MULTIDIMENSIONAL DIVERGENT SELECTION AND LOCAL ADAPTATION

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Abstract

During ecological speciation, populations diverge by adapting to local environmental selection pressures. The number of divergent selection pressures that populations adapt to can be described as the 'dimensionality of divergent selection'. This property of dimensionality is thought to be a key determinant of patterns of divergence, local adaptation, and speciation. However, understanding of the precise mechanisms through which this can occur is under-developed, and there is little empirical evidence for a role of dimensionality in these processes. To develop a deeper understanding of how dimensionality impacts patterns of divergence, in this thesis, I combine theoretical, simulation, experimental evolution, and genomic approaches. I re-examine existing conclusions regarding dimensionality and find the evidence base to be of insufficient depth to support them. I highlight areas of unclarity regarding how the dimensionality of the environment, of traits, and of genomes map together, and hypothesise how these might affect divergence. I test some of these hypotheses using a quantitative genetics simulator, finding that, whilst the dimensionality of divergent selection *per se* is a relatively arbitrary concept, it can impact local adaptation and extrinsic isolation via the overall strength of selection, the number of loci under selection, and through transgressive segregation. I perform an experimental evolution study using an evolve and resequence genomic approach in which I expose populations of the monogonont rotifer, *Brachionus plicatilis*, to unidimensional and multidimensional divergent selection pressures. By tracking trajectories of local adaptation over time, I show that the speed and eventual strength of local adaptation vary by dimensionality. The results of this evolution experiment, comprising both local adaptation and genomic data, indicate that dimensionality influences the balance between the contribution of generalist and specialist alleles to (local) adaptation.

Declaration

I, Nathan Joseph White, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (*www.sheffield.ac.uk/ssid/unfair-means*). This work has not been previously been presented for an award at this, or any other, university.

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Table of Contents

Chapter 1: Introduction

General introduction

Understanding the processes that create, maintain, and erode biological diversity is one of the most complex and enduring missions undertaken in the whole of scientific research (Levene, 1953). Key among these processes is the evolution of local adaptation; the property of multiple populations which have on average higher relative fitness under their local environmental conditions than would other populations (Kawecki and Ebert, 2004). The term 'local adaptation' can be used interchangeably to describe both the pattern of populations exhibiting this property, and the process of becoming locally adapted from an ancestral state (Kawecki and Ebert, 2004; Whitlock, 2015). Local adaptation, as I will explore in this first thesis chapter, is central to the generation and maintenance of biodiversity, and often acts as a first step on the road to the creation of new species; allowing populations to decouple their evolutionary trajectories from one another and embark on increasingly independent futures (Rundle and Nosil, 2005; Nosil, Harmon and Seehausen, 2009; Nosil, 2012). Yet for such a fundamental process, apparently driven by straightforward evolutionary forces, there remain many questions surrounding the build-up and maintenance of local adaptation (Butlin *et al.*, 2012; Tigano and Friesen, 2016).

The dimensionality of divergent selection is expected to affect the evolution of local adaptation and consequently diversification and progress towards ecological speciation (Nosil, Harmon and Seehausen, 2009; Butlin *et al.*, 2012; White and Butlin, 2021). Varying this dimensionality, the number of environmental selection pressures acting on a population, could conceivably act either to drive or to constrain the evolution of local adaptation depending on a variety of conditions being met. For instance, increased dimensionality might increase overall selection and drive local adaptation, or might dilute selection over many loci which may constrain divergence in the face of gene flow. However, the question of how dimensionality affects the evolution of local adaptation remains broadly under-studied (Nosil, Harmon and Seehausen, 2009; Butlin *et al.*, 2012). This is likely, in part, due to over-interpretation of early experimental work (Rice and Hostert, 1993), combined with a lack of clarity regarding multidimensionality and the various levels of organisation at which it plays a role (White and Butlin, 2021). Additionally, although there is a growing number of studies investigating multidimensionality in natural populations (e.g. Egea-serrano *et al.*, 2014; Stuart *et al.*, 2017; Aguirre-Liguori *et al.*, 2019; Hu *et al.*, 2019), this has long been a challenging task, requiring accurate environmental measurements not just contemporarily but back through ancestral time (Hereford, 2009; Ravinet *et al.*, 2017; White, Snook and Eyres, 2020).

In this thesis, I combine theoretical, simulation, experimental evolution, and genomic approaches to test how dimensionality impacts the evolution of local adaptation and so contributes to extrinsic isolation and progress towards ecological speciation. Combining these approaches allows understanding of the way in which dimensionality may drive or constrain local adaptation and experimental testing of the underlying theory (Fry, 2009; Kawecki *et al.*, 2012). My approach begins with the theoretical and transitions towards the experimental, zeroing-in from broad generalisable patterns to specific experimental tests and the idiosyncrasy of natural systems.

Local adaptation

Adaptation to spatially heterogeneous environments is a key process in generating diversity and differentiating populations (Rundle and Nosil, 2005; Nosil, 2012; Savolainen, Lascoux and Merilä, 2013). Where populations are distributed across a range of environmental conditions, divergent selection can drive adaptation only to the specific conditions encountered by each population independently, generating local adaptation.

Determinants of local adaptation

The ability of divergent selection to drive adaptation is dependent upon a balance of evolutionary forces. Paramount is the force of divergent selection which acts as the driver of local adaptation, although its effects may vary according to its strength, periodicity and, as I shall explore in this thesis, dimensionality. Other evolutionary forces act to strengthen or constrain the ability of divergent selection to drive local adaptation. Perhaps the most important antagonist to divergent selection is gene flow arising from migration (Garant, Forde and Hendry, 2007; Yeaman and Whitlock, 2011; Tusso *et al.*, 2021). The level of adaptive divergence between populations is predicted to reflect the balance between the strength of divergent selection and the homogenising force of gene flow (Lenormand, 2002; Garant, Forde and Hendry, 2007; Guillaume, 2011; Yeaman and Otto, 2011). Where populations are strongly isolated by distance or physical barriers, gene flow is minimal and locally adaptive alleles are favoured regardless of their fitness effects in other populations, leading to highly specialised genotypes and local adaptation (Kassen, 2002; Bono *et al.*, 2017). However, even low levels of gene flow can provide a strong countervailing force against divergent selection, exposing locally adaptive alleles to a wider range of environments and reducing differentiation at locally adaptive loci (Akerman and Bürger, 2014). At higher levels of gene flow, generalist alleles which have, on average, higher fitness than locally adaptive alleles are favoured by selection, and local adaptation decreases (Lenormand, 2002).

The consequences for local adaptation stemming from this balance established between divergent selection and gene flow may be modified by other evolutionary or demographic factors (Blanquart *et al.*, 2013). For instance, variability in environmental conditions is predicted to undermine the ability of divergent selection to produce local adaptation by purging genetic variation within isolated demes (North *et al.*, 2010). Another consequence of environmental instability can be the evolution of phenotypic plasticity: the ability for a given genotype to produce variable phenotypes in response to variable environmental conditions (Reed *et al.*, 2010; Fox *et al.*, 2019). Plasticity itself can also reduce the ability for divergent selection to produce local adaptation, although this is not a straightforward outcome and there are cases in which plasticity enables local adaptation, such as via the creation of distinct phenotype groups that begin to reduce gene flow (Gao *et al.*, 2018; Reger *et al.*, 2018; Öhlund *et al.*, 2020).

Genetic drift is another evolutionary force which may act to constrain local adaptation by depleting the available additive genetic variation within populations, stochastically fixing non locally adaptive alleles within populations and reducing the effectiveness of selection (Kawecki and Ebert, 2004). The relative strength of genetic drift depends on the effective population size; where populations are small, genetic diversity is likely to be low and drift can produce strong effects, resulting in the loss of locally adaptive alleles either through stochastic processes or via negative selection on linked alleles (Blanquart, Gandon and Nuismer, 2012). Where drift is strong, migration may conversely promote

local adaptation by providing a supply of new foreign alleles into the local deme (Garant, Forde and Hendry, 2007). Equally, in small populations or populations with low genetic diversity, gene flow may promote local adaptation by countering the effects of inbreeding depression, which may otherwise act as a barrier to local adaptation or endanger population persistence (Ebert *et al.*, 2002; Garant, Forde and Hendry, 2007).

The strength and transience of local adaptation are also dependent upon the nature of alleles under selection (Yeaman and Whitlock, 2011; Anderson *et al.*, 2013). By definition, locally adaptive alleles must provide a fitness benefit in the local environment, however their effects can differ elsewhere (Tigano and Friesen, 2016). Gene flow connecting demes plays an important role here, broadening the range of environments encountered by an allele. The extent of local adaptation is very much determined by the fitness of these alleles in the away environment, which may be positive (generalist alleles), neutral (specialist, conditional neutrality) or negative (specialist, antagonistic; Bono *et al.*, 2017). For instance, if there is a migration rate of 1% between demes, each allele experiences local conditions reflecting one environment 99% of the time, and the other environment only 1% of the time. As a result, the non-local environments is unlikely to have much of an effect on the fitness benefit of this adaptive allele.

Reproductive isolation

The biological species concept is underpinned by the idea that populations can be reproductively isolated from one another, i.e. that there are barriers preventing members of one population mating with members of another to produce fertile offspring (Mayr, 1942). The establishment of stable local adaptation between demes, restricting effective gene flow relative to migration, is often viewed as a crucial first step on the route to the evolution of distinct ecotypes and the onset of ecological speciation (Rundle and Nosil, 2005; Nosil, 2012; Butlin *et al.*, 2014; Thompson, 2016). Progress towards ecological speciation requires increasing adaptive divergence and restriction of gene flow between diverging populations. Local adaptation is itself a form of reproductive isolation as migrants and their offspring will, on average, have lower fitness than the native population, reducing gene flow relative to the migration rate (Abbott *et al.*, 2013). However, local adaptation would have to be infeasibly strong and stable if it were the only form of reproductive isolation required for speciation (Roughgarden, 1976; Slatkin, 1984).

Typically, for the progression and completion of speciation in the face of gene flow, multiple barriers may need to be coupled together to produce a stronger overall barrier (Butlin and Smadja, 2018). Secondary barriers may be manifest as assortative mating; the tendency to mate more frequently with homospecifics over conspecifics, or Dobzhansky-Muller incompatibilities (DMI; Dobzhansky, 1936, 1937; Muller, 1942) in hybrid offspring (Fishman and Willis, 2001; Dettman, Anderson and Kohn, 2008; Wang, White and Payseur, 2015). Secondary barriers further reduce the fitness of migrants and hybrids, such as via intrinsic fitness with DMIs or via sexual selection with divergence in mating-related traits, creating additional barriers to gene exchange. The coupling of secondary barriers with local adaptation may, in turn, enable local adaptation to be stronger at selection-migration equilibrium, or enable other adaptive processes such as reinforcement. This coupling of barrier effects requires the establishment of linkage disequilibrium among underlying genetic loci. This could occur either as a by-product of stochastic events such as their simultaneous evolution (Wright *et al.*, 2013) or coincidental changes in distribution range (Hewitt, 1996), or by adaptive coupling such a reinforcement (Butlin, 1987; Servedio and Noor, 2003; Matute, 2010a; Butlin and Smadja, 2018).

Dimensionality

In Chapter 2, I provide an in-depth introduction to the concept of dimensionality and the many ways in which it could affect local adaptation and speciation. Therefore, I will only present a short introduction here, covering some of the more salient points with respect to local adaptation.

The term 'niche' is notorious among ecologists and evolutionary biologists as an exceedingly confusing and ill-defined concept (Chase and Leibold, 2003). One view of the niche is Hutchinson's verbal model of niche dimensionality which states that every species occupies a parameter space defined by some number of environmental axes (Hutchinson, 1957). On each axis there is a region spanning two points within which a given species can survive and reproduce. For two environmental axes this parameter space forms an area, the shape of which depends on axis interactions (e.g. rectangular if axes are independent). By adding a third axis, the space forms a volume, and so on at higher dimensionalities forming a multidimensional hypervolume. In reality, a species' niche always forms a multidimensional hypervolume: it is hard to even conceptualise a truly unidimensional niche. One could apply this niche dimensionality concept to the example of a common garden experiment which fails to account for the full range of environmental conditions; one might describe this as failing to capture the full niche dimensionality of the field environment within the artificial laboratory.

For a given species all relevant ecological variables could be added to define a species' fundamental niche with the surface representing its ecological limits, rather than geographic range. In this way, the niche is differentiated from physical space, or 'biotope' (Hutchinson, 1957; Colwell and Rangel, 2009). When thinking about local adaptation, this mapping of niche space needs to be expanded to consider more than one population. With increasing multidimensionality there are more axes along which disruptive selection may act. Resultantly, there is a greater potential that two populations adapt to occupy niches that are delimited along at least one axis, thus limiting the likelihood of correspondence between niche spaces (Hutchinson, 1957). Without niche differentiation, two diverging populations require physical isolation, else one may outcompete the other according to the competitive exclusion principle (Gause, 1934; Hardin, 1960).

In addition to the total number of selection pressures affecting populations, the number of ecological variables leading to divergent selection can be thought of as the dimensionality of divergent selection, or 'divergence dimensionality'. Varying the dimensionality of divergent selection can have various consequences for the evolution of local adaptation (White and Butlin, 2021). As I describe in Chapter 2, the outcome for local adaptation likely depends on additional conditions such as the demographic context and genomic composition of diverging populations. For instance, when comparing one system with another, greater dimensionality of divergent selection may be associated with greater overall strength of divergent selection, yielding lower fitness for migrants and hybrids (MacPherson, Hohenlohe and Nuismer, 2015), or be associated with more loci, capturing more additive genetic variation and enabling more rapid divergence (Barrett and Schluter, 2008; Flaxman *et al.*, 2014). On the other hand, increasing the dimensionality of divergent selection may constrain divergence, particularly if overall selection does not increase in proportion with dimensionality (Nosil, Harmon and Seehausen, 2009; White and Butlin, 2021). In this case, increasing dimensionality may have the effect of spreading divergent selection over many more loci, reducing selection per-locus, and making adaptive divergence more susceptible to the homogenising force of gene flow (Nosil, Harmon and Seehausen, 2009; Yeaman, 2015).

Although the effects of dimensionality on local adaptation and extrinsic isolation have been considered theoretically in some detail (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014; MacPherson, Hohenlohe and Nuismer, 2015; Thompson, Osmond and Schluter, 2019; Yamaguchi and Otto, 2020), empirical tests are generally absent. Attempts to quantify the effects of dimensionality on local adaptation and reproductive isolation are challenging in natural populations, although meta-analyses have highlighted environmental dimensionality as an important variable in explaining variance in local adaptation (Hereford, 2009; MacPherson, Hohenlohe and Nuismer, 2015). No experimental test of this major outstanding question has yet been attempted, perhaps due to experimental complexity and declining popularity of experimental evolution in the genomics era (Fry, 2009; White, Snook and Eyres, 2020). Research presented in this thesis attempts to fill that gap, providing the first direct empirical test of how dimensionality can impact local adaptation.

Studying local adaptation

Quantification

Local adaptation has classically been estimated by comparing measurements of population mean fitness in either reciprocal transplant assays or common garden experiments (Turesson, 1922; Kawecki and Ebert, 2004; Gibson *et al.*, 2016; Johnson *et al.*, 2021). The former, most commonly performed as a field transplant, is the most robust means by which to measure local adaptation, as fitness is assayed directly in the environments to which populations have been adapted, but may be problematic for practical or legal reasons (Johnson *et al.*, 2021). The latter, also referred to as an 'explant' experiment, attempts to recreate the environmental conditions within a laboratory, but can sometimes fail to capture the range of environmental factors yielding natural selection (Kawecki and Ebert, 2004; Hoeksema and Forde, 2008). The purpose of both types of assays is to identify whether the fitness of a population varies by the environment it is assessed in; that is, is there a population x environment interaction effect on fitness?

To calculate the magnitude of local adaptation, several local adaptation contrasts have been designed. The first, and the most conceptually straightforward of these is the sympatric-allopatric (SA) contrast. The SA contrast calculates the difference between the average fitness of all demes within a metapopulation in their home environment (sympatric combinations of deme and environment) and the average fitness of all populations across all away environments (allopatric combinations of deme and environment; Blanquart *et al.*, 2013). This metric is appealingly straightforward because it yields a single value of local adaptation for the overall metapopulation, accounting for variation among both demes and environments (Blanquart, Gandon and Nuismer, 2012). However, it faces two key problems. The first is that, in yielding a single value for the entire metapopulation, there is no scope for significance testing unless multiple parallel metapopulations can be assessed. The second problem is that it relies on successful estimation of all combinations of demes and environments; the number of which increases quadratically with number of demes (Blanquart *et al.*, 2013; Johnson *et al.*, 2021).

To solve the problem of significance testing, two more granular metrics can be used that provide estimates of local adaptation for each deme or environment, yielding variation around an average (Blanquart *et al.*, 2013). The home-away (HA) contrast calculates local adaptation as the extent to which a focal population has higher fitness under 'home' environmental conditions than on average under 'away' conditions. In contrast, the local-foreign (LF) contrast uses a focal environment, calculating the difference between fitness of the 'local' deme and the average fitness of all 'foreign'

demes when assayed there. Notably, Blanquart *et al.*, (2013) do propose a method of significance testing using the SA contrast, but as it relies on estimation of individual (genotype) fitness, it is beyond the scope of this thesis.

Both the HA and LF contrasts are only able to deal adequately with large variation in either population fitness or environmental quality, but not both. As shown through simulation by Blanquart *et al.* (2013), the power of local-foreign estimates decreases along a sigmoidal curve given an increasingly large standard deviation of deme quality whilst the power of home-away estimates remains unaffected. Likewise, the power of home-away estimates decreases along a sigmoidal curve given an increasingly

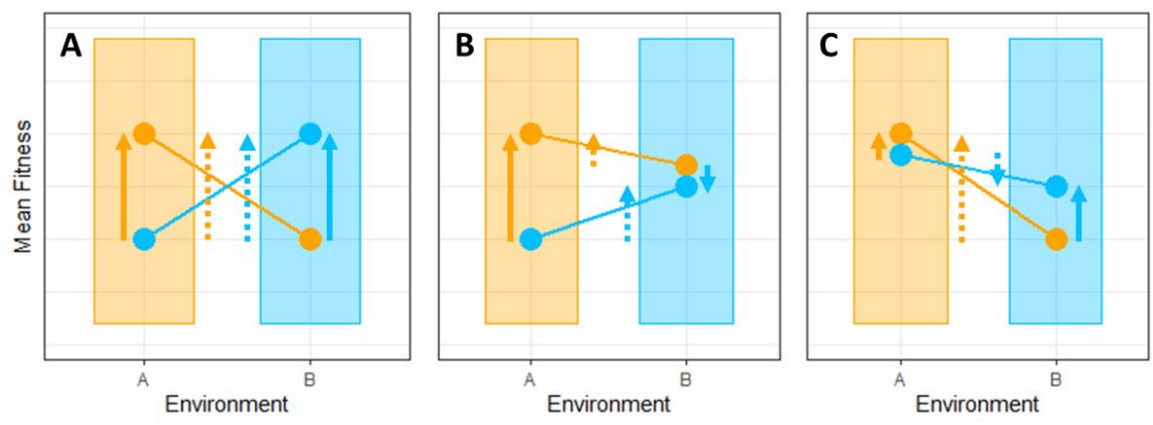

Figure 1: Adapted, with permission from Blanquart et al. (2013).

Box 1: Local adaptation contrasts

In Figure 1, two hypothetical populations (orange vs blue dots/lines) inhabit separate environments (A vs B; orange vs blue regions respectively). A reciprocal transplant experiment is done to assess fitness for all 4 combinations of population and environments to measure local adaptation. Panels A-C present 3 different outcomes for this hypothetical experiment. In each panel, the SA criterion for local adaptation is met, but contributions of HA (dashed arrows) and LF (solid arrows) local adaptation vary.

The results in panel A satisfy all measures of local adaptation. Both populations have higher fitness in their home environment than their away environment, and fitness is higher in each environment when the local population is measured rather than the foreign population. In panel B, the HA local adaptation criterion is satisfied (dashed arrows), however only one metapopulation satisfies the LF criterion (solid arrows). The consequence is that the orange population is always of higher fitness than the blue population, and gene flow will occur asymmetrically with the orange population acting as a source of globally advantageous alleles. Panel C depicts the opposite scenario with global LF locally adaptation, but only one of two populations meets the HA criterion for local adaptation. Here, environment B is more stressful for both populations, but assuming environments have limited carrying capacities, ecological specialisation will nonetheless form.

Therefore, we can measure local adaptation using the SA contrast, but it is important to recognise that it will only form a barrier to reciprocal gene flow if each sub-population satisfies the LF criterion. In meeting this criterion of LF local adaptation, a crossover between lines as shown in panels A and C is always produced (Kawecki & Ebert 2004).

large standard deviation of habitat effect, with no impact on the power of local-foreign estimates. In both cases, the sympatric-allopatric contrast (using the individual-based method referred to above) is unaffected, demonstrating its ability to handle substantial variation in both deme and habitat quality. As such, it provides the most accurate quantification of local adaptation, and is the 'gold standard' contrast if the measurements allow.

However, there remains debate as to what the criteria should be for determining the presence of local adaptation. There are arguments against using the sympatric-allopatric criterion, along the lines that it fails to account for local adaptation resulting from strongly asymmetric fitness within each sympatric and/or allopatric component (Kawecki and Ebert, 2004; Box 1). The local-foreign contrast is oriented towards identifying variation in the fitness of populations in a constant environment. Furthermore, whilst environmental quality may vary widely, intrinsic fitness of populations tends not to. For this reason, Kawecki & Ebert (2004) argue that the key comparison in determining local adaptation is the local vs foreign test, as this focuses on the fitness of populations within each environment, not the performance of a population over multiple environments. Therefore, Kawecki & Ebert argue that a metapopulation is locally adapted only when each deme satisfies the LF criterion; that the fitness of each deme is higher than the average fitness of all other demes under the local deme's environment.

However, this is an extremely strict criterion which becomes increasingly difficult to satisfy with more demes; as Blanquart et al (2013) note, it has the unwelcome property of reducing statistical power with increasing sample size. Moreover, by definition, local adaptation requires a comparison of local (home) conditions with other (away) conditions; all adaptation is in some way 'local' (Whitlock, 2015). Although the local-foreign contrast is the better criterion for establishing local adaptation as a barrier to gene flow, it neglects the spatial heterogeneity implied by local adaptation. Finally, whilst SA produces a unified measurement of local adaptation that can be compared across all levels of taxonomy or throughout the evolutionary history of a metapopulation (Nuismer and Gandon, 2008; Johnson *et al.*, 2021), it is difficult to use Kawecki & Ebert's (2004) binary LF definition comparatively. These issues are challenging to navigate for natural systems comprised of multiple demes and environments. However, in systems comprised of only two demes, as used in simulation and experimental studies in this thesis, these problems are mostly avoidable.

Approaches

In the course of this thesis, I use these population-level metrics of local adaptation in combination with a two-pronged approach to test how dimensionality impacts local adaptation. Firstly, I use a forward-in-time simulator, Nemo (Guillaume and Rougemont, 2006), to test how the dimensionality of divergent selection and niche dimensionality can impact the evolution of local adaptation over 100,000 generations *in silico*. Then, I experimentally evolve populations of the monogonont rotifer, *Brachionus plicatilis*, under treatments that expose populations to either unidimensional or multidimensional divergent selection. The combination of simulations with experimental evolution creates a powerful means by which to explore this research area in a highly controlled setting. The merits of these approaches are discussed individually, and in combination, in Chapters 2 and 4. In brief, simulations allow identification of the broad patterns, produced by variable dimensionalities, including identifying rare but important effects, whilst experimental evolution allows testing of these processes *in vivo* whilst maintaining the highly controlled environment. The power of this approach is increased further with the integration of genome sequencing with experimental evolution in the 'evolve and resequence' approach (Schlötterer *et al.*, 2015), as is explored further in Chapter 4. Key to

both simulation and experimental evolution approaches is a high level of replication, allowing multiple evolutionary trajectories to be compared and significance testing of the patterns of local adaptation produced.

Study system- *Brachionus plicatilis*

In this thesis I use populations of a species of monogonont rotifer (Rotifera: Monogononta), *Brachionus plicatilis*. *B. plicatilis* is a powerful model system which has been applied to a wide range of research foci, including ageing (Enesco, 1993; Snell, 2014), the evolution of sex (Becks and Agrawal, 2012), ecotoxicology and speciation (Gomez *et al.*, 2002). In this section, I discuss the biology of monogonont rotifers in general and their applications, with particular attention to evolutionary and experimental studies.

Taxonomy, anatomy and ecology

Monogononta is the largest class within Rotifera, comprising around 2000 species. The phylogeny of Rotifera, and its related groups has been the subject of much debate, however it is believed that Monogononta diverged from its sister class, Bdelloidea, around 100 million years ago (Poinar and Ricci, 1992; Waggoner and Poinar, 1993; Welch, 2000; Welch and Meselson, 2001; Mark Welch *et al.*, 2004).

Monogononts are bilaterally symmetrical microscopic aquatic metazoans (Fontaneto and De Smet, 2015). They are oviparous and eggs develop through spiral cleavage (Hejnol, 2010). Rotifers are eutelic, with no larval form, and hatchings emerge with their full complement of somatic cells; any subsequent growth is due to cell enlargement, not mitosis. The body is divided by transversal folds into three pseudosegements; the head, trunk and foot (Clément and Amsellem, 1989). At their anterior, rotifers possess a ciliated structure known as the 'corona', which is used for locomotion and filter-feeding. Food is passed via the corona into the mouth, through an intestine and excreted from the cloacal opening. Eggs are produced in the ovarium, pass through the vittelarium and also emerge through the cloaca (Fontaneto and De Smet, 2015; Wallace, Snell and Smith, 2015). To resist osmotic change, rotifers possess two protonephridia which pass fluid from the pseudocoel into the bladder and filter it. Most species of monogononts (including *B. plicatilis*) exhibit strong sexual dimorphism, with males being many times smaller than females. Monogononts are not desiccation-resistant in any adult stage but are as resting eggs (Gilbert, 1974; Ricci, 2001).

Due to the desiccation-resistant dormancy stages in the life cycle of monogonont rotifers (see reproductive biology below), monogononts have substantial potential for passive dispersal. As such, it has been proposed that they may possess a near-cosmopolitan distribution across the world (Fontaneto *et al.*, 2012). However, data to support this claim are extremely limited and, to quote Fontanteo and De Smet (2015), "our biogeographical knowledge reflects the distribution of rotifer scientists more than that of rotifers themselves".

Reproductive biology

The clearest distinguishing difference between Monogononts and Bdelloids is the presence of a facultative sexual cycle in the former (Gilbert, 1974; Ricci, 2001). The reproductive cycle of monogonont rotifers comprises periods of rapid asexual reproduction via parthenogenesis, interspersed with occasional sexual cycles (Carmona, Gómez and Serra, 1995).

Sex is determined via a haplodiploid system; diploidy produces females, haploidy produces males. When reproducing in the asexual phase, (diploid) females produce diploid eggs via parthenogenesis: egg production is mitotic (Carmona, Serra and Miracle, 1993; Carmona, Gómez and Serra, 1995; Aparici, Carmona and Serra, 2001). These eggs are genetically identical to the mother and hatch into daughter clones which may begin asexual reproduction within as little as 24 hours. Females often produce several eggs concurrently, and as they can survive typically for over two weeks, generations overlap significantly, enabling rapid population growth. Asexual reproduction will proceed unless the chemical cue for mixis is at sufficiently high concentration, amictic females will continue to produce amictic females (Carmona, Gómez and Serra, 1995).

However, once this chemical cue surpasses a given threshold, mictic diploid females will be produced that are able to reproduce sexually (Gilbert, 1974; Aparici, Carmona and Serra, 2001). Mictic females produce haploid eggs by meiosis and are amenable to mating with a male. Unfertilised eggs remain haploid and once hatched will develop into males. Typically, this is common during the early stages of sexual reproduction, as by definition fewer males have been produced for females to mate with. Males are a degenerate form of the female, lacking a functional gut or the means by which to feed. They are morphologically distinct due to their significantly smaller size and locomotive pattern. Males possess a single testis and prostate gland. They may mate with mictic females, resulting in fusion of haploid sperm with haploid egg to produce a genetically distinct diploid female embryo.

The product of sexual reproduction is commonly referred to as a 'resting egg'. After a short period of development, the embryos halt development and enter a state of diapause (Gilbert, 1974). Therefore, the common label 'resting egg' is technically incorrect, as by the time they are observable, they are no longer truly eggs but rather diapausing embryos (Pourriot and Snell, 1983). Whilst in this state of diapause the embryos are desiccation resistant and can remain dormant potentially for decades, until they receive the cues for hatching and become an amictic diploid female that can resume asexual reproduction. Resting eggs are distinct from asexual eggs in both colour (a deep orange/brown in contrast to translucent grey) and density, sinking to the bottom of the culture, making them easy to identify and isolate (Pourriot and Snell, 1983). This combination of easy-identification and arrested development make them a powerful feature as a model organism, enabling the experimenter to separate out and control the asexual and sexual contributions to reproduction.

Applications as a model organism

Monogononts have the required attributes expected of any classically 'experimentally evolvable' model organism. They have short generation times (asexually 1-2 days, sexually ~2 weeks) and are easy to culture to high population density. The *B. plicatilis* genome is small (diploid size of roughly 200 Mb), which is beneficial for evolve and resequence work, and there are three assemblies available (NCBI 2021), the most recent of which is assembled to scaffold-level [716 scaffolds, 5950 contigs, 106.939 Mb] (Han *et al.*, 2019). Monogononts are experimentally amenable, for instance, local adaptation can be assayed simply by a home vs away environment asexual growth assay. Additionally, unlike alternative systems such as yeast, rotifers are facultative sexual metazoans and so exhibit mating behaviour, thereby enabling the analysis of behavioural phenotypes such as assortative mating in speciation experiments (Gómez and Serra, 1995).

In addition, the production of diapausing eggs grants the experimenter a powerful degree of control over experimental manipulations. Mictic eggs sink to the bottom of the culture and can remain in

diapause from between a week and decades, depending upon culture conditions. Therefore, the experimenter may control precisely what proportion of each generation originates from sexually or asexually-produced eggs (Smith and Snell, 2012, 2014). The hardy nature of these eggs allows the experimenter to archive samples at regular intervals throughout an evolution experiment and then resurrect these bygone generations at a later stage. The value of this is twofold. Firstly, it provides a practical robustness, so that in the event of an experimental catastrophe (fire, flooding, critical loss of power), the experiment can be re-started from the most recent collection of eggs. Secondly, and surely more significantly, once the experimenter sequences ancestral and final populations, they have the ability of 'looking back in time' to observe the genomic landscape at any given collection date.

Thesis structure

Chapter 1: Introduction

Chapter 2: Multidimensional divergent selection, local adaptation, and speciation

In Chapter 2, I critically examine the existing literature surrounding ideas of dimensionality. I discuss how the concepts of environmental, phenotypic, and genomic dimensionality map onto one another. I emphasise the need for distinction between overall niche dimensionality and the dimensionality of divergent selection. I re-visit previous predictions regarding how the dimensionality of divergent selection impacts speciation, and argue that there is insufficient evidence from theory, nature or experiments to support them. In doing so, I highlight several assumptions made regarding multidimensional selection and discuss the consequences of these not holding in all cases. Finally, I map out the potential routes by which multidimensionality might produce local adaptation and barriers to reproduction.

Chapter 3: Multidimensionality and extrinsic isolation

In Chapter 3, I use the quantitative forward-time genetically explicit evolutionary simulator, Nemo, to simulate some of the processes discussed in Chapter 2 relating to local adaptation and extrinsic isolation such as increasing overall selection and increasing the number of loci under selection. I show that there is no single property of multidimensionality *per se* that drives these barriers to reproduction. Rather, it is the correlated effects, such as increasing the overall strength of divergent selection or increasing the number of loci under selection that yields reduced fitness in migrants and hybrids. However, increasing the dimensionality of the overall environment does yield reduced fitness for hybrids in dimensions not under divergent selection. By measuring the direction and magnitude across an adaptive landscape, I compare the loss of fitness due to overall dimensionality (fitness costs imposed by stabilising selection) to that of divergence dimensionality.

Chapter 4: The past and future of experimental speciation

In Chapter 4, I review the existing literature on experimental speciation, i.e. the application of experimental evolution to speciation research. I contrast experimental speciation to comparative approaches for studying speciation using natural populations and highlight areas of complementarity between the two. I recommend the integration of evolve and resequence strategies with experimental speciation, and the use of gene flow in the form of reciprocal migration as two important tools for exploring speciation questions. I describe how these two tools come as a pair, as without gene flow it is impossible to identify barrier loci or examine patterns of differentiation across the genome. I highlight key areas in which experimental evolution can be applied effectively to answer outstanding

questions in speciation research, with an emphasis on the genomic conditions likely to allow speciation, the effect of gene flow and the various facets of selection. For each, I discuss key questions that could be approached effectively with experimental evolution, the latter category including how the dimensionality of divergent selection impacts local adaptation and reproductive isolation.

Chapter 5: Experimental evolution of local adaptation under unidimensional and multidimensional selection

In Chapter 5, I describe the first experimental test of how the speed and magnitude of local adaptation is affected by the dimensionality of divergent selection. Using a species of monogonont rotifer, *Brachionus plicatilis*, I expose populations to either unidimensional or multidimensional divergent selection, whilst keeping the overall strength of divergent selection constant. I use the blueprint for experimental speciation as described in Chapter 4. I find that local adaptation evolves in both treatment groups, although the patterns over time vary widely between treatments. Whilst unidimensional selection produces slow but ultimately stronger local adaptation, multidimensional selection produces rapid but transient local adaptation. By decomposing local adaptation into its constituent home vs away fitness measurements, I propose an explanation for these contrasting patterns, considering how dimensionality may affect the behaviour of alleles under selection.

Chapter 6: Dimensionality and genomic differentiation: an evolve and resequence experiment

In Chapter 6, I examine evolve and resequence pool-seq genome data from the *B*. *plicatilis* populations used for the experiment described in Chapter 5. Using populations from the start and end of experimental evolution, I extract and sequence the DNA of all experimental demes. I use the population genomics outlier detection software, BayPass, to identify significantly differentiated SNPs between broad stressor treatments and within individual metapopulations. I test how the patterns of differentiation within these comparisons differ between unidimensional and multidimensional divergent selection treatments, and test for differences in the ratio of generalist to specialist outliers between dimensionality treatments. I find no significant effect of dimensionality on the patterns of differentiation, but do find that unidimensional divergent selection yields a stronger response for generalist alleles than multidimensional divergent selection.

Chapter 7: Discussion

In Chapter 7, I summarise the main findings within this thesis and contextualise them within current research. I synthesise findings and discuss what effects dimensionality is likely to have for driving local adaptation and ecological speciation in nature. I conclude by suggesting new directions for future research by adopting an increasingly integrated approach to outstanding questions.

Chapter 2: Multidimensional divergent selection, local adaptation and speciation

Abstract

Divergent selection applied to one or more traits drives local adaptation and may lead to ecological speciation. Divergent selection on many traits might be termed 'multidimensional' divergent selection. There is a commonly held view that multidimensional divergent selection is likely to promote local adaptation and speciation to a greater extent than unidimensional divergent selection. We disentangle the core concepts underlying dimensionality as a property of the environment, phenotypes and genome. In particular, we identify a need to separate the overall strength of selection and the number of loci affected from dimensionality *per se*, and to distinguish divergence dimensionality from dimensionality of stabilising selection. We then critically scrutinise this commonly held view that multidimensional selection promotes speciation, re-examining the evidence base from theory, experiments and nature. We conclude that the evidence base is currently weak and generally suffers from confounding of possible causal effects. Finally, we propose several mechanisms by which multidimensional divergent selection and related processes might influence divergence, both as a driver and as a barrier.

Introduction

Populations adapt in response to natural selection to optimise fitness in their native environment. Any number of environmental pressures might drive adaptation, either through stabilising, directional, or divergent selection. Interactions between the environment and the traits of an organism determine fitness; typically, many selection pressures and traits contribute to fitness, some operating independently, whilst others interact (Débarre, Nuismer and Doebeli, 2014). Divergent selection generates local adaptation, potentially leading to speciation, by driving the divergence of populations toward distinct fitness optima in a heterogeneous environment (Kawecki and Ebert, 2004; Rundle and Nosil, 2005; Nosil, 2012). For any pair of locally-adapted populations, many shared environmental variables are likely to generate stabilising selection in both habitats (Gavrilets, 1997; Langerhans and Riesch, 2013) but there is one axis in niche space that separates them (Yamaguchi and Otto, 2020). This axis of separation between habitats, might be dominated by a single environmental variable, or might impose selection on a single trait, in which case divergent selection could be described as 'unidimensional'. Alternatively, one might identify differentiation in multiple environmental variables or traits, generating 'multidimensional' divergent selection (Rice and Hostert 1993; sometimes called 'multifarious selection', e.g. Feder and Nosil 2010).

There is a broad consensus that the higher the dimensionality of divergent selection the more likely it is to generate local adaptation and speciation. This stems from classic reviews (Rice and Hostert, 1993; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009) and is often repeated (Smadja and Butlin, 2011; Butlin *et al.*, 2012; Langerhans and Riesch, 2013; Ravinet *et al.*, 2017). However, nearly three decades on from the original proposal, there remains little clear evidence supporting this hypothesis. Furthermore, the multiple mechanisms by which increased dimensionality might influence local adaptation and speciation have not been fully distinguished, either theoretically or in empirical

studies. Here, we argue that the proposed effects of increased dimensionality of divergent selection might instead be attributed to increased overall strength of divergent selection, increased dimensionality of stabilising selection, increased number of loci under selection, or to other possible correlates of dimensionality. We highlight the need for theoretical and empirical work to test the impact of dimensionality on local adaptation and speciation in ways that help to understand the mechanisms of action.

Defining dimensionality

Whilst we are mainly concerned about response to divergent selection, it helps to begin by considering the dimensionality of selection in a single habitat. This requires an understanding of the mapping between environmental, phenotypic and genetic variation.

Overall dimensionality

First, there is the dimensionality of the environment. The habitat occupied by a population can be described by measuring many environmental variables. For each environmental variable there is a range within which the population can survive and reproduce and this defines a hypervolume that describes the population's niche (Hutchinson, 1957). Since the environmental variables are likely to be correlated, the effective dimensionality of this volume is lower than the number of measurable variables. The leading eigenvectors of the matrix of environmental variables define a set of orthogonal environmental axes and the complexity or dimensionality of the environment can be described by the number of these axes required (Paula-Souza and Diniz-Filho, 2020).

Phenotypic variation can be described in a similar way. A large number of possible phenotypic traits can be measured but correlations among traits mean that the dimensionality of phenotypic variation is likely to be much lower (Kirkpatrick and Meyer, 2004; McGuigan, Chenoweth and Blows, 2005). Therefore, phenotypic variation can be described by a smaller number of orthogonal phenotypic axes. The dimensionality of phenotypic variation depends, in part, on the underlying pattern of genetic variation. This can be described in quantitative genetic terms by the genetic variance-covariance matrix (G-matrix; Guillaume 2011) which also has a limited number of orthogonal axes that may differ from the major axes of the phenotypic matrix. Alternatively, genetic variation can be described at the level of individual genetic variants and their patterns of linkage disequilibrium. The forms of the genetic and phenotypic variation will depend on the history of selection on the population, and so on the environment (Yamaguchi and Otto, 2020).

The dimensionality of selection depends on the interaction between environmental and genetic variation (Kirkpatrick and Meyer, 2004). This is implicit in Hutchinson's niche definition because the environmental variables that matter are those that impose limits on the region that the population can occupy. There are environmental variables that have little or no effect on these limits and, similarly, there are genes and phenotypes whose variability does not influence fitness within the given habitat. Tenaillon (2014 p.194) defines complexity as 'a quantitative measure that reflects the number of variationally quasi-independent traits an organism is exposing to the action of natural selection in a given environment'. This definition arises from consideration of Fisher's Geometric Model of selection towards an environmental optimum (Fisher, 1930). Tenaillon emphasises that its value is labile, as environments, phenotypes and genotypes evolve, and depends on the time-scale under consideration, typically being lower for shorter durations. It can be thought of as the number of orthogonal phenotypic or genetic axes on which there is effective stabilising selection. The number of

environmental axes that impose appreciable stabilising selection may be similar but the mapping between the two sets of axes may not be simple.

Divergence dimensionality

In order to consider local adaptation, it is necessary to extend this thinking to two habitats, each of which can be represented by Fisher's Geometric Model with a single optimum that differs between habitats. Therefore, there is stabilising selection around each optimum, and divergent selection between habitats. Making the simplifying assumption that the same set of environmental or phenotypic axes underlies selection in both environments, these optima are two points in the same multidimensional space. Clearly they can be connected by a single axis, which we will refer to as the axis of divergent selection. In this sense, divergent selection is always unidimensional (unless there are more than two habitats under consideration). However, the axis of divergent selection might be aligned with a single axis in the environmental or phenotypic space, or even with a single underlying environmental variable or phenotypic trait (Figure 1). This might be considered unidimensional divergent selection in contrast to cases where the divergent selection axis implies selection on multiple phenotypic traits or axes in response to multiple environmental variables or axes. Unfortunately, the literature on the role of multidimensional selection in local adaptation and speciation rarely makes these distinctions (see below).

MacPherson et al (2015) provide an example to illustrate their model of local adaptation in a metapopulation. Femur length and head width are phenotypes in the cricket, *Gryllus firmus* (Bégin and Roff, 2001) that are genetically correlated (G-matrix, covariance is positive). MacPherson et al. also envisage some environmental selection on head width and on femur length. We might imagine, for the sake of argument, that selection is due to two environmental variables; a resource variable and a predation risk variable that impose selection on the two phenotypes, respectively. Rather than varying independently across demes, there is a positive correlation between these environmental variables (described by an E-matrix). The strengths of the environmental or genetic correlations influence the effective dimensionality: if they are strong, dimensionality is close to one, if weak it is close to two. Since the genetic and environmental correlations need not be the same, dimensionality depends on the viewpoint. The dimensionality of selection depends on the interaction between genetic and environmental variation: for example, if head width and femur length were perfectly genetically correlated, the dimensionality of selection would be one, regardless of the correlation between environmental variables. MacPherson et al. (2015) show that local adaptation increases with dimensionality in their model (see below) but the increase is greater if the G and E-matrices are aligned, illustrating this interaction.

This example can be extended to show the distinction between divergence dimensionality and the overall dimensionality of the selective environment. Suppose that the crickets also vary in colour and that matching to the background colour of the environment influences fitness, but that this background colour does not vary among patches. The dimensionalities of the environment and of the phenotype are increased but the divergence dimensionality is not. Finally, the crickets might vary in bristle pattern, which has no influence on fitness in this environment, and the habitats might vary in sward height, over a range that has no impact on cricket survival and reproduction. These variables would increase phenotypic or environmental dimensionality but not the dimensionality of selection. A 'roadmap' of the perspectives of overall vs divergence dimensionality and their correlated effects is provided in Figure 1.

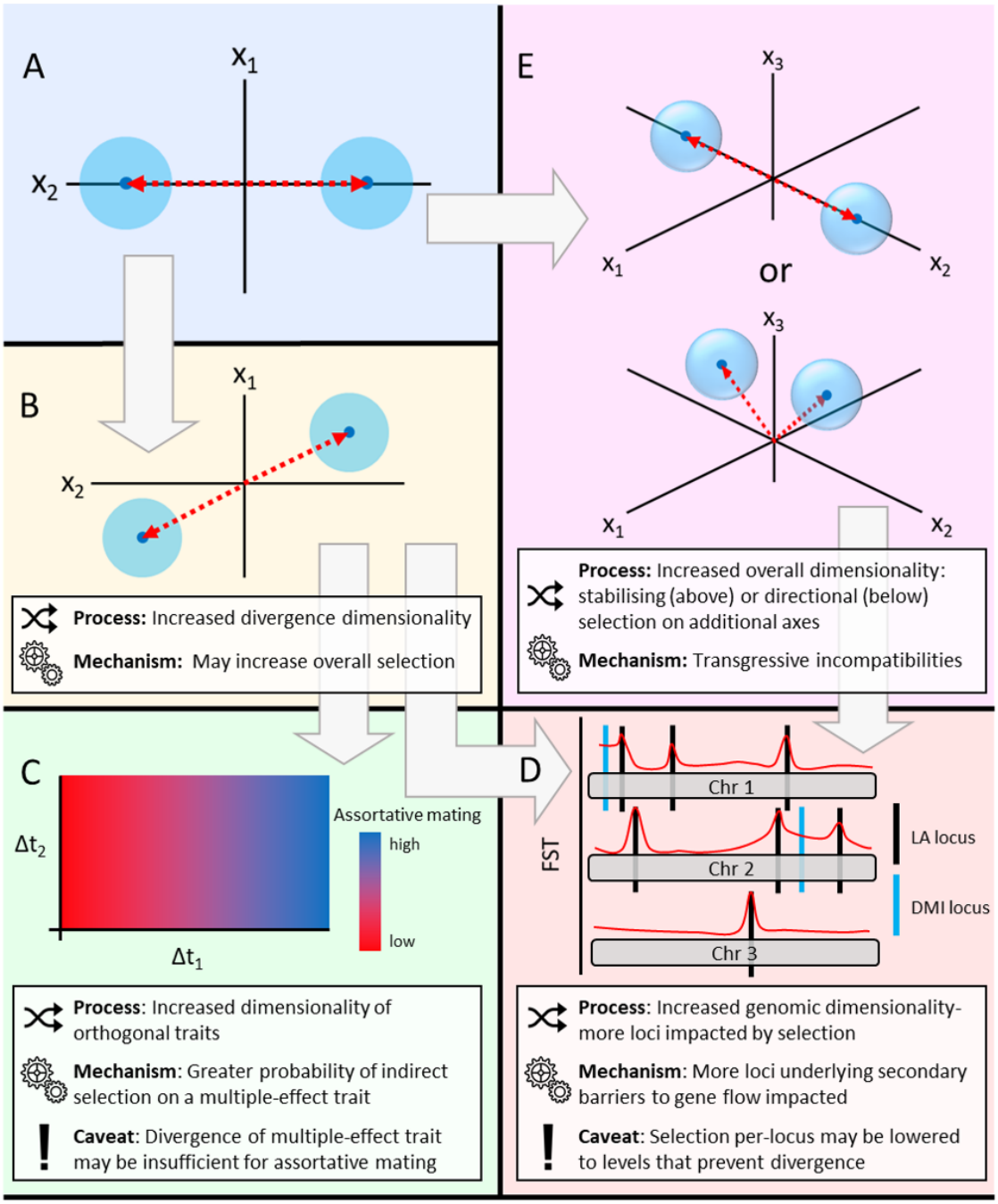

Figure 1: A roadmap of multidimensional processes and their effects on barrier mechanisms

Top-left (A): All niches are highly dimensional environments. Here, two niches for diverging populations are represented in two dimensions (light blue circles) within which there is an optimum (dark blue dot). These optima are separated along one environmental axis (x_2) with stabilising selection along a different axis (x_1) creating unidimensional divergent selection between multidimensional niches. We might envisage that an ancestral population evolved from the origin to fill these niches via the trajectories shown by the red dashed arrows. Transitioning to the middle-left plot (B), divergent selection is now applied over two environmental axes (x_1 & x_2). There is still a single axis in multidimensional environment space separating the optima, but as two axes are now divergent, there is the potential for stronger divergent selection as optima become more distantly separated.

(Figure 1 continued) Multidimensional divergent selection may have correlated responses. The example in the bottom-left plot (C) shows two orthogonal traits have now diverged: t_1 in response to selection on x_1, and t_2 in response to selection on x_2. By chance, t_1 is a multiple-effect trait and greater divergence in t_1 produces assortative mating. This additional barrier would not have arisen without multidimensional divergent selection. An additional barrier mechanism is shown in the bottom-right plot (D). Multidimensional divergent selection has produced regions of divergence, as measured by F_{ST}, around multiple locally adaptive loci (LA loci). Linkage between locally adaptive loci and DMI loci produces correlated divergence in the DMI loci, generating an additional barrier to gene flow.

Furthermore, returning to the initial two-dimensional niche representation, overall dimensionality might increase from two-dimensional (A) to three-dimensional (D) via the introduction of a third environmental axis (x_3). This additional axis might provide either stabilising or directional selection, but regardless will increase transgressive incompatibilities as hybrid fitness deviates from the optimum along the additional axis.

Multidimensionality and extrinsic isolation

Why should multidimensional selection increase local adaptation and the chances of progress towards speciation, compared with unidimensional selection? If a full picture of the effects of dimensionality *per se* is to be achieved, there are several potentially co-varying effects that must be addressed. Here we distinguish three of these possible effects; 1) The intensity of divergent selection increases with divergence dimensionality, 2) Other components of selection increase with overall dimensionality, and 3) The genetic dimensionality increases with divergence dimensionality.

Stronger divergent selection

Considering divergent selection between two distinct habitats, overall selection for local adaptation can be conceptualised as the distance between environmental optima in multidimensional space (defined by orthogonal phenotypic axes). For simplicity, the axes can be scaled so that fitness in each habitat follows a Gaussian decline, equally in all directions from the habitat optimum, and we can assume equal fitness for well-adapted phenotypes in the two habitats. In reality, this scaling might not be possible across two different habitats but this complexity does not influence our arguments here. The fitness of an individual depends on its phenotype and the habitat in which it is found. Here, we will focus on the fitness of individuals that are well adapted to one habitat when they are either in their home environment or the alternative environment, and on the fitness of hybrids in the habitat in which they have higher fitness (cf. Thompson et al. 2019, for example). The Euclidean distance between environmental optima then determines both the fitness of a migrant between habitats, and the reduction in fitness of a hybrid whose phenotype is at the mid-point between optima (Figure 2).

In this framework, there is no necessary relationship between the intensity of divergent selection and either the dimensionality of divergence or the dimensionality of the trait space. With increasing dimensionality of divergent selection, i.e. where the two optima differ on a greater number of orthogonal phenotypic axes under selection, there are two possible extreme modelling assumptions. The first is that overall selection increases with the number of selection pressures, as each selection pressure contributes an additional fitness reduction for migrants or hybrids. Alternatively, overall selection might remain constant but be spread over more axes, implying weaker selection per-axis (Nosil et al. 2009b). In the first scenario, selection is ‘additive’ across dimensions while in the second

it is 'diluted'. We will use these terms to refer to the two modes of multidimensional selection while recognising that there is a continuum of intermediate possibilities (Figure 2). One might expect to find cases throughout this continuum in nature and the empirical challenge is to measure both divergence dimensionality and the overall intensity of selection if their effects are to be separated. Attempting to predict *a priori* how stressors will interact is a significant challenge, mired by ecological and temporal complexity, although significant strides are being made (Galic *et al.*, 2018; Birk *et al.*, 2020; Orr *et al.*, 2020).

Increased overall dimensionality

Hybrids between populations adapted to two distinct habitats have reduced fitness because of their intermediate phenotypes, which fall between two adaptive optima. However, they also have reduced fitness due to segregation of alleles that influence other phenotypic axes, i.e. because their phenotypes fall away from the line directly connecting the two optima resulting in further fitness reduction (Figure 2). This fitness reduction is known as 'transgressive incompatibility' (Chevin et al. 2014). It increases as overall dimensionality, not divergence dimensionality, increases (Chevin et al. 2014, Thompson et al. 2019). It is also more dependent on the history of the populations that on their current separation in phenotypic space, even being experienced by populations in identical environments. This is because the set of loci at which two populations differs depends on the evolutionary trajectory by which they have reached their current state. Transgressive incompatibilities can increase the barrier to gene flow between populations and this might increase local adaptation as well as making speciation more likely. Therefore, although the effect is not dependent on divergence dimensionality it can produce a positive relationship between overall dimensionality and local adaptation. It is possible to distinguish this fitness cost experimentally, for example by measuring the fitness of hybrids between populations independently adapted to similar environments as well as those adapted to distinct environments (e.g. Johansen-Morris and Latta 2006; Van Der Sluijs et al. 2008), or how hybrid fitness varies by phenotype and environment (Arnegard *et al.*, 2014).

Increased genetic dimensionality

There is potentially a correlation between the divergence dimensionality and the number of loci under divergent selection. In turn, the number of loci, or other aspects of genetic dimensionality determined by covariance among loci, might influence the potential for local adaptation and speciation. The number of loci under selection is a measure of genomic dimensionality, but the extent to which each locus represents an independent dimension is modulated by pleiotropy, because an allele can influence multiple traits, and by genetic architecture, because nearby loci do not evolve independently due to linkage disequilibrium. Asexual organisms have fewer dimensions of genomic variability as loci are locked together, whilst unlinked loci in sexual organisms are more orthogonal as they can be inherited independently. The independence of loci is also impacted by epistasis; alleles that interact in their effects on fitness will tend to be inherited together. Decomposition of genomic variation via principal component analysis is a familiar concept when analysing genomic divergence (for recent examples, see e.g. Hu et al. 2019; Morales et al. 2019; Tusso et al. 2021). The genetic variance-covariance matrix (the G-matrix) describes standing genetic variation and can also be decomposed into a smaller number of orthogonal axes. There is evidence that widespread pleiotropy makes the dimensionality of the G-matrix lower than the number of measurable traits, but also that pleiotropy causes fitness effects of unmeasured traits to influence the selection observed (e.g. Sztepanacz and Blows 2017a,b). The dimensionality of the G-matrix needs to be considered in the context of the

fitness landscape in order to determine the dimensionality that is relevant here: there will be genomic axes of variation that are neutral, as well as those that are relevant to selection but not to local adaptation. The G-matrix is both a product of mutation and (multidimensional) selection (e.g. Matuszewski et al. 2014) and a determinant of the short-term response to selection (MacPherson et al. 2015).

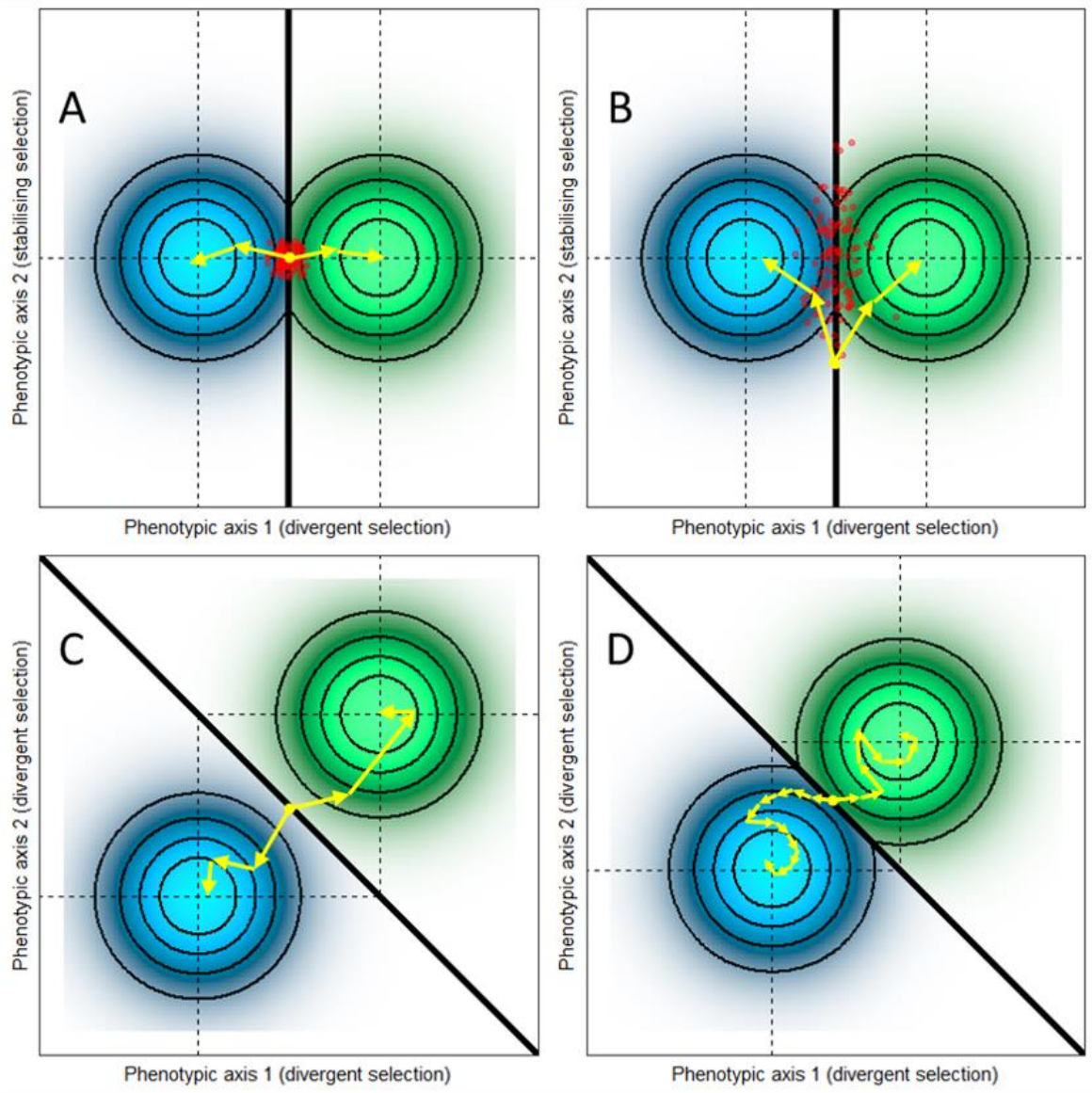

Figure 2: Concepts in adaptation to a multidimensional landscape

Panels depict overlays of 2-dimensional adaptive landscapes in two different environments (separated by thick black lines). Environments have distinct fitness optima (intersection of dashed black lines) which may vary along one or both phenotypic axes. Selection over the two environments may therefore be either stabilising (single optimum) or divergent (two optima) for each phenotypic axis. Contours represent maximum fitness of a given phenotypic combination over both environments. In all cases, we assume that phenotypes are scaled such that fitness surface is Gaussian with equal variances in both phenotypes and no covariance between phenotypes.

(Figure 2 continued) The adaptive landscapes of panels A-B are identical, only phenotype 1 is under divergent selection with two adaptive optima; phenotype 2 is under stabilising selection. Comparison of this landscape with C-D shows the two modes of multidimensional selection. Here, both phenotypes are under divergent selection. Under additive multidimensionality (comparison of A/B with C), the per-axis divergence is held constant, producing a greater Euclidean distance between fitness optima and deeper fitness valley (contours), lowering the fitness of migrants and hybrids respectively. Under diluted multidimensionality (comparison of A/B with D) overall selection remains constant: the distance between multidimensional optima is equal to the unidimensional case. The divergence of individual phenotypes (distance between dashed lines) is smaller than under unidimensional divergent selection (A-B) or additive 2-dimensional selection (C).

In all panels, adaptation proceeds from an ancestral point to adaptive optima via mutations (yellow arrows). Each mutation has pleiotropic effects on both phenotypic axes. Local adaptation can proceed via few large-effect mutations (C) or via many small-effect mutations (D), irrespective of mode or dimensionality. With more mutations, there is an increased probability of producing a constitutive incompatibility. Theory states that at higher overall environmental dimensionality, more mutations are required for adaptation to a local optimum (Chevin, Decorzent and Lenormand, 2014). Panels A-B also depict the effect of transgressive incompatibilities. In panel B, the mutational trajectory is less closely aligned with the axis between optima in phenotype space than in panel A. These off-axis mutational effects produce "segregation variance" in hybrid offspring, as phenotypes (red dots) vary widely along axes that are orthogonal to the discriminant axis separating optima (e.g. axis 2 in panels A-B; Chevin et al. 2014; Thompson et al. 2019). Off-axis variance in hybrid phenotypes is predicted to decrease with the alignment seen in panel A and to increase with the overall environmental dimensionality

In the long-term, measures of genomic dimensionality are dynamic because genomic architecture can evolve. Where multiple selection pressures are correlated and gene flow is present, high levels of recombination may be costly as haplotypes containing adaptive alleles for different selection pressures are disrupted (Felsenstein, 1981; Kirkpatrick and Barton, 2006). As the cost of recombination between co-adapted alleles increases, genetic architectures that reduce recombination and thereby lower genomic dimensionality are likely to be favoured (Yeaman, 2013). The extreme of this would be the formation of supergenes where many previously independent loci underlying a set of coevolving traits become tightly associated (Thompson and Jiggins, 2014). This has the effect of re-writing the genetic variance-covariance matrix and re-defining the dimensionality of orthogonal traits (Svensson *et al.*, 2021). Alternatively, it may be beneficial to increase genomic dimensionality and break associations between loci, for instance if adaptation to a newly available multidimensional niche requires separation of two associated phenotypes into independent traits.

The genomic dimensionality relevant to local adaptation is likely to increase with divergence dimensionality, but this relationship is not necessarily strong. A broad genomic response, involving many loci (polygenic), could be termed 'multidimensional' while selection on a single locus might be termed 'unidimensional' (Kinsler, Geiler-Samerotte and Petrov, 2020), regardless of the number of selection pressures to which they respond. On average, one might expect that multidimensional divergent selection elicits multidimensional genomic responses (Nosil, Funk and Ortiz-Barrientos, 2009), but this is not guaranteed in every case. A single large-effect locus might pleiotropically affect adaptation of multiple phenotypes to multiple environmental axes, as with the cricket 'body size' (= head width + femur length) example (MacPherson, Hohenlohe and Nuismer, 2015). In contrast, many

loci might contribute to divergence on a single phenotypic axis. If the divergence axis is multidimensional in a space defined by orthogonal axes of genetic variation, then the genetic basis of divergence must be more complex than for unidimensional selection in this space.

The extent to which the mapping of loci to traits influences local adaptation has been explored in studies of restricted pleiotropy (Chevin, Martin and Lenormand, 2010; Le Nagard, Chao and Tenaillon, 2011; Kinsler, Geiler-Samerotte and Petrov, 2020; Yamaguchi and Otto, 2020). MacPherson et al. (2015) show that local adaptation increases more strongly with dimensionality if genetic and environmental axes of variation are correlated. Strong selection on a few loci might overcome gene flow more readily but divergence due to many loci of small effect is possible and may, ultimately, result in a stronger barrier to gene flow (Flaxman *et al.*, 2014; Nosil *et al.*, 2017). Therefore, an impact of dimensionality on local adaptation could be mediated by its effect on genetic or genomic complexity but the extent of this contribution remains largely an open theoretical and empirical question.

Deconstructing current conclusions

An alternative view on experiments

Experimental speciation (experimental evolution of diverging populations) is a direct way to test these proposed effects on local adaptation and speciation, and yet no study has explicitly varied the dimensionality of divergent selection. The view that experimental studies support the role of multidimensional selection in promoting speciation dates back to a classic review of speciation experiments (Rice and Hostert, 1993), and is repeated in a later review of niche dimensionality (Nosil and Harmon, 2009). However, we argue that there was not, nor is there currently, any strong experimental evidence to support this conclusion.

Experimental speciation studies test aspects of ecological speciation by varying the environmental conditions for adaptation (Fry, 2009). Therefore, they are well-suited to examine how environmental dimensionality shapes the speciation process. This is in contrast to artificial selection studies in which the experimenter determines fitness based on traits (e.g. Koopman 1950), and so might examine trait dimensionality. Unfortunately, the overwhelming majority of experimental speciation studies have selected along only one axis (though, as established above, selection along one environmental axis might produce divergence in multiple traits). Just five (Kilias, Alahiotis and Pelecanos, 1980; Rice, 1985; Rice and Salt, 1988, 1990; Rundle, 2003) out of 59 studies reviewed by Nosil and Harmon (2009) used multidimensional selection. That these studies were associated with higher levels of reproductive isolation has been taken as evidence that multidimensional selection promotes speciation (Rice and Hostert, 1993; Nosil and Harmon, 2009).

However, all five of these multidimensional studies used *Drosophila* species and three involved the same experimental setup; Rice's 'habitat maze' (Rice, 1985; Rice and Salt, 1988, 1990). This design deliberately selects for multiple-effect traits (traits that are under divergent selection and impact on other components of reproductive isolation - sometimes referred to as 'magic traits'; Smadja and Butlin 2011) because habitat choice is experimentally tied to mate choice. Note that here, in the context of multiple-effect traits, we use the term 'traits' in the sense of individual phenotypes rather than orthogonal phenotypic axes. In these experiments, multiple-effect traits are significantly more likely to have driven reproductive isolation than multidimensional selection (Fry, 2009; White, Snook and Eyres, 2020). This conclusion is reinforced by a recent experimental speciation study of the parasitic feather louse, *Columbicola columbae,* which produced rapid reproductive isolation via

unidimensional divergent selection (via host body size) on a multiple-effect trait (louse body size; Villa et al. 2019). Since the Nosil and Harmon review, few studies have imposed divergent selection (Sharon *et al.*, 2010; Castillo *et al.*, 2015; Markov *et al.*, 2016; Bush *et al.*, 2019) and hence there remains little experimental evidence for the role of multidimensional divergent selection in local adaptation and speciation (White, Snook and Eyres, 2020).

Evidence from nature

In natural populations, several studies cite divergence in multiple phenotypes (Nosil and Sandoval, 2008; Gompert *et al.*, 2013; Egea-serrano *et al.*, 2014; Stankowski, Sobel and Streisfeld, 2015; Stuart *et al.*, 2017; Aguirre-Liguori *et al.*, 2019). This is to be expected: any two habitats will often generate multiple different demands on an organism though the extent to which these represent orthogonal axes of selection is unclear. For example, the marine to freshwater transition in sticklebacks changes the osmoregulatory environment, food availability and predation pressure (Hendry *et al.*, 2013) while the coastal to inland transition in monkey-flowers alters water availability, season length and competition (Lowry and Willis, 2010). In *Littorina* winkles, environmental axes of selection can be combined in different ways, but each axis imposes selection on multiple traits (Morales *et al.*, 2019). There are examples where single traits dominate divergence, such as coloration in *Timema* stick insects (Sandoval and Crespi, 2008) or beach mice (Steiner, Weber and Hoekstra, 2007), or heavy metal tolerance in plants (Singh *et al.*, 2016). There are good examples of studies in which divergence in environments, traits and genomes have been parsed. For instance, a study of *Phrynocephalus* lizards, identified divergent clusters along the first principal component axis from 9 environmental variables, and along the first principal component axis of 11 phenotype measurements. These major axes of environment and phenotype were significantly correlated, indicating divergence along one major environmental/trait axis (Hu *et al.*, 2019). However, systematic comparisons among studies to identify associations between dimensionality and patterns of divergence are rare.

Perhaps the strongest comparison across studies of variable dimensionality is a meta-analysis in which the dimensionality of environmental divergence (1-4 traits) was estimated consistently across 35 plant reciprocal transplant studies (MacPherson, Hohenlohe and Nuismer, 2015). The degree of local adaptation was significantly correlated with the dimensionality of divergent selection, which accounted for 20% of the variance. Only 4% of the variance in local adaptation was explained by the overall extent of divergence in environmental measurements, using the same set of sites (Hereford, 2009). However, this should not be interpreted as a separation between dimensionality and total selection: MacPherson et al. (2015) acknowledge that the main driver of the dimensionality effect that they observe is likely to be an increase in total selection with more environmental dimensions, with the response to selection probably aided by alignment of environmental and genetic axes. A study of *Scutellaria* plants similarly concluded that speciation-with-gene-flow and species-coexistence are facilitated by multidimensional selection, on the basis that no single environmental factor among 71 variables assessed could account for niche separation. Rather, multidimensional selection partitioning niches along several axes was more consistent with the observed niche distribution among species (Huang *et al.*, 2017).

Quantifying dimensionality of present-day environmental divergence is a difficult issue but it is also necessary to reconstruct it at the time of divergence (Orsini, Spanier and De Meester, 2012; Pfrender, 2012). Whilst there are good examples of quantification for the present, the past is rarely considered (Öhlund *et al.*, 2020). This is problematic because theory shows that where multiple

species coexist at evolutionary equilibrium, a single key change (unidimensional divergence) can destabilise evolutionary equilibria and so cause co-evolutionary ripple effects that lead to multidimensional divergence in response to biotic selection pressures, conflating cause and effect (Gilman, Nuismer and Jhwueng, 2012; Chevin, Decorzent and Lenormand, 2014; Débarre, Nuismer and Doebeli, 2014; Yamaguchi and Otto, 2020). A study of present-day conditions could thus implicate multidimensional selection, whereas this was not the primary cause of divergence (Öhlund *et al.*, 2020). Furthermore, it is important to consider that present-day divergence on one environmental/trait axis might be the end point of many possible adaptive trajectories with implications for genomic parallelism and transgressive incompatibilities (Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019).

Theory and simulation

Upon first impression, most modelling/simulation studies addressing this question indicate that multidimensional selection promotes speciation. However, these have not yet made important distinctions between effects of the number of traits, the overall strength of selection and the genomic basis of adaptation. For instance, a model of a mosaic metapopulation with complex spatial structure and environmental heterogeneity showed that local adaptation is strongly correlated with dimensionality, and that its impact increases with migration (MacPherson, Hohenlohe and Nuismer, 2015). However, the effects of multidimensionality cannot be separated from overall selection as there was no test of the diluted alternative. Since the model was not genetically explicit it could not test the effects of number of loci. Furthermore, models generally make the simplification that selection and trait dimensionalities are equal (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019) which prevents separation, and that loci are universally pleiotropic.

Beyond local adaptation, three simulation studies have examined reproductive isolation upon secondary contact following divergent selection of variable dimensionality in allopatry. Each study used orthogonal traits where each trait corresponded to fitness in a respective environmental dimension, hence dimensionality was varied along joint environment-trait axes. In one study, axes of divergent selection were added sequentially, with total selection increasing under the additive assumption (Nosil and Harmon, 2009). Reproductive isolation, measured as the decrease in the average fitness of hybrids compared to perfectly-adapted parental individuals, increased with higher dimensionality. The authors noted that the same effect might be achieved by increasing separation of optima on a single axis, but they did not attempt to isolate the effects of dimensionality from those of overall selection strength. The number of loci was held constant, with all loci influencing all traits. Therefore, selection per-locus also increased at higher dimensionality.

Two subsequent simulations based on Fisher's geometric model probed these issues more deeply (Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019). The overall dimensionality of selection was varied, rather than only divergent selection. The authors made an important distinction between sources of hybrid incompatibilities. Chevin et al. (2014) show that transgressive incompatibilities arise as recombination creates new combinations of alleles causing hybrid phenotypes to vary, not just along the axis separating adaptive optima but also on other axes, reducing fitness under stabilising selection. Alternatively, 'constitutive incompatibilities' can arise as some combinations of derived alleles are incompatible for reasons unrelated to the environment, further reducing hybrid fitness. Both transgressive and constitutive incompatibilities fit the

Dobzhansky-Muller incompatibility (DMI) model in a general sense because they depend on negative interactions between derived alleles. Thompson et al. (2019) extend this to include the angle of divergence between two populations' adaptive trajectories, finding that transgressive phenotypes are more common with greater angles of divergence.

The transgressive incompatibility component depends on the set of mutations accumulated during divergence (Figure 2). This is determined by the evolutionary trajectory of the two populations and not the distance between optima. As the dimensionality of the environment increases, more combinations of alleles in hybrids can generate phenotypes of low fitness under stabilising selection. Thus, reproductive isolation increases at higher overall environmental dimensionality when all else is equal. Furthermore, under these assumptions, mutation accumulation between two diverging populations increases with the number of environmental dimensions. If some proportion of these mutations produce environment-independent fitness reductions when combined in hybrids, then constitutive incompatibilities are also expected to increase with the environmental dimensionality (Barton, 1983; Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019).

Both the Chevin et al. and Thompson et al. models are sophisticated and provide an elegant picture of how environmental dimensionality can affect hybrid fitness in a variety of ways. However, there are important limitations. They do not vary divergence dimensionality; only the overall dimensionality of the environment. Critical assumptions (particularly many loci, each capable of influencing all traits and with free recombination) may be violated, potentially leading to trait-specific effects or an impact of genomic architecture. Furthermore, gene flow between diverging populations has not yet been considered in any model of dimensionality (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019). Most significantly, however, no model has yet distinguished the contribution of increasing genomic dimensionality from the effects of dimensionality of divergent selection.

Finding the way forward in multiple dimensions

Are there ways in which divergence dimensionality might impact local adaptation and speciation other than through increased overall selection, transgressive incompatibility or increased genetic complexity of adaptation? Divergent selection can drive evolution beyond local adaptation towards speciation. Speciation may result purely from increasing local adaptation, perhaps enhanced by ecological character displacement, resulting in low fitness of migrants and hybrids, and so strong extrinsic barriers to gene exchange. However, for the progression and completion of speciation, secondary barriers to gene flow are likely to be required. How might multidimensionality affect the evolution of these additional barriers?

One possibility is that divergence along more phenotypic axes increases the chance that a trait under divergent selection also contributes to another component of reproductive isolation, such as assortative mating, i.e. that the set of traits includes a multiple-effect trait. Multiple-effect traits reduce gene flow both by reducing the production of hybrids and by reducing hybrid fitness and so they generate strong barriers to gene flow that are less prone to disruption by recombination (Gavrilets, 2004; Servedio *et al.*, 2011; Smadja and Butlin, 2011). Other barriers may arise as pleiotropic effects or via indirect selection on loci linked to locally adaptive loci, including DMIs and prezygotic barriers such as assortative mating (Rundle and Nosil, 2005; Maan and Seehausen, 2011). Alternatively, these barriers might arise as an adaptive response, as in reinforcement (Smadja and

Butlin, 2011). Coupling of these different barriers can produce strong reproductive isolation (Butlin and Smadja, 2018; Kulmuni *et al.*, 2020) that can feed back to increase local adaptation by reducing the effect of gene flow. The likelihood that these additional barriers evolve is higher when overall selection is strong, hence they may be more likely to arise under additive multidimensional selection, but this is also affected by the dimensionality of the genomic response. With more loci under selection, it is more likely that loci underlying secondary barriers are impacted by divergent natural selection (as described for constitutive DMIs in Chevin et al. 2014).

Linkage disequilibrium between local adaptation loci and loci underlying secondary barriers is a key component of ecological speciation. Some of these effects have been described by unidimensional divergent selection baseline models of divergence hitchhiking that explore the impact of selection on multiple loci on divergence at a neutral locus. Where dimensionality is additive (fixed per-locus selection) more loci under selection lead to greater divergence in neutral loci (Feder and Nosil, 2010; Flaxman, Feder and Nosil, 2012), but under a diluted mode, the opposite is true as selection is applied more weakly across loci, many of which are unlinked with the neutral locus (Barton, 1983; Flaxman, Feder and Nosil, 2012). Understanding how this aspect of 'genomic dimensionality' interacts with the dimensionality of divergent selection may radically alter our conclusions about multidimensional selection. However, thus far it has not been addressed, with models favouring the simplifying assumption that a fixed number of loci underlie adaptation regardless of environmental dimensionality (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014).

Divergence in the presence of gene flow, and the consequences of gene flow following secondary contact must also be considered since both are common in nature (Nosil, 2008a, 2008b; Seehausen *et al.*, 2014; Schilling *et al.*, 2018). In the presence of gene flow, divergent selection per-locus must be strong enough to build or maintain differences in allele frequencies in order to create local adaptation and reproductive isolation. If high dimensionality is associated with high numbers of loci and weak selection per-locus, it may impede local adaptation with gene flow. Gene flow also makes the spread of alleles with constitutive incompatibilities more difficult, because the incompatibilities are exposed to selection (Bank, Bürger and Hermisson, 2012). However, gene flow also provides the opportunity for recombination, enabling genetic architecture to influence the evolution of local adaptation and speciation and it tends to align the G-matrix with environmental variability (Beldade, Koops and Brakefield, 2002). A greater number of loci increases the potential for linkage disequilibrium to build among loci to a point where indirect selection contributes to divergence and creates a strong barrier to gene flow (Flaxman *et al.*, 2014; Nosil *et al.*, 2017). It also increases the chances for linkage disequilibrium between loci under divergent selection and those underlying secondary barriers to gene exchange (Blanquart, Gandon and Nuismer, 2012; Akerman and Bürger, 2014; Seehausen *et al.*, 2014; Tigano and Friesen, 2016), so enhancing coupling and overall reproductive isolation (Butlin and Smadja, 2018). Different demographic scenarios, such as divergence in allopatry, secondary contact, island models with migration or divergence along a cline, along with different genetic architectures, might interact to a greater or lesser extent with divergence dimensionality to alter the probability of local adaptation and speciation.

Arriving at satisfactory understanding of these issues will require more theoretical work, more comparative work and meta-analyses performed using systems whose environmental dimensionality can be accurately measured (MacPherson, Hohenlohe and Nuismer, 2015; Muschick *et al.*, 2020). However, to tease apart the processes and mechanisms of multidimensional selection, experimental

speciation studies are also critical, taking up where Rice and others started. Using an experimental speciation approach, total strength of selection, number of dimensions of selection, migration and population structure can be either controlled or manipulated under laboratory conditions (Fry, 2009; White, Snook and Eyres, 2020). It is less feasible to control for genomic effects, though evolve and resequence strategies (Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015) can be used to characterise them (Michalak *et al.*, 2019) and it may be possible to choose traits that are likely to have more or less complex genetic architectures.

Concluding remarks

Moving forwards, there are several issues that require greater clarity. Firstly, it remains necessary to arrive at a consistent concept and language of dimensionality. Taking any case in isolation, the additive vs diluted argument is irrelevant; stabilising selection occurs over *n* dimensions and habitats vary on *m* of them, generating divergent selection with some total strength. However, when *the effect* of dimensionality is discussed, what is really being addressed? Are local adaptation and speciation more likely for greater *n* (niche dimensionality), or greater *m* (divergence dimensionality), all else being equal? Does genetic dimensionality matter? Alternatively, is it only the overall strength of divergent selection that matters? The available theory confirms that the overall strength of divergent selection is important, mainly through extrinsic isolation, and also shows that niche dimensionality can contribute to reproductive isolation through the transgressive and constitutive components of incompatibility (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019). However, it does not address whether divergence dimensionality or genetic dimensionality (i.e. the numbers of loci available for local adaptation or the dimensionality and orientation of the G-matrix) can also contribute. Covariation between these factors may be common, but it is not inevitable. Full understanding of mechanisms requires the isolation of each of these possible effects. Evidence from experiments, natural populations and modelling all point to the significance of multidimensional selection but there is work to do to understand the mechanisms involved.

Chapter 3: Multidimensionality and Extrinsic Isolation

Abstract

Understanding the dimensionality of selection, defined as the number of selection pressures impacting a population, is key to understanding patterns of evolution. Of particular interest is the dimensionality of divergent selection, which has previously been implicated as a determinant of local adaptation and extrinsic isolation. However, important theoretical concepts, such as how the dimensionality of selection interplays with the overall strength of selection and the number of quantitative loci it impacts, have not been decoupled and analysed in isolation. In this study, we use the genetically-explicit forward simulator, Nemo, to test three ways in which dimensionality can impact local adaptation and extrinsic isolation. Throughout, we find that there is no effect of the dimensionality of divergent selection *per se*. However, increasing this divergence dimensionality will drive local adaptation and extrinsic isolation if there is a commensurate increase in the strength of divergent selection. Increasing divergence dimensionality might also increase the number of quantitative loci under selection, which can increase the rate of local adaptation and its strength at equilibrium with gene flow, although this association depends on the variance of mutational effects. Furthermore, increasing the dimensionality of overall selection drives extrinsic isolation via increased segregation variance in F2 hybrids, and interacts with the number of quantitative loci under selection.

Introduction

Models of quantitative trait evolution tend to use the enduring metaphor of an adaptive landscape (Gavrilets, 1997; Arnold, Pfrender and Jones, 2001; De Lisle and Bolnick, 2020) where fitness is represented as a function of *m* dimensions, each representing a different orthogonal trait. Some models consider only a single trait, although there is a large body of theory on multidimensional adaptive dynamics (Lande, 1979; Lande and Arnold, 1983). Whilst all populations likely have high, perhaps infinite, trait dimensionality (Kirkpatrick and Meyer, 2004), relevant trait variation can often be summarised on 2-3 orthogonal axes representing correlated variation across some larger number of traits (Laughlin, 2014). In simple models, there is usually a single fitness peak, or 'local optimum' per environment which arises from this function of traits, with some trait combination resulting in the highest fitness. Adaptation proceeds by mutation and recombination generating allele combinations whose frequency changes shift a population's average traits up a fitness gradient towards a local multidimensional adaptive peak (Gavrilets, 1997).

Where a population is spread over two or more environmentally heterogenous localities, adaptive peaks can vary in their trait values producing peaks in different places on the multidimensional adaptive landscape (Gavrilets, 1997). This environmental heterogeneity forms a source of divergent selection. Where multiple selection pressures vary on the landscape, *n* out of *m* total trait dimensions can be under divergent selection, yielding a 'dimensionality of divergent selection' (White and Butlin, 2021). Divergent selection leading to local adaptation is an important first step in driving progress towards ecological speciation via multiple mechanisms of reproductive isolation (Rundle and Nosil, 2005; Nosil, 2012). It has frequently been hypothesised that there is an association between

multidimensionality of divergent selection and increased reproductive isolation (Rice and Hostert, 1993; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009; Smadja and Butlin, 2011; Butlin *et al.*, 2012; Langerhans and Riesch, 2013; Ravinet *et al.*, 2017). However, there remains a disconnect between mechanisms of reproductive isolation and several facets of dimensionality, such as the strength of divergent selection, the number of loci under selection, and the effects of overall dimensionality (White and Butlin, 2021).

Ecological speciation could occur through local adaptation alone. If divergent selection is sufficiently strong, migrants and hybrids may have extremely low fitness such that gene flow is severely restricted. However, barriers due to extrinsic isolation alone are likely to be inherently unstable (Lenormand, 2012), depending on the maintenance of environmental differences and the possibility of generalist genotypes arising (Melo *et al.*, 2014). For this reason, extrinsic isolation is often viewed as a 'first step', rather than an end point of speciation (Christie and Strauss, 2018). Models of multidimensional divergent selection generally make the assumption that higher dimensionality of divergent selection is associated with stronger overall strength of selection (Nosil and Harmon, 2009; MacPherson, Hohenlohe and Nuismer, 2015). However, this relationship is not well-studied, and the compound effect of multiple stressors is extremely challenging to predict in nature (Galic *et al.*, 2018; Birk *et al.*, 2020; Orr *et al.*, 2020).

Increased dimensionality of divergent selection may also be associated with a higher number of loci under divergent selection. Indeed, if each dimension of selection impacts an orthogonal trait, this is evident by definition; orthogonal traits can evolve independently as there is no genetic covariance (Arnold *et al.*, 2008; MacPherson, Hohenlohe and Nuismer, 2015), hence an additional orthogonal trait is underlain by an independent locus or set of loci. By impacting more loci, multidimensional divergent selection is likely to encompass a wider range of standing genetic variance and total mutational variation over time. However, this effect may trade-off against allele effect size, resulting in population differentiation in many small effect alleles which may be vulnerable to the homogenising effects of gene flow (Nosil, Harmon and Seehausen, 2009).

In additional to the dimensionality of divergent selection, the overall environmental dimensionality (also known as 'niche dimensionality') plays an important role through transgressive incompatibilities in hybrids (Rieseberg, Archer and Wayne, 1999; Rieseberg *et al.*, 2003). Regardless of the dimensionality of divergent selection, some environmental variables will remain constant, producing stabilising selection around an optimum. During adaptation to a new optimum on one axis, beneficial mutations or combinations of alleles have a net positive effect on fitness, but nonetheless may pleiotropically produce small fitness reductions on other axes under stabilising selection (Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019). Transgressive incompatibilities are a form of Dobzhansky-Muller incompatibility (DMI; Dobzhansky, 1936, 1937; Muller, 1942) manifest through increased segregation variance in hybrid phenotypes under stabilising selection (Chevin, Decorzent and Lenormand, 2014). In a single population, the sum of these effects is predicted to be negligible, as alleles at different loci combine to generate phenotypes close to the local optimum. However, hybridisation of two populations evolving independently is predicted to release this cryptic variation producing transgressive phenotypes that are significantly more variable than the parent populations and have lower fitness due to divergence from the optimum on axes under stabilising selection (Johansen-Morris and Latta, 2006; Van Der Sluijs *et al.*, 2008). In this way,

both the dimensionality of divergent selection, and the overall dimensionality of selection, contribute to extrinsic isolation.

In this simulation study we construct model populations that experience various selection regimes, targeting one or more traits underpinned by few or many loci. With this model, we examine mechanisms by which multidimensional selection can affect local adaptation and extrinsic isolation. Firstly, we test the hypothesis that varying the dimensionality of divergent selection will have different outcomes for local adaptation and extrinsic isolation depending on whether it is independent from, or associated with, the strength of overall selection. Secondly, assuming that each dimension of divergent selection impacts some loci, we test the hypothesis that increased divergence dimensionality will impact these barriers according to the number of loci under selection. We test the concept that by spreading selection over many dimensions and hence many loci, local adaptation may be underlain by many weak-effect loci and hence vulnerable to homogenisation from gene flow. Finally, we quantify the impact of transgressive incompatibilities, incurred via increased overall dimensionality, and compare this with the effects of divergence dimensionality. Throughout, we test these mechanisms of reproductive isolation under demographic conditions of allopatry followed by secondary contact.

Model and Results

Simulation Methods

All simulations were performed using the forward-time, individual-based stochastic programming framework 'Nemo' (Guillaume and Rougemont, 2006). Data processing and analysis were performed using R (v4.0.3; R Core Team, 2020). All simulations were run over 100,000 generations with ten replicates derived from a burn-in simulation of 20,000 (10*N*) generations under weak stabilising selection for all traits and varied only by number of quantitative loci and per-locus variance of mutational effetcs where applicable. We modelled a two-patch island model with 1000 adults per patch and two sexes (see Table 1 for a full list of parameters). Unless otherwise stated, the two patches existed in total allopatry for 50,000 generations (m = 0), followed by 50,000 generations of secondary contact (m = 0.001). Individuals were sexual diploids and mated monogamously and randomly once per-generation within their patch to produce some number of offspring drawn randomly from a

Table 1: Parameters used in the simulations and their values. Values in bold used throughout, values not in bold used as default but may vary as specified in the main text.

Notation	Description	Value
α	variance of mutational effects per quantitative locus per axis	0.05
d	Euclidean distance between optima under dilution multidimensionality	8
N	number of diploid individuals	**1000**
m	m, overall dimensionality of selection	5
n	dimensionality of divergent selection	**1-4**
q	number of quantitative loci	10
s	selection variance (diagonal of S)	**100**
μ_n	mutation rate for neutral loci	**$1x10^{-4}$**
μ_q	mutation rate for quantitative loci	**$1x10^{-5}$**

Poisson distribution with mean of 4. Adults then died. Migration, if present, occurred prior to selection so that migrants were exposed to selection associated with their adult, rather than natal patch. Viability selection was then applied to offspring based on quantitative traits (see below). Surviving offspring became adults and were removed at random to resize the population to carrying capacity (i.e. soft selection with non-overlapping generations).

Traits were assumed to match environmental axes that impose independent stabilising selection with the same strength, producing an isometric Gaussian fitness surface in each patch (optimal values provided in Table 2). The values of each quantitative trait varied continuously and were determined by the sum of each allele effect at each locus on the relevant traits, with no interactions or dominance effects. Loci were fully pleiotropic, hence each allele could potentially influence all traits. Mutations were drawn randomly from a multivariate Normal distribution and their effects were added to the existing allele value for each trait. Our simulations used zero mutational correlation between allele effects, hence the mutational effect on each trait was independent but genetic correlations could arise due to selection. In each simulation, a variable number of axes, *n*, were under divergent selection, with other axes, *m*-*n*, under stabilising selection. The fitness of an individual in a specified environment was calculated from all quantitative traits as;

$$W(z_i)_j = \exp\left[-\frac{1}{2}\left(z_i - \theta_j\right)^T S^{-1}\left(z_i - \theta_j\right)\right] \quad (1)$$

Where z_i represents the vector of trait values for individual *i*, θ represents the vector of local trait optima in environment *j*, *S* is the variance-covariance matrix of selection and *T* specifies matrix transposition (Guillaume and Rougemont, 2006).

Equation 1 can be simplified via matrix multiplication to express individual fitness as the product of all per-trait fitness values.

$$W(z_i)_j = \prod_{k=1}^{m} \exp\left[-\frac{1}{2s}\left(z_{ik} - \theta_{ijk}\right)^2\right] \quad (2)$$

Where *k* represents the dimension index ranging from 1 to *m* and *s* represents the selection variance (the diagonal values of *S*). Given no interaction among traits, fitness only depends on the selection

Table 2: Absolute values of local optima (ϑ_m) per-trait (*m*) and Euclidean distance between optima (*d*) for each combination of mode and dimensionality (*n*) with an overall dimensionality (*m*) of 5 (for *m* = 10/20 simulations, $\sigma_{6\text{-}20} = 0$). For each pair of patches, one has positive values of θ whilst the other has negative values of θ.

Mode	*n*	$\lvert\theta_1\rvert$	$\lvert\theta_2\rvert$	$\lvert\theta_3\rvert$	$\lvert\theta_4\rvert$	$\lvert\theta_5\rvert$	*d*
N.A	1	4	0	0	0	0	8
Additive	2	4	4	0	0	0	11.314
	3	4	4	4	0	0	13.856
	4	4	4	4	4	0	16
Dilution	2	2.828	2.828	0	0	0	8
	3	2.309	2.309	2.309	0	0	8
	4	2	2	2	2	0	8

variance (*s*) and the Euclidean distance from the optimum (*d*), as $d^2 = \sum_{i=1}^{m}(z_i - \theta_{ij})^2$ (Guillaume and Rougemont, 2006).

Local adaptation was calculated over the two patches as the 'sympatric-allopatric' (SA) contrast (Kawecki and Ebert, 2004; Blanquart *et al.*, 2013) where *W* is the average fitness of deme *A/B* in environment *a/b*.

$$SA_{AB} = \frac{W(A_a) + W(B_b)}{2} - \frac{W(A_b) + W(B_a)}{2} \quad (3)$$

Average fitness for each deme was measured by calculating the fitness of all adults within a deme using equation 2 and taking the average. These measurements were taken every 5,000 generations throughout each simulation (plus generations 10, 100 and 1,000 to capture early responses at higher resolution, and generation 49,990 to capture the transition to secondary contact).

Each simulation tracked 45 neutral loci and a variable number (*q*) of quantitative loci with free recombination among all loci. Neutral loci had 10 alleles and with each mutation, a given allele *k* was able to mutate to allele *k*+1 or *k*-1 at random with reflexive boundaries (i.e. 10 + 1 = 1). F_{ST} was calculated among all neutral loci (Weir and Cockerham, 1984).

Hybrid fitness was calculated individually from trait data collected prior to selection for each sampled generation and the trait optima of the hybrid's native patch (i.e. before the opportunity for migration). F1 hybrids were defined as the offspring of one native and one immigrant parent. F2 hybrids were defined as any hybrid with one or more parents that were themselves F1 hybrids. Hybrid data were collected every 10,000 generations from the point of secondary contact.

Finally, to quantify the multidimensional effects of individual phenotypes or alleles, their effects were calculated as vectors across the multidimensional landscape. For each phenotype/allele vector, we calculated the multidimensional effect size (magnitude of the vector) as the square root of the sum of the squared per-axis effect sizes. We note that as a vector on an isometric landscape, the effect size is unaffected by start/end position, although the effect on fitness would be affected. Furthermore, using Euclidean geometry, we calculated the angle between each phenotype/allele vector and the vector that describes the 'discriminant axis' between patches (i.e. the vector that connects θ_1 with θ_2). We describe this angle as the 'angle of deviance' (ψ).

These two parameters respectively illustrate the size and alignment of each allele/phenotype, and therefore its importance for fitness under multidimensional divergent selection and stabilising selection. To calculate this explicitly, we calculated the size of two other vectors; the 'along-axis distance' which quantifies the effect of each allele/phenotype in parallel with the discriminant axis (and so affects fitness only under divergent selection) and the 'off-axis deviation' which quantifies the effect of each allele/phenotype perpendicular to the discriminant axis (and so affecting fitness only under stabilising selection). These were calculated using Euclidean geometry, as together with the multidimensional effect size, these three vectors form a right-angled triangle, with the angle between the multidimensional effect size and the along-axis distance forming ψ. Finally, when considering the fitness of hybrids, it can be more illustrative to consider the deviation from the optimum in parallel with, and perpendicular to, the discriminant axis. This latter property is already calculated as the off-axis deviation. Loss of fitness in parallel with the discriminant axis was also calculated and is described as the 'along-axis deviation'. These geometric concepts are illustrated in Box 1.

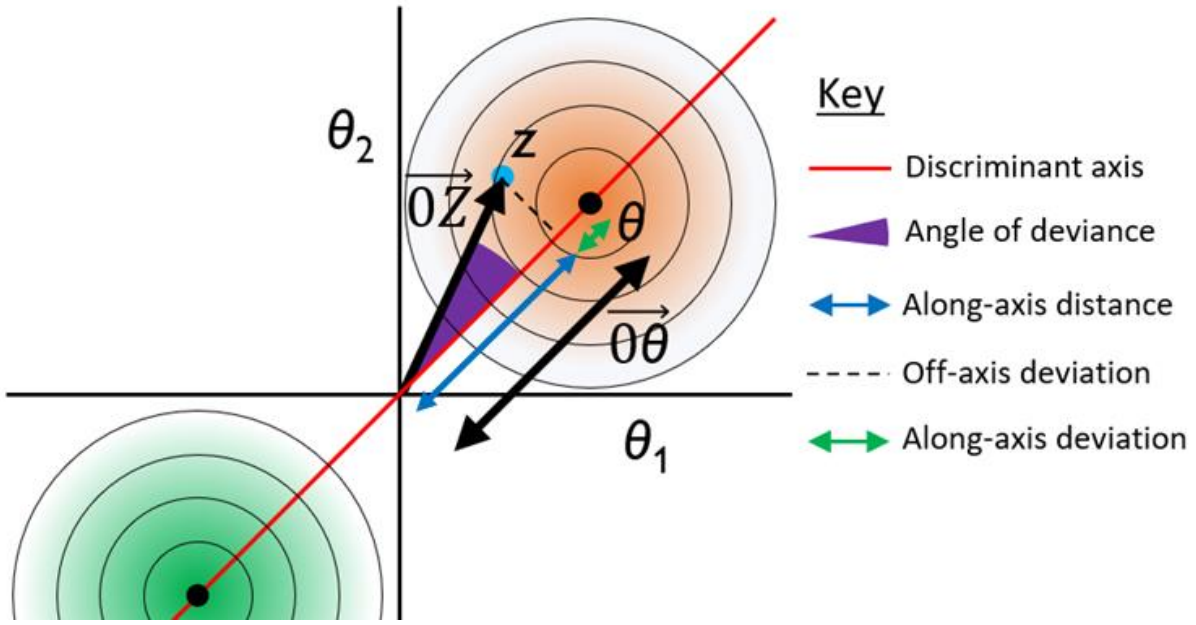

Figure 1: Geometry of a phenotype/allele vector on a multidimensional landscape.

Box 1: Multidimensional geometry

Figure 1 displays a two-dimensional landscape (m = 2). Two environments (green/orange contour shading) have optima which are divergent in both dimensions. The discriminant axis separating these optima is shown by the red line.

The vector $\overrightarrow{0Z}$, shown here as a black single-headed arrow, describes the multidimensional vector of either an allele or a phenotype. The effect size (magnitude) of any such allele or phenotype can be calculated as its Euclidean distance;

$$\left|\overrightarrow{0Z}\right| = \sqrt{\sum_{k=1}^{m} {z_k}^2}$$

The vector $\overrightarrow{0\theta}$, spans from 0 to the local trait optima and is parallel with the discriminant axis. The angle of deviance (ψ) between allele/phenotype vector $\overrightarrow{0Z}$ and $\overrightarrow{0\theta}$ can be calculated as;

$$\psi = arccos\left[\frac{\overrightarrow{0Z} \cdot \overrightarrow{0\theta}}{\left|\overrightarrow{0Z}\right| \times \left|\overrightarrow{0\theta}\right|}\right]$$

The Euclidean distance can be thought of as the sum of two other vectors; the path from 0 to *z* moving along the discriminant axis and the path from and perpendicular to the discriminant axis to point z. These vectors have distances described as the 'along-axis distance' and 'off-axis deviation' respectively. Using the Euclidean distance and angle of deviation of $\overrightarrow{0Z}$, these distances can be respectively calculated as; $\cos(\psi) \times \left|\overrightarrow{0Z}\right|$ and $\sin(\psi) \times \left|\overrightarrow{0Z}\right|$.

Hypothesis 1- the effect of multidimensionality on local adaptation and extrinsic isolation depends on its association with strength of selection

We first examined the effects of varying the dimensionality of divergent selection: overall dimensionality was held constant (m = 5) within which the number of dimensions of divergent selection varied (n = 1-4). As the dimensionality of divergent selection increased from 1 to 4, the overall strength of divergent selection against migrants and hybrids was either held constant, and so the optima of each divergent trait were less divergent, or increased with dimensionality as a result of using the same optimum value for all divergent traits irrespective of divergence dimensionality. These simulations are comparable to dilution and additive modes of multidimensional divergent selection *sensu* White & Butlin (2021).

Given that selection is Gaussian with equal variance per axis and no covariance, fitness follows a Gaussian decline with distance from each local optimum uniformly in all directions in multidimensional trait space. Therefore, individual fitness depends only on d, the Euclidean distance between multidimensional trait values and local optima. The additive mode used a fixed distance between optima per axis, hence the Euclidean distance through multidimensional space increased proportionately with the square root of the dimensionality of divergent selection (n). Alternately, the dilution mode kept this Euclidean distance constant, resulting in progressively smaller distances separating trait optima on each axis, thereby holding overall selection constant (Table 2).

Against this design and computation experiment, we found that the effect of divergence dimensionality on local adaptation varied by mode of multidimensionality (Figure 2). This was true for both the endpoint and rate of local adaptation. Under the dilution assumption, where total selection is held constant against migrants and hybrids, divergence dimensionality had no effect. However, under additive multidimensionality where overall selection increased with divergence dimensionality, local adaptation built more rapidly and reached a higher strength at equilibrium under allopatry. Upon secondary contact, local adaptation also remained stronger at selection-migration equilibrium with higher divergence dimensionality. Local adaptation thus varied by the overall strength of selection, not the dimensionality of divergent selection *per se*. (Figure 2, top panels).

Closer inspection of home vs away fitness revealed that all populations reached an equilibrium point of 'home fitness' prior to secondary contact which was unaffected by dimensionality under either mode. Although this equilibrium point was reached increasingly later under additive multidimensionality, this was simply a function of the increased distance between ancestral trait values and new optima (Figure 2, middle panels). This link between additive multidimensionality and local adaptation/extrinsic isolation has been described previously (Nosil and Harmon, 2009; MacPherson, Hohenlohe and Nuismer, 2015). However, it was yet to be made clear that this link does not hold unless d, and therefore overall divergent selection, increases.

In line with patterns of local adaptation, the fitness of both F1 and F2 hybrids decreased with increasing dimensionality under additive multidimensionality but not dilution multidimensionality (Figure 3). This effect was seen most severely in F1s but was still observed in the F2 generation. Whilst in allopatry, there was no effect of dimensionality on the barrier to neutral gene exchange, measured as F_{ST} of all neutral loci averaged across the genome: the stronger selection on quantitative loci at higher additive dimensionalities did not impact neutral divergence. Upon secondary contact, F_{ST} was greatly reduced globally, but was maintained at a higher level at higher additive multidimensionality,

with stronger local adaptation acting as a barrier to reduce gene flow relative to migration rate, despite free recombination (Figure 2, bottom panels).

In summary, the extrinsic effects of multidimensional divergent selection can create a stronger local adaptation barrier to gene flow and reduced fitness of hybrids only via increasing the overall strength of selection. Our simulations using a dilution mode of multidimensionality show that there is no detectable effect of divergence dimensionality *per se* on local adaptation or extrinsic isolation under the assumptions of this simple model. To further demonstrate this effect, we ran simulations using

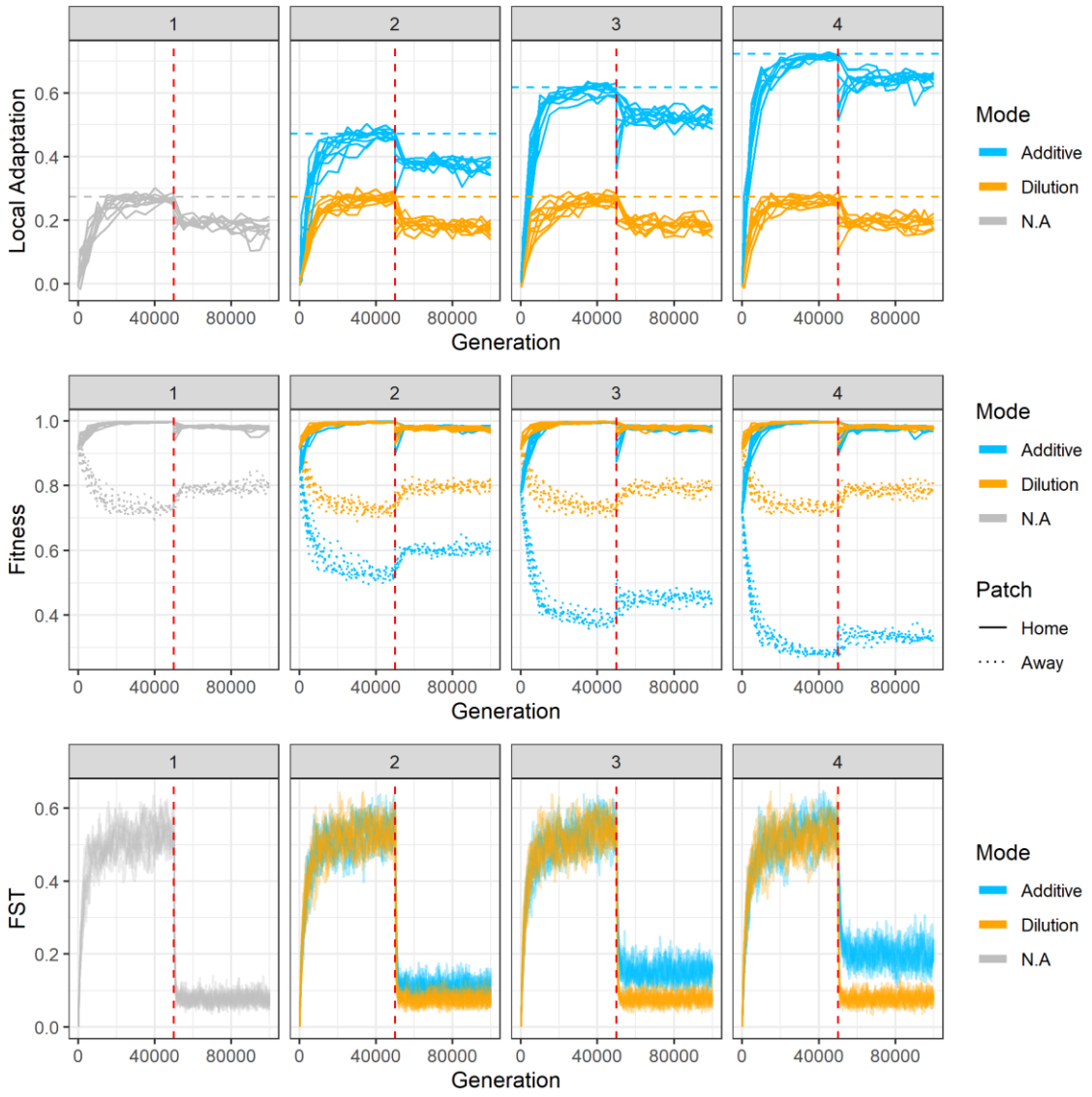

Figure 2: Local adaptation over 100,000 generations under additive or dilution modes of multidimensional divergent selection. Panels 1-4 are dimensionalities of divergent selection; red vertical dashed line represents the transition from allopatry to secondary contact.

Top panels show how local adaptation, measured by the SA contrast, builds and is maintained over time. Horizontal dashed lines represent the theoretical local adaptation of two perfectly adapted populations at each dimensionality. Middle panels show how the constituent home (sympatric) and away (allopatric) average fitnesses of the two populations over time. Bottom panels show how F_{ST} of the two populations varies over time.

divergent selection along only one of five axes, but with increased *d* between optima to match that of our additive multidimensionality simulations. The patterns of local adaptation and F_{ST} were indistinguishable from those under additive multidimensional effects (Figure S1). We also confirm this by showing that the angle of deviance between the discriminant axis and the average multidimensional phenotype vector within each deme is unaffected by divergence dimensionality in a dilution mode; whatever the divergence dimensionality, phenotype vectors evolve to align with the discriminant axis (Figure S2).

Hypothesis 2- the effect of multidimensionality on local adaptation and extrinsic isolation depends on the number of quantitative loci and their mutational effects

A second route by which multidimensionality might influence local adaptation is by impacting a wider range of quantitative loci. In this section, we use the assumption that for each dimension of divergent selection, a given number of universally pleiotropic quantitative loci are under selection. Here, we test whether increasing the number of quantitative loci with the dimensionality of divergent selection impacts the speed and strength of local adaptation and the strength of extrinsic isolation under secondary contact. Furthermore, we tested how this effect varied depending on whether variance of mutational effects remains 'fixed' or decreases with the number of quantitative loci such that overall

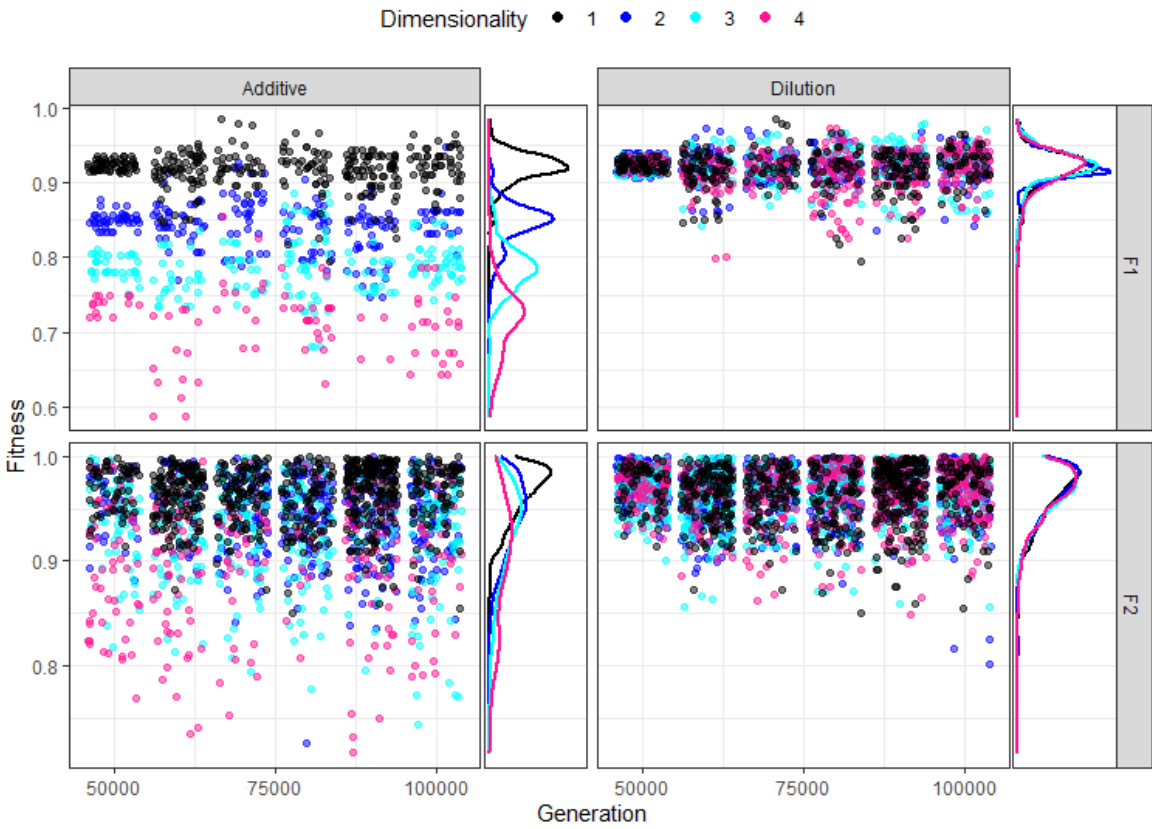

Figure 3: Hybrid fitness. Individuals sampled at 10000-generation intervals from generation 50000 to 100000 (points distributed along the x axis using the *geom_jitter()* function). Top panels show overall fitness per hybrid. Bottom panels show the fitness of F2 hybrids. Each panel has a corresponding side panel displaying the density of data points across all sampled generations along the y axis to highlight the distribution of fitness values per dimensionality. Side plots created using the *ggside* R package (Landis, 2021).

mutational variance is 'balanced' between per locus variance and number of loci. Having isolated the effect of overall strength of selection, in this section, simulations only use the dilution mode of multidimensionality where the overall strength of divergent selection is unchanged (*d* is unchanged by dimensionality).

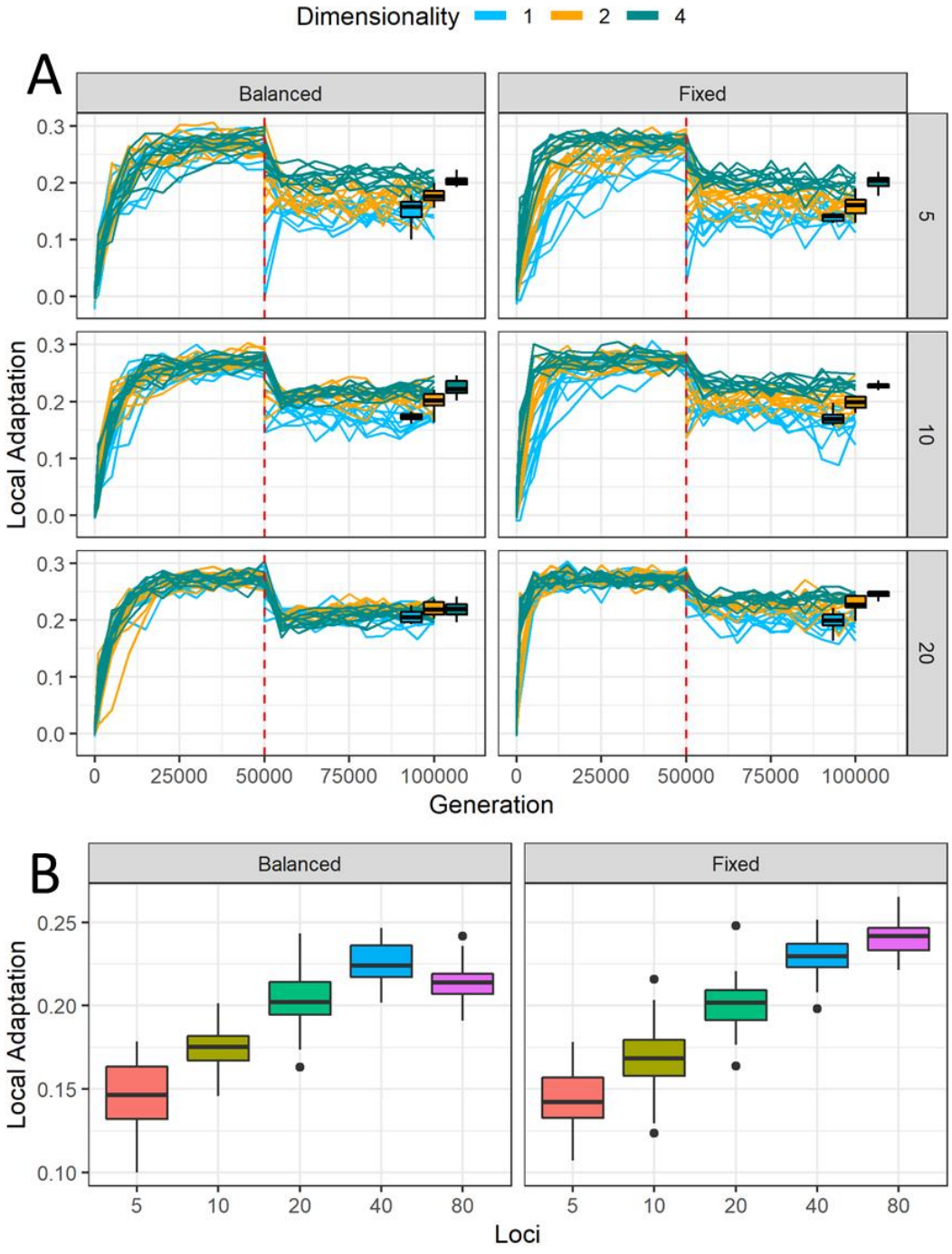

Figure 4: Local adaptation varies by number of quantitative loci. Plot A displays the pattern of local adaptation over 100,000 generations according to its mode of mutational variance (horizontal panels) and number of quantitative loci per dimension of divergent selection (q/n) (vertical panels). Superimposed boxplots show a snapshot of local adaptation at generation 100,000. Plot B displays the pattern of local adaptation by number of loci regardless of divergence dimensionality at generation 100,000.

To evaluate this hypothesis, we varied the number of quantitative loci, *q*, alongside the number of dimensions under divergent selection such that each divergent dimension corresponds to either 5 (5-20 total), 10 (10-40 total) or 20 loci (20-80 total). We then simulated two scenarios for comparison. In the first 'fixed' mode, the variance of mutational effects remained constant, such that the total mutational variance introduced per generation increased with the number of loci. In the second 'balanced' mode, the variance of mutational effects (α) decreased with the number of loci (q) such that the mutational variance remained equal ($2Nq\alpha = 0.5$). Starting populations were derived from burn-in simulations as before, but with corresponding values of q and α. A comparison of these two modes can reveal how the effect of more loci under selection at higher dimensionality affects extrinsic isolation, and how this depends on the availability of mutational variance.

We find that the rate at which local adaptation builds in allopatry is affected by the dimensionality of divergent selection (and hence q) under a fixed, but not a balanced, variance of mutational effects

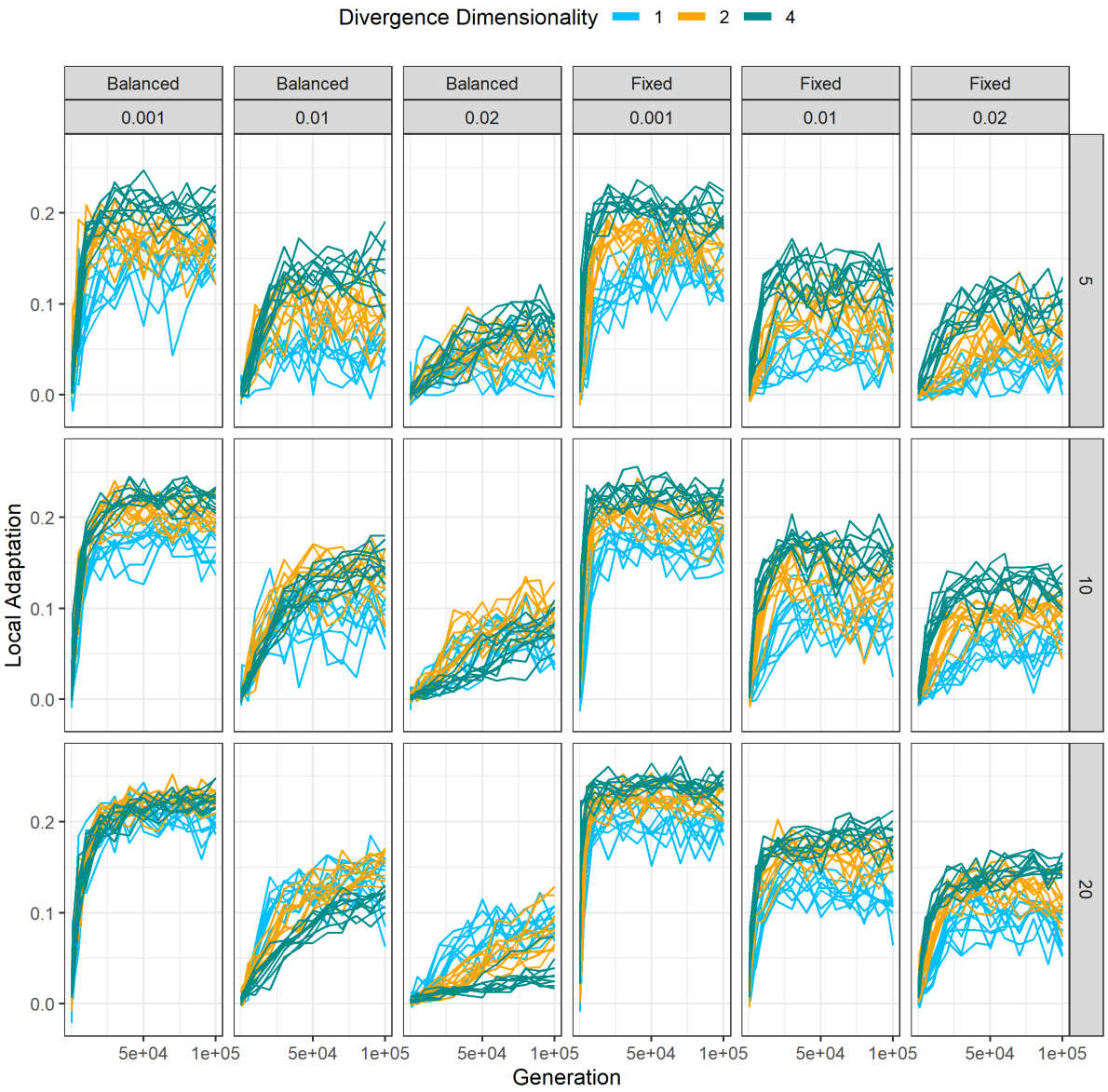

Figure 5: Local adaptation with variable divergence dimensionality, number of loci per dimension of divergent selection (vertical panels), and variable rates of ongoing migration (horizontal panels).

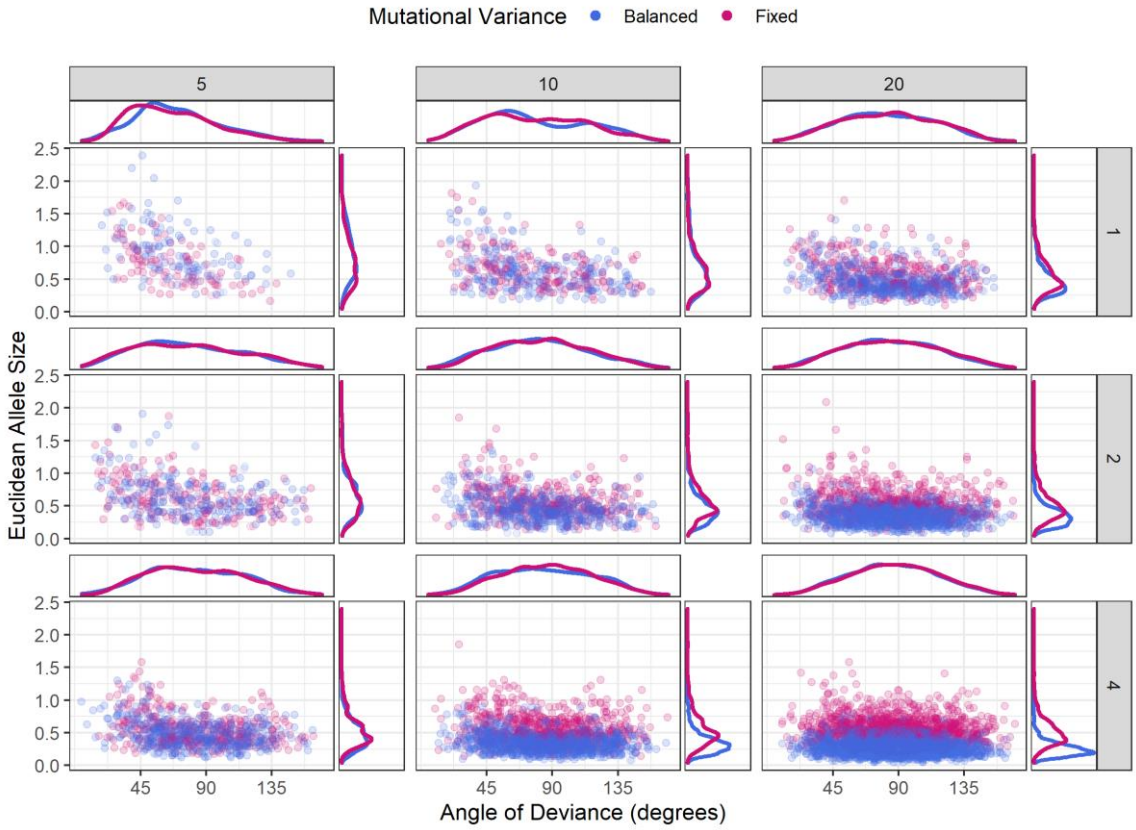

Figure 6- Distribution of alleles' Euclidean distances (effect size) and angle of deviance at generation 100,000. Horizontal panels represent loci per dimension of divergent selection, vertical panels represent dimensionalities of divergent selection (panels on the bottom left-top right diagonal have equal numbers of loci). The distribution of both allelic parameters is displayed in side panels (Landis, 2021).

(Figure 4A). The effect of divergence dimensionality is strongest when there are few loci per dimension; with fixed variance of mutational effects and low dimensionality of divergent selection, simulations did not reach a locally adaptive equilibrium before entering secondary contact. This did not occur under balanced variance of mutational effects whereby the lack of additive genetic variation was compensated for by higher per-locus variance of mutational effects. Following secondary contact, local adaptation was generally maintained at a higher level with greater divergence dimensionality (and hence greater *q*). However, at high numbers of loci under a balanced mode, this relationship was less clear. That this relationship was strong under a fixed mode, but not a balanced mode suggested having more alleles increases the barrier to gene flow, but if they have increasingly small effects then that barrier may begin to weaken. By excluding divergence dimensionality (which has no effect *per se*) and examining only the number of quantitative loci, this pattern is more clearly visible (Figure 4B).

To test whether local adaptation due to a greater number of small-effect alleles (via increased divergence dimensionality) would become increasingly permeable to gene flow, we altered the rate and period of migration. We repeated the simulations, but instead of using allopatry followed by secondary contact, we used three variable levels of ongoing migration (*m* = 0.001, 0.01, 0.02). Where variance of mutational effects remained fixed, the pattern of stronger local adaptation with greater divergence dimensionality was maintained across all rates of migration. However, where variance of

mutational effects was balanced against number of quantitative loci, this pattern reversed at higher migration rates and with more loci per dimension (Figure 5). With increased divergence dimensionality and hence more loci under selection, local adaptation became increasingly vulnerable to the homogenising force of gene flow under balanced variance of mutational effects.

To confirm that these different patterns of local adaptation were associated with differences in the distributions of allele effects, we calculated the multidimensional effect size and angle of deviation for all alleles at generation 100,000. We find that under a fixed mode of mutational variance, regardless of number of quantitative loci or divergence dimensionality, there is a consistent small cluster of large-effect low-angle alleles, with all other small-effect alleles forming a roughly normal distribution across the range of possible angles (Figure 6). However, under a balanced mode of mutational variance, the average effect size is inversely proportional to the number of quantitative loci, with larger allele effects at low loci and dimensionality, and a narrow distribution of small alleles at high loci and dimensionality. This composition of many small-effect alleles may be stable over time, or there might be very slow evolution of larger effect sizes as rare large-effect mutations occur, or small-effect mutations accumulate at the same locus, becoming 'stacked' (Yeaman and Whitlock, 2011; Yeaman, 2015; Rafajlović *et al.*, 2016). As for hypothesis 1, there was no effect of divergence dimensionality *per se*.

Finally, we find that the number of adaptive loci has no effect on the mean fitness of either F1 or F2 hybrids (Figure S3). Aside from the first generation of F1s having reduced variation in fitness, due to both parental populations being near to their respective optima prior to secondary contact, in neither mode does the fitness of hybrids vary with generation, divergence dimensionality or the number of loci per dimension. Additionally, the differences in local adaptation do not provide sufficient barriers to gene flow to produce consistent, observable differences in F_{ST} (Figure S4).

Hypothesis 3- overall dimensionality interacts with divergence dimensionality to impact transgressive segregation

Previous studies (Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019) have demonstrated that increased overall dimensionality, not necessarily the number of divergent dimensions, can affect extrinsic isolation through 'transgressive incompatibilities'. These are manifest in hybrids between diverging populations whereby phenotypes lie off the main discriminant axis of divergent selection; i.e. reductions in hybrid fitness due to variance in phenotypes under stabilising selection. Fitness loss is predicted to be small in F1 generations, but large in F2 hybrids due to transgressive segregation.

In this section, we test how varying the dimensionality of overall selection impacts the fitness of hybrids and how it interacts with other variables examined in this study. We compared the results in the previous simulations from a 5-dimensional niche (m = 5) with those in a 10-dimensional (m = 10) and 20-dimensional (m = 20) niche by adding 5 or 15 extra dimensions of stabilising selection.

Firstly, we tested how increased overall dimensionality interacts with multidimensional divergent selection. We confirm that higher overall dimensionality reduces fitness strongly in F2 hybrids but weakly in F1 hybrids (Figure 7). Again, there was no effect of dilution multidimensionality, nor interaction with overall dimensionality in determining hybrid fitness. The effect of increased additive divergence dimensionality, as observed in section 1, does not vary by overall dimensionality: there is no observable interaction between divergence and overall dimensionality. We find that 'along-axis'

phenotypic deviation from the optimum increases with additive divergence dimensionality steeply in F1 hybrids and to a lesser extent in F2 hybrids (Figure 8, top panels). However, there is no observable effect of overall dimensionality on this metric. The 'off-axis' phenotypic deviation is much more strongly affected by overall dimensionality, with increased overall dimensionality producing much higher and less variable deviation for both F1 and F2 hybrids (Figure 8, middle panels). However, the effect of divergence dimensionality on off-axis deviation varies by hybrid generation. Whilst off-axis deviation decreases with additive divergence dimensionality in F1 hybrids, in F2s this pattern is reversed, such that higher additive divergence dimensionality produces greater deviation. To assess the relative importance of each metric for hybrid fitness, we took the ratio of 'along-axis' to 'off-axis' deviation (Figure 8, bottom panels). This shows that the 'along-axis' deviation is responsible for much higher fitness loss than 'off-axis' deviation in F1 hybrids, but there is greater parity in F2 hybrids. Additionally, the ratio increases with divergence dimensionality in F1 hybrids, but remains relatively

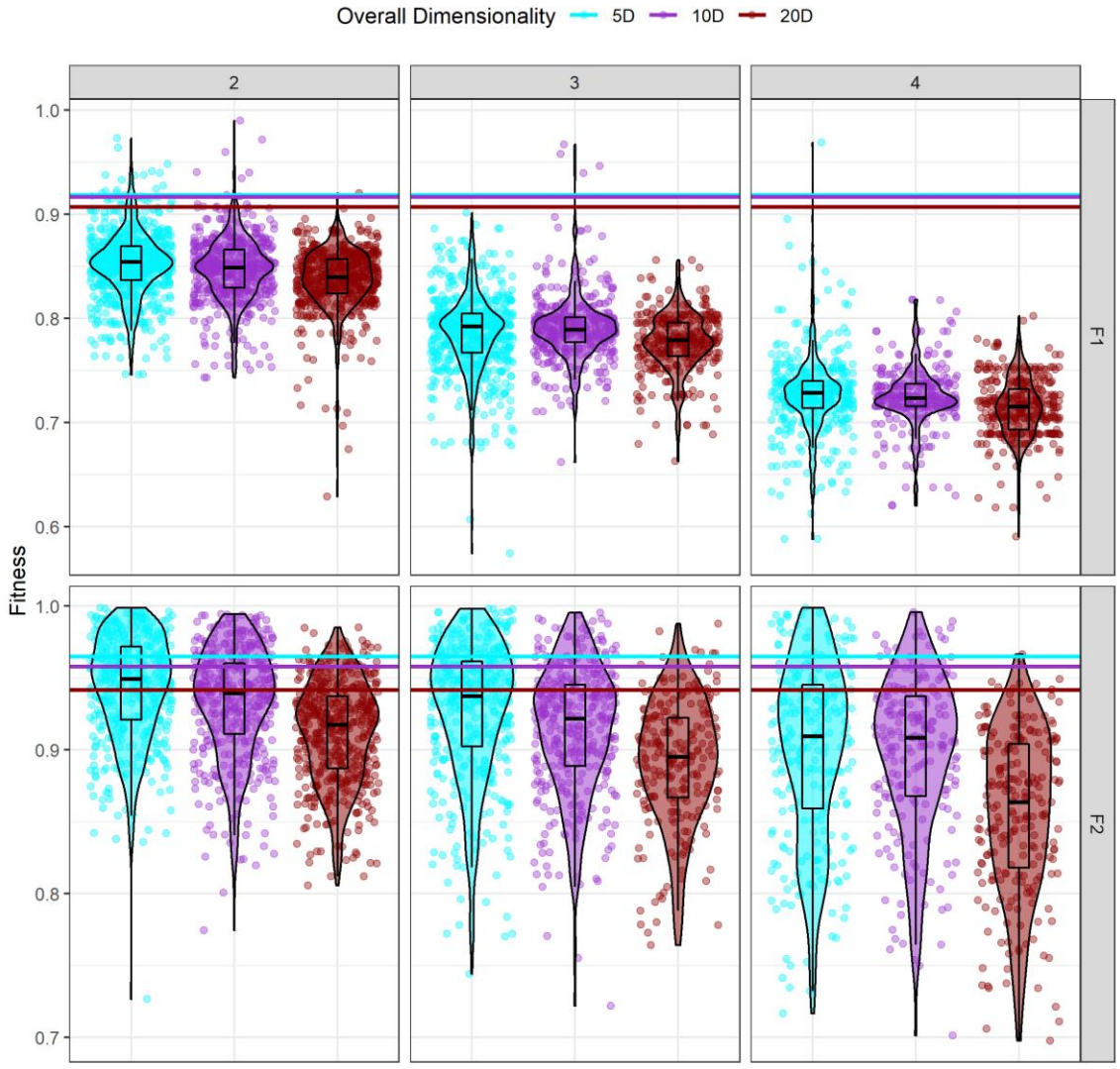

Figure 7: Fitness of F1 and F2 hybrids under variable overall dimensionality and additive divergence dimensionality. Horizontal lines represent average values under a Dilution mode (unaffected by dimensionality).

stable with respect to divergence dimensionality in F2 hybrids; divergence dimensionality affects each deviation proportionally.

Secondly, we tested how overall dimensionality interacts with the number of loci per dimension of divergent selection when the per-locus mutational variance remains fixed, or is balanced against the number of quantitative loci, as in section 2.

As above, increased overall dimensionality decreases the fitness of F1 and F2 hybrids, with the effect in F2 hybrids again being stronger than in F1 hybrids. In F1 hybrids, this loss of fitness due to overall dimensionality is extremely consistent; it does not vary according to whether mutational variance is fixed or balanced, or by the divergence dimensionality and number of quantitative loci per dimension (Figure S5). However, in F2 hybrids, this more substantial loss of fitness varies by number of quantitative loci and divergence dimensionality under a balanced, but not a fixed mode of mutational variance. Where mutational variance is balanced against number of quantitative loci, low numbers of

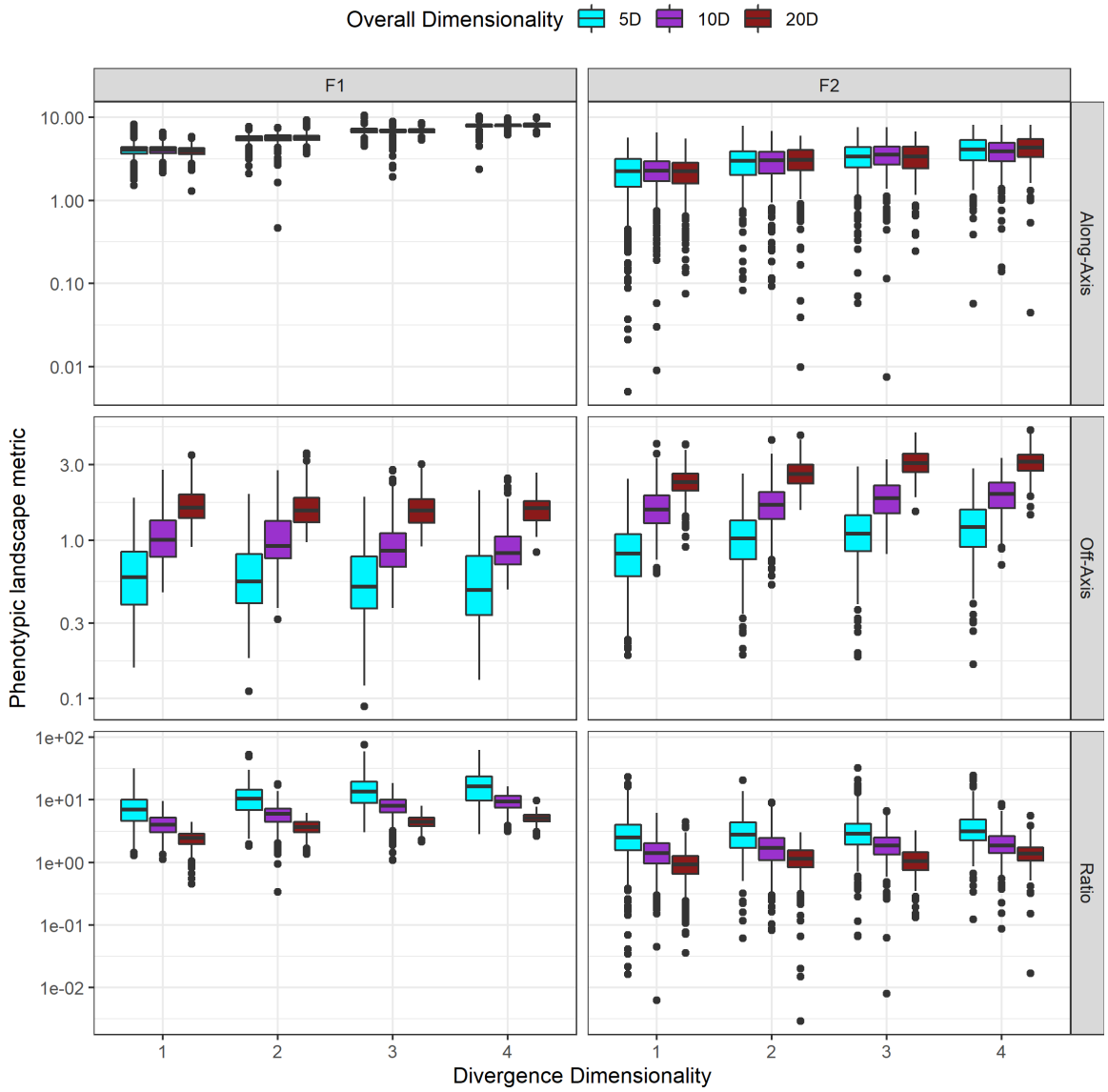

Figure 8- Distances of along-axis and off-axis phenotype deviation from a local optimum, and the ratio of the two, for simulations using Additive multidimensionality.

quantitative loci produce high fitness losses with overall dimensionality, and high numbers of loci produce low fitness losses (Figure 9). Increased overall dimensionality produces a small increase in along-axis deviation in F2, but not F1, hybrids with no interaction with any other conditions. As above, off-axis deviation is higher with increased overall dimensionality, and higher in F2s than in F1s (Figure 10). Under a fixed mode of mutational variance, this effect is consistent across numbers of quantitative loci. However, under a balanced mode, the effect of overall dimensionality on off-axis deviation decreases with number of quantitative loci. The reduction in off-axis deviation at high loci per dimensionality and overall dimensionality correlates with the loss of fitness described above.

This suggests that segregation variance in the F2 generation is inversely proportional to the number of quantitative loci under a balanced mode of mutational effects. This effect is not present in the fixed mode of mutational effects because the number of loci that significantly contribute to local adaptation does not vary as much due to the availability of large-effect alleles. This effect of the number of quantitative loci, and its sensitivity to the mode of mutational variance, was not observed in the previous section (m = 5) due to low overall dimensionality and limited opportunity for transgressive segregation.

Discussion

In this study, we have presented a collection of simulations that highlight key mechanisms by which various forms of dimensionality can affect ecological speciation. Here, we discuss these findings in the context of existing work and future directions.

Key findings

The first outcome of our study supports a key argument made in White & Butlin (2021) that increased divergence dimensionality *per se* does not increase local adaptation. Instead, multidimensionality is a means by which the strength of divergent selection can increase (when trait optima remain fixed with respect to dimensionality), and so produce faster and stronger local adaptation. Under a dilution mode, where overall selection remained constant, local adaptation neither builds faster nor stabilises at a stronger level with increased multidimensionality. There is also no impact of divergence dimensionality on other forms of extrinsic isolation such as F1 hybrid fitness or transgressive F2 phenotypes. In this context, under this set of assumptions, it would be incorrect to say that multidimensionality itself drives the evolution of reproductive barriers, but it may do so by impacting correlated variables.

Where there is no constraint on the M-matrix (mutational effects of a given magnitude are equally likely in any direction) and no covariance structure present in the G-matrix at the start of the simulation (traits are genetically independent), the 'dimensionality' of divergent selection becomes an arbitrary concept (Bégin and Roff, 2001; Arnold *et al.*, 2008). In other words, trait optima of two environments are always separated by a single principal component. Naturally, this would not be true in the case of three or more populations, but for any pair, there can only ever be a single axis of divergent selection. Any number of environmental variables might be aligned with this axis to a greater or lesser degree and may be entirely independent of one another in the wider environmental context, but for any two-population comparison must combine to form a single discriminant axis.

The lack of an inherent effect of divergence dimensionality is unsurprising and somewhat artificial. However, it is the first formal confirmation, via population genetic simulations, showing how the

dimensionality of selection is decoupled from overall selection. It highlights the geometric fact that, irrespective of the dimensionality of divergence, there is a single discriminant axis separating two points in trait space. Under this model of dilution multidimensionality, the distance between these points remains fixed, and as if we assume that selection follows an isometric Gaussian distribution in all directions, the divergence dimensionality has no impact. In nature, these contrasting modes of multidimensional divergent selection presented in hypothesis 1 represent points on a spectrum of ecological stressor interactions ranging from highly antagonistic (i.e. a dilution mode) to additive (Galic *et al.*, 2018; Orr *et al.*, 2020). In ecological reality, this spectrum extends beyond our additive mode to forms of synergistic stressor interactions whereby multiple stressors combine to yield selection which is stronger than the sum of their parts (Folt *et al.*, 1999; D. Vinebrooke *et al.*, 2004).

Our second key finding is that increased multidimensionality can increase the speed at which local adaptation builds by impacting more quantitative loci, although again, divergence dimensionality has no effect *per se*. Furthermore, the impact of multidimensionality on the number of quantitative loci may also affect the genetic composition of local adaptation, potentially making it vulnerable to homogenisation due to gene flow.

In allopatry, our results imply that the overall input of mutational variance ($V_m = 2N\mu_q q\alpha$) is the determinant factor for the rate of local adaptation. The fixed mode of per-locus mutational variance meant that overall mutational variance increased with quantitative loci number, and duly local

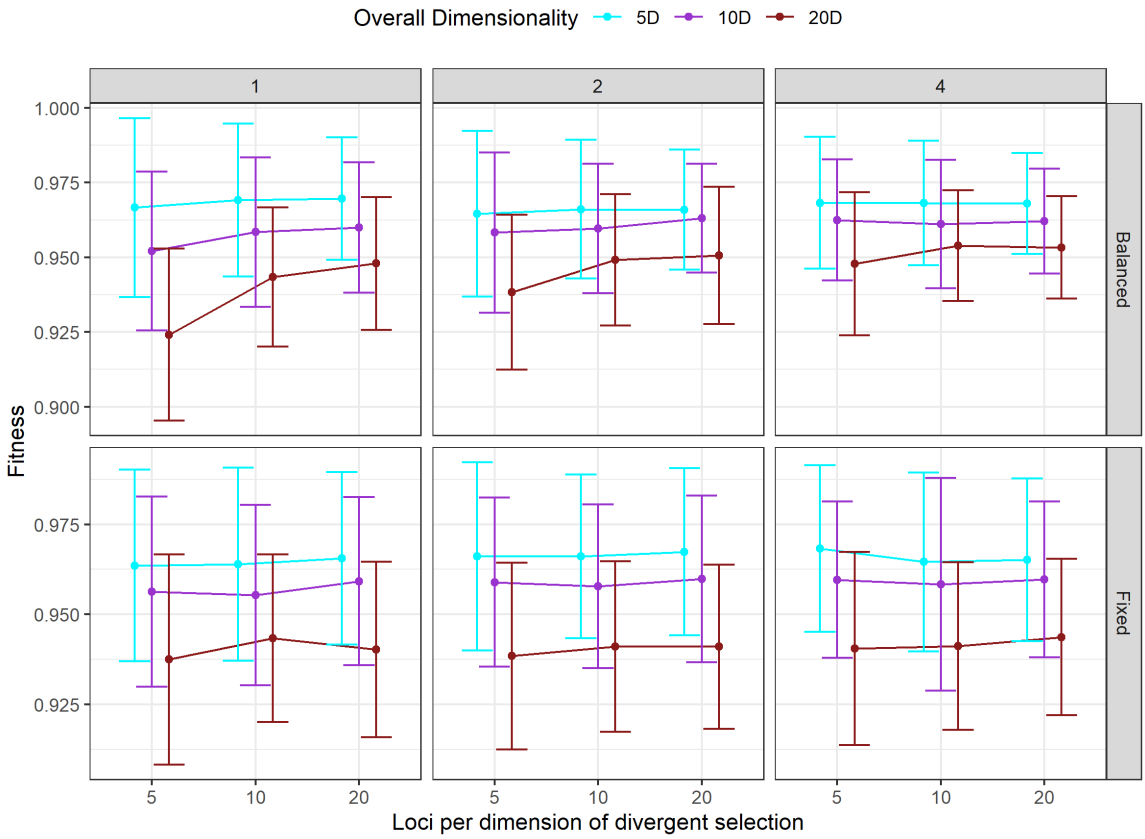

Figure 9: Fitness of F2 hybrids under variable dimensionalities of overall selection. Horizontal panels represent the dimensionality of divergent selection. Vertical panels display the mode of mutational variance. Points show mean values for F2 hybrid fitness ± 1 standard deviation.

adaptation was faster. This effect was not present in the balanced mode; the speed of local adaptation was unaffected by whether the composition of genetic variance was underlain by few large-effect loci or many weak-effect loci. However, under secondary contact or ongoing migration, the equilibrium level of local adaptation is less dependent on overall mutational variance. Under a fixed mode of mutational variance, local adaptation is consistently underpinned by few large-effect alleles which are closely aligned to the discriminant axis, whilst the remaining small/medium-effect loci, seemingly contribute little to adaptation overall. With more loci, adaptive optima can be reached more quicky and accurately due to increased resolution. Under a balanced mode of mutational variance, there is a trade-off between more loci generating more mutations, and the lower variance of mutational effects narrowing their distribution. With many loci but small mutational variance, large-effect alleles arise more rarely, therefore small-effect adaptive alleles accumulate and reduce the requirement for large-effect alleles to achieve high fitness, hence local adaptation is underlain by many small-effect. If phenotypes are highly polygenic, i.e. many loci per dimension, this result shows that increased divergence dimensionality can make local adaptation more vulnerable to homogenisation from gene flow.

This property of divergence dimensionality whereby a fixed quantity of selection is distributed over few vs many loci was previously discussed in Nosil et al. (2009)'s 'weak multifarious' hypothesis. As presented by Nosil *et al.*, at higher dimensionalities of divergent selection, selection does become diluted over many loci. However, this does not necessitate a shift towards divergence being maintained by many small-effect loci which may be vulnerable to the homogenising effects of gene flow. As seen in the fixed mode of mutational variance, and in other studies (Yeaman and Whitlock, 2011), the average allele size may decrease as the number of loci increases, but there remain enough large-effect alleles to sustain strong local adaptation. Therefore, this effect of higher divergence dimensionality is only likely to be manifest where mutational variation is low.

Our final result demonstrates a clear and quantifiable effect of overall (niche) dimensionality on the fitness of hybrids, with F2s significantly affected due to transgressive segregation. This effect is much weaker than the effect on fitness due to stronger divergent selection under additive multidimensionality. This is partly specific to this study and the trait optima values used here, but will still only strongly affect hybrids beyond the F1 stage. The transgressive incompatibility effect slightly develops our existing conclusions regarding how additive or dilution divergence dimensionality impacts extrinsic isolation. Fitness of F1 hybrids depends on the variation within populations, which in turn depends on the strength of selection. Fitness of F2 hybrids depends more on segregation of between-population differences, and therefore on the number and effect size of alleles responsible for local adaptation. The effect of overall dimensionality is strongest when local adaptation is underlain by few, strong-effect loci, as this produces the widest transgressive segregation (off-axis deviation) in F2 hybrids. If local adaptation is underlain by many small-effect alleles, hybrid fitness loss due to overall dimensionality is comparably small, compounding their vulnerability to homogenisation. Although we identify effects and interactions between the strength of divergent selection, the overall dimensionality of selection, and the number of quantitative loci, there remains no effect of divergence dimensionality *per se*.

Limitations

The major limitation to our simulations is that alleles are unconstrained with regard to dimensionality and hence act pleiotropically on all axes; mutations conferring adaptation along one axis can have

positive or negative fitness consequences for potentially all other axes. In reality, this universal pleiotropy is relatively uncommon, as seen in knock-down experiments (Nichols *et al.*, 2011; Wagner and Zhang, 2011), with the genotype-phenotype map instead split into modules whereby genes affect a subset of traits without global ramifications (Welch and Waxman, 2003; Wagner and Zhang, 2011; Kinsler, Geiler-Samerotte and Petrov, 2020; Yamaguchi and Otto, 2020). This universal pleiotropy may have affected the ease with which selection can align alleles with the discriminant axis, resulting in an unrealistically high proportion of large-effect alleles that provide adaptation in all dimensions. More restricted pleiotropy would require recombination between alleles that provide adaptation to individual dimensions of selection to produce well-adapted genotypes. Universal pleiotropy also impacts the interpretation of transgressive incompatibilities, as with lower pleiotropy the 'off-axis' variance is predicted to decrease (Nosil and Harmon, 2009).

A further limitation is that this study only considers populations which diverge in antiparallel directions. There is no possibility for an allele to be a 'generalist' allele and provide adaptation to both

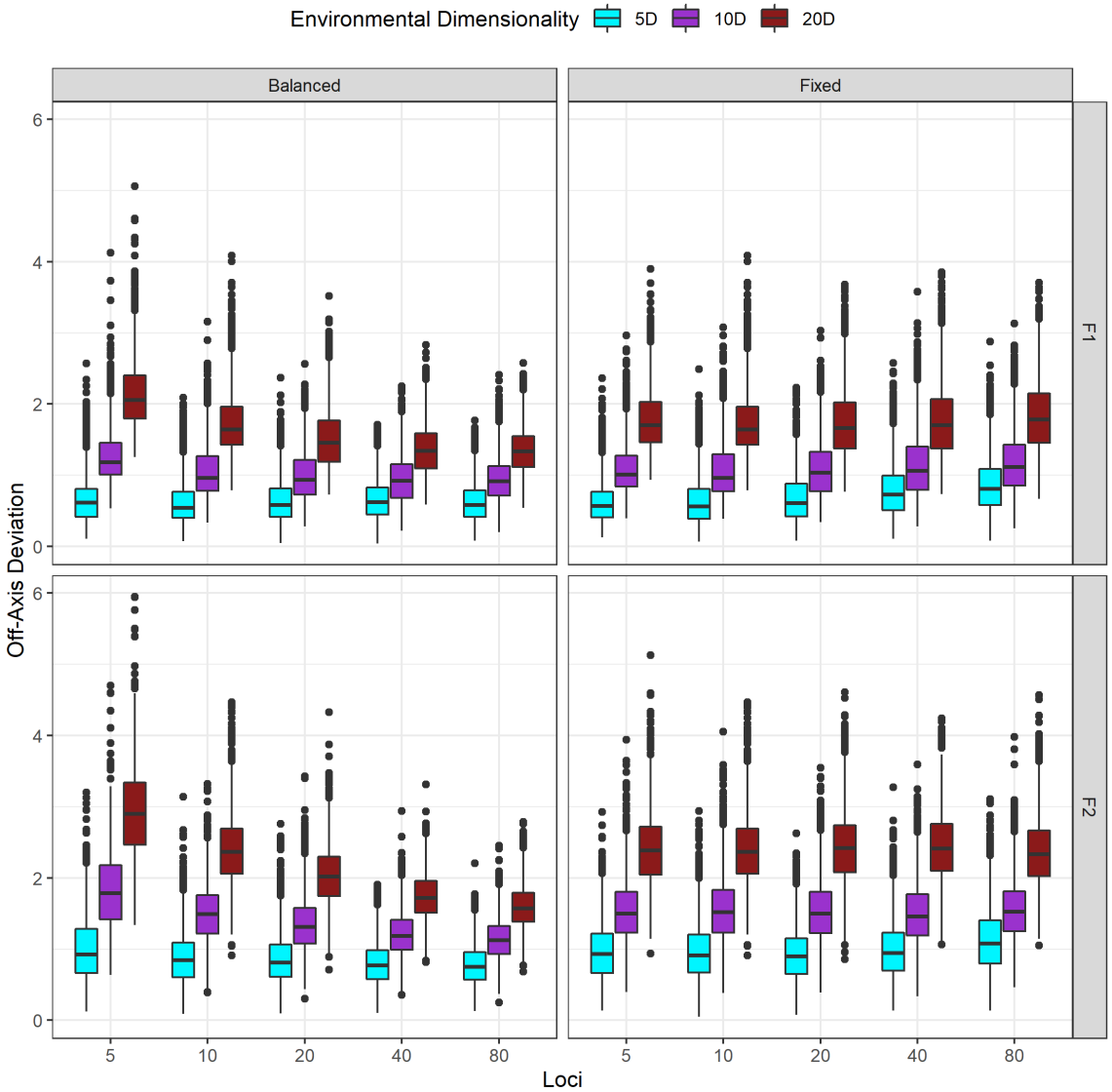

Figure 10: Off-axis deviation of F1 and F2 hybrids under a fixed or balanced mode of mutational variance. Loci number plotted along x axis regardless of divergence dimensionality.

environments. Other simulations studies have considered the effects of adaptive trajectories with different lengths and degrees of parallelism, and in general find that the magnitude of transgressive incompatibility increases with initial distance from the adaptive optima and angle of divergence (Chevin, Decorzent and Lenormand, 2014; Thompson, Osmond and Schluter, 2019).

Summary & Extensions

This is the first study to attempt to isolate any effects of dimensionality as a distinct parameter from the overall strength of selection (MacPherson, Hohenlohe and Nuismer, 2015; White and Butlin, 2021). Exactly how environmental variables interact to form a multidimensional fitness landscape remains both difficult to quantify or predict in natural populations. Future simulation work might consider how a spectrum of multistressor interactions ranging from antagonism to synergism might impact these reproductive barriers (D. Vinebrooke *et al.*, 2004; Galic *et al.*, 2018). Another extension of this study would be to consider more than two populations (e.g. MacPherson, Hohenlohe and Nuismer, 2015). As established here, any two-populations can only ever be separated by a single discriminant axis; divergent selection may involve multiple environmental variables or selection pressures, but theses merely constitute two points on a single axis in multidimensional space.

In natural populations, whereas multidimensional selection will likely elicit some response along the spectrum between additive and dilution modes in natural populations, there is scarce mechanism to explain how multidimensionality would affect the variance of individual mutational effects. Therefore, under the assumption that fixed mutational variance is a better model of evolution in nature, it is unlikely that the spreading of selection over more loci will make locally adaptive loci increasingly vulnerable to homogenisation. However, this remains an open question, and future studies may consider how effects beyond the scope of this study such as linkage and epistasis might impact the dimensionality of the genome and shape the genomic responses to multidimensional selection.

This study brings together and displays some of the key processes by which multidimensional divergent and overall selection can impact local adaptation and extrinsic isolation. We highlight two routes by which multidimensional divergent selection can act; stronger overall selection and more quantitative loci. Furthermore, we show a clear effect of increased overall dimensionality, and although there is no interaction with divergence dimensionality *per se*, we describe the interactions between overall dimensionality and these two routes. In exploring how dimensionality drives the evolution of reproductive barriers and progress towards ecological speciation, further exploration of genetic effects is likely to be a productive avenue for future research.

Chapter 4: The past and future of experimental speciation

Abstract

Speciation is the result of evolutionary processes that generate barriers to gene flow between populations, facilitating reproductive isolation. Speciation is typically studied via theoretical models and "snap-shot" tests in natural populations. Experimental speciation enables real-time direct tests of speciation theory and has been long-touted as a critical complement to other approaches. We argue that, despite its promise to elucidate the evolution of reproductive isolation, experimental speciation has been underutilised and lags behind other contributions to speciation research. We review recent experiments and outline a framework for how experimental speciation can be implemented to address current outstanding questions that are otherwise challenging to answer. Greater uptake of this approach is necessary to rapidly advance understanding of speciation.

Forward and reverse approaches to study speciation

The progression and outcome of speciation depend on interactions between evolutionary forces (Ravinet *et al.*, 2017) that act with varying importance over space and time to either facilitate or impede the evolution of reproductive isolation (RI; Abbott *et al.*, 2013). RI may arise through the action of genetic drift and/or divergent natural selection, may depend on gene flow via continuous migration or secondary contact, is impacted by population size and structure, and influenced by genomic properties such as mutation and recombination rates (Ortiz-Barrientos and James, 2017). Understanding the relative contributions of these processes to the evolution of RI is the focus of speciation research. A classic and highly successful approach to studying speciation involves identifying a phenotypically divergent trait and testing its association with the level of RI between extant populations (McKinnon *et al.*, 2004; Huber *et al.*, 2007; Le Pennec *et al.*, 2017). The increasing application of high-throughput genomic data to address speciation genomics questions (Box 1) is used to reconstruct population history (e.g. demography) and infer the evolutionary processes leading to speciation, often over a long timescale (Ortiz-Barrientos and James, 2017; Ravinet *et al.*, 2017; Wolf and Ellegren, 2017). This approach is analogous to the use of forward genetics to study the function of a gene, but applied to the study of RI. Here the study of speciation begins with a phenotype (RI) and proceeds to identify the potential evolutionary processes that caused RI to build up between diverged populations. Many studies support the success of this approach [see examples in 1,4–7]. However, this forward method of studying speciation is actually backward looking, reflecting a static snapshot of the processes that contributed to divergence. Realistically, signals of early barriers to gene flow are likely erased or over-written as speciation progresses. Thus, such studies are challenged to deduce the action of multiple evolutionary processes impacting phenotypic and genomic factors that influence speciation, either sequentially or simultaneously, either in the same or different directions, inferred over long evolutionary histories.

Laboratory experimental evolution (EE) experiments can address these challenges by manipulating evolutionary processes thought to generate RI over many generations and then testing the outcome on the evolution of RI. Experimental speciation (ES) is analogous to the use of reverse genetics to study

gene function. It begins with the putative evolutionary processes and proceeds to identify the conditions leading to and maintaining RI. This approach is experimental and therefore directly identifies the evolutionary processes and circumstances for the evolution of RI. ES complements snapshot studies (Table 1) but is also a standalone powerful approach because it reveals speciation processes in real-time. ES has been implemented for several decades and when its influential contribution was last reviewed, 10 years ago by Fry (2009), the technique seemed poised to exponentially accelerate understanding the evolution of RI. Fry also outlined neglected speciation questions that ES was well-suited to answer. Since Fry's review, speciation theory has advanced to incorporate more sophisticated ideas on genomic conditions and constraints impacting the evolution of RI. Snapshot studies have widely adopted a genetic approach to identifying signatures of RI. However, these conventional studies are vexed with inference problems, limiting understanding of speciation (Noor and Bennett, 2009; Cruickshank and Hahn, 2014; Westram and Ravinet, 2017). ES provides a potent method to test speciation theory by controlling and/or testing genomic factors and environmental conditions thought to influence speciation, factors that forward speciation approaches cannot disentangle (see section "A selection of new challenges that experimental speciation can address").

Here we review ES studies over the past decade to examine progress on Fry's original neglected speciation questions. We identify areas of speciation research that have progressed since that review, such as speciation-with-gene-flow models and genomic conditions impacting speciation, but which ES studies have not been applied. We provide a framework for using ES combined with genomics to enable rapid advances in understanding speciation.

Another decade of experimental speciation

Fry's review suggested ES could address: the relative efficacy of selection and drift in generating RI, the relative rates of evolution of different types of reproductive barriers, the feasibility of sympatric and parapatric speciation, and the feasibility of reinforcement (Fry, 2009). We summarize the relatively limited progress on these topics in the past decade, identify new areas in which ES has been employed, and argue that since Fry's review, two fundamental shifts in speciation theory and approach have occurred that have been ignored in an ES framework.

The relative efficacy of selection and drift

To maintain differences between populations, barriers to gene flow must emerge and generate RI. Barriers can act at the prezygotic (premating and postmating, prezygotic) and/or postzygotic stage, and can be influenced by extrinsic isolation and/or intrinsic isolation. Initial ES studies found relatively strong support for divergent natural selection generating RI in allopatry, even on arbitrary traits with no clear link to an isolating mechanism (Fry, 2009). However, under sympatric conditions, disruptive selection did not generally lead to RI, likely because many of the divergently selected traits had little relevance to fitness (Fry, 2009). Since Fry's review, few ES studies have altered conditions for local adaptation and then tested for the evolution of RI. Most studies tested the role of sexual selection and sexual conflict in generating RI (Parker and Partridge, 1998; Gavrilets, 2014). Fry found equivocal support for sexual selection generating RI (Fry, 2009). Subsequent work on sexual selection and speciation continues to fail to find significant RI (Gay *et al.*, 2009; Plesnar-Bielak *et al.*, 2013; Reding, Swaddle and Murphy, 2013; Debelle, Ritchie and Snook, 2016) even when manipulating genetic variation and population size to increase the likelihood of response (Gay *et al.*, 2009) and assessing

different RI barriers (Plesnar-Bielak *et al.*, 2013). One species, *Drosophila melanogaster*, has been tested independently in two laboratories but only one study found RI (Wigby and Chapman, 2006; Syed *et al.*, 2017). Theory suggests that different components of sexual selection may interfere with the evolution of RI (Ritchie, 2007) and one ES study supports this interpretation. In *D. pseudoobscura*, experimental sexual selection drove divergence in female choice for divergent male courtship traits (Debelle, Ritchie and Snook, 2014), which should generate assortative mating. However, males from the high sexual selection lines always outcompeted males from the enforced monogamy lines (Debelle, Ritchie and Snook, 2016). Overall, surprisingly, experimental sexual selection by itself does not seem to generate RI.

Box 1: Speciation Genomics

The reduced cost of genomics has expanded the ability to address outstanding questions in speciation (McKinnon *et al.*, 2004; Huber *et al.*, 2007; Falk *et al.*, 2012; Ravinet *et al.*, 2017). Of interest is how barrier loci are distributed across genomes and how they evolve during population divergence. Predicted genomic patterns are based on whether speciation proceeds between geographically separated populations without gene flow, or with gene flow occurring either during initial divergence or following secondary contact. In allopatry, divergence is not substantially constrained by the extent of genetic linkage and recombination relative to the strength of either selection or drift producing reproductive isolation (RI). In contrast, during speciation-with-gene-flow, selection for divergence is opposed by the processes of both gene flow and recombination that erode associations between genes under selection (Nosil and Feder, 2012). The genic view of speciation-with-gene-flow posits that speciation is initiated by selection acting against gene flow at specific targets of selection, and speciation genomics is interested in how barriers to gene flow initiate and facilitate (through the build-up of linkage disequilibrium) RI, including subsequent genomic divergence that is dependent on genomic architecture (Wu, 2001; Turner, Hahn and Nuzhdin, 2005; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009). Patterns of divergence are predicted to be different depending on whether gene flow is primary or secondary (Richards, Servedio and Martin, 2019).

Speciation genomics has begun to address these issues by identifying barrier loci evolving in response to selection or drift, their effect sizes, genomic distribution and associations, and how this builds up as RI increases, along with inferring demographic history and gene flow (Ellegren *et al.*, 2012; Nadeau *et al.*, 2012; Smadja *et al.*, 2012; Marques *et al.*, 2016). However, there are well-reviewed confounding factors influencing genome heterogeneity that are unrelated to speciation (e.g. population history, gene flow over time, variation in strength and timing of selection; Noor and Bennett, 2009; Cruickshank and Hahn