutes. We calculated survival rate as described in the Main Text and fit a linear model whereby incubation duration, stressor and their interaction were used to explain the variance in survival rate. We interpolated from these fits to identify the predicted duration that would produce 45% survival.

All stressors displayed a negative linear relationship between incubation duration and survival (Figure S1). The durations predicted to produce 45% survival are presented in Table S1. These durations were used as the incubation periods for each shock in experimental evolution.

Unidimensional vs multidimensional divergent selection

To test the hypothesis that local adaptation would vary with the dimensionality of divergent selection, we experimentally evolved populations of *B. plicatilis* under two selection treatments: 'unidimensional' divergent selection and 'multidimensional' divergent selection. Unidimensional divergent selection, as defined here, imposed selection using pairs of environmental stressors, each differing from the ancestral environments in one way. Multidimensional divergent selection, as defined here, imposed selection using paired combinations of three environmental stressors each of which differed from the ancestral environment. The two alternative environmental conditions of a stressor are hereafter referred to as 'stressor pairs' (e.g. unidimensional high and low salinity are a stressor pair; Table 1). Stressors in a pair may not generate strictly antiparallel selection and a single stressor might require adaptive responses in more than one trait. Therefore, our unidimensional treatments may not impose divergent selection on a single environmental or phenotypic axis. Nevertheless, our design contrasts low dimensionality of divergent selection in the unidimensional treatment with higher dimensionality in the multidimensional treatment.

In order to test the effects of dimensionality *per se*, rather than the effects of specific environmental variables, we nested four different stressor pairs (or stressor pair combinations) within each treatment. Our unidimensional treatment was replicated four times using the stressor pairs: 'pH' (acid vs alkali), 'Salinity' ("Sal" = high vs low salinity), 'Insecticide' ("Ins" = neurotoxin vs digestive inhibition) and 'Miscellaneous' ("Misc" = hot vs ethanol). Our multidimensional treatments were also replicated four times using the four unique combinations of three of these four stressor pairs: 'Sal-Ins-Misc', 'pH-Ins-Misc', 'pH-Sal-Misc' and 'pH-Sal-Ins' (Table 1).

Each pair of stressors was applied over a pair of cultures such that each culture was exposed to a unique stress or stress combination and the two cultures in the pair were linked by migration (see below). We will refer to the individual cultures as 'demes' and the pairs as 'metapopulations'. Each stressor pair was replicated four times: experimental demes were populated by sampling from pools of the 500 clones described above, over four successive weeks. Therefore, in total there were 16 unidimensional and 16 multidimensional metapopulations (four pairs of stressors per dimensionality x four culture replicates; Figure 1a).

Experimental cycle

Metapopulations experienced a weekly cycle of growth, selection, passaging and migration (Figure 1b). Demes were established at 6 rotifers ml^{-1} (asexual females; 400ml cultures, hence an expected population of 2400 individuals). After 6 days of asexual growth each deme was exposed to selection via a shock stressor designed to produce a large reduction in population density from pre-shock to 24 hours post-shock. For brevity, we refer to this reduction in population density metric as 'survival',

477 although it may include some asexual reproduction. All shock stressors were calibrated in pilot studies
478 to produce 45% survival relative to the control at the outset of experimental evolution, i.e. before any
479 evolutionary response, using an identical source population (Supplementary Information; Figure S1).
480 In this way, the strength of selection was standardised across all metapopulations, including between
481 unidimensional and multidimensional treatments.

482 To deliver each shock, rotifers were removed from culture by filtration through a 50µm mesh bag that
483 was rapidly transferred to a shock medium for the specified duration (Table S1). Rotifers were then
484 washed back into their original culture medium. For multidimensional selection, prior to filtration,
485 each deme was split into three equally sized cultures, each of which was filtered independently and
486 exposed to a different shock. Cultures were merged after the shock so that, in the long term, the whole
487 population experienced all three shocks. This strategy made it possible to impose shocks that did not
488 interact in their effects on survival and, therefore, to ensure that each stressor contributed equally to
489 selection on the deme, with overall strength equal to the unidimensional treatments. This would not
490 have been possible with simultaneous shocks. In nature, it is often the case that selection pressures
491 do not impact all individuals in all generations and yet adaptation to multiple challenges is necessary
492 for long-term success. Therefore, our experimental strategy is not actually unlike the selection
493 experienced by natural populations.

494 At day 7 (24 hours post-shock), population density was measured, and demes were passaged to form
495 new cultures. Following passage, exactly 24 of the approximately 2,400 rotifers were reciprocally
496 transferred between demes (1% migration). Migration did not use filtration; hence the microbiota was
497 also shared between demes. A 1% migration rate was selected based on experimental evolution
498 guidance [71,72] to achieve homogenisation of neutral genomic regions ($Nm > 1$) without impeding
499 response to moderate selection pressures per locus.

500 With this passaging routine, there is no contribution from sexual reproduction. To include the effects
501 of recombination arising from sexual reproduction, the remaining non-passaged culture was retained
502 for three more days to allow for additional sexual reproduction. On day 10, diapausing embryos were
503 collected from culture sediment and incubated in 1.2ml 50 g/L artificial seawater at 4°C in the dark for
504 a 2-week dormancy period, after which they were hatched via incubation in 6 g/L artificial seawater
505 at 25°C under constant illumination. 24 hatchlings per deme were transferred to tubes containing
506 30ml 12g/L seawater for a further 4 days, then added to the experimental demes coinciding with
507 weekly passage, to form a 1% sexually produced contribution of new clonal genotypes.

508 ***Population density estimates***

Population density was determined by counting the number of rotifers in four 1ml samples under a stereo microscope. This was done at regular intervals throughout the experiment for use in fitness estimation, passaging at a consistent population size and monitoring adaptation to the laboratory and experimental regime. Densities at day 6 were also determined at 4-week intervals in line with local adaptation assays, before shocks were delivered. These enabled comparisons of growth rates without the additional effect of the stressors. Densities at day 7 were determined each week, although these are influenced by both growth rate and shock survival.

Local adaptation assays

To measure the evolution of local adaptation over time, we performed classic 'home vs away' reciprocal transplant assays at regular intervals every four passages throughout the experiment (Figure 1c). During passaging, a reciprocal 'away' deme was established in addition to the experimental 'home' deme. Whilst the experimental home deme received the same stressor (or stressor combination) treatment as usual, the away deme received the alternate stressor/combination within the stressor pair. Thus, both demes in the metapopulation were assayed under each stressor. Stressors were delivered as described above with unidimensional populations receiving a single shock and multidimensional populations being split into thirds to receive separate shocks before being re-combined. Population density was counted pre-shock on day 6, and 24-hours post-shock on day 7 (after re-combining the samples exposed to different shocks for multidimensional populations) to calculate a 24-hour survival rate for each combination. Because selection was delivered via short-term exposure to stressors, and all cultures were maintained in the same conditions, no separate acclimation period was needed before these assays.

Quantification and Statistical Analysis

Quantifying local adaptation

Survival at 24 hrs was used as a measure of fitness in response to a given shock. A quantitative measure of local adaptation describes the fitness interaction between population and environment, ideally capturing both population-level and environment-level sources of variation [49]. The measure which achieves this is known as the 'sympatric-allopatric' (SA) contrast. At 4-cycle intervals and where all local adaptation assays were successfully performed within a metapopulation, we calculated an SA contrast per metapopulation as;

$$SA_{AB} = \frac{w(A_a) + w(B_b)}{2} - \frac{w(A_b) + w(B_a)}{2}$$

where w is fitness $\left(\frac{density\ day\ 7}{density\ day\ 6}\right)$, A/B are demes of the AB metapopulation and a/b are the local environments for A and B respectively.

Statistical methods

Statistical analyses were performed in R v1.4.1106[73]. We modelled local adaptation variables using linear mixed-effects models with random slopes and intercepts per metapopulation to account for longitudinal non-independence and nesting of replicates within stressor pairs.

Do the speed and magnitude of local adaptation vary by dimensionality?

To test whether the speed and magnitude of local adaptation (SA) varied by the treatment (dimensionality) over time, we fitted a fixed effects structure in which SA could vary by a second order polynomial of cycle (i.e. duration of experimental evolution) that varied among stressor pairs;

$$SA \sim poly(cycle, 2) * dimensionality + random(poly(cycle, 2) \mid metapopulation)$$

We tested the fixed effects using Type II sums of squares F tests with Kenward-Roger adjustment for degrees of freedom defined by the *Anova()* function in the *car* package for R[74]. Model comparisons via significance testing of fixed effects and data visualisation indicated that the polynomial was justified. This polynomial also was used in the random effect structure.

Could identified effects be explained by differences among stressor pairs alone, or by other factors that bias the SA contrasts?

We repeated the above sequence of model testing using stressor pair as a predictor variable instead of dimensionality;

$$SA \sim poly(cycle, 2) * stressor\ pair + random(poly(cycle, 2) \mid metapopulation)$$

To confirm the non-linear evolution of local adaptation (SA) and its dependence on dimensionality, we defined *a priori* contrasts of estimated marginal means from this model between unidimensional stressor pairs and multidimensional stressor pairs at three timepoints (passages 5, 21 & 41) using the *emmeans* package for R[75]. These three contrasts correspond to the prediction that, if the trajectories of SA over time varied by dimensionality, we would expect to observe differences between stressor pairs grouped by dimensionality at the start, midpoint or end of the experiment.

For validation, we repeated this statistical analysis using a 'local-foreign' (LF) measure of local adaptation. The 'local-foreign' (LF) contrast[1,49] quantifies differences between the fitnesses of two populations when in the same environment. It is therefore a measure of local adaptation for a specific environment, rather than across a metapopulation. These were calculated as;

$$LF_a = w(A_a) - w(B_a), \qquad LF_b = w(B_b) - w(A_b)$$

We repeated our above statistical analyses using LF rather than SA as response variable.

571 Finally, SA measures of local adaptation can be positive because of large asymmetry between the two
572 estimates of LF within a metapopulation, in which case there would be increased gene flow from one
573 deme to another[1,49]. To test whether LF asymmetry might be driving patterns of SA, we also fitted
574 models for the absolute difference between LF statistics within each metapopulation (LF Asymmetry
575 = |LFa – LFb|).

576 *What components of home vs away fitness are responsible for any observed differences?*

577 SA is a contrast between fitness of 'home' and 'away' combinations of demes and environments.
578 Patterns of local adaptation (measured as SA) over time can be due to variation in home fitness, away
579 fitness, or some combination. Therefore, to identify the cause(s) of the patterns in SA, we modelled
580 home and away fitness estimates separately. This used the same fixed and random effects structure
581 as above, but using fitness in either home or away environment as the response variable instead of
582 SA. Mean fitness over both demes within each metapopulation, rather than the fitness of each deme
583 individually, under home or away conditions was used in this analysis. This was to control for non-
584 independence of the two demes within a metapopulation due to reciprocal migration

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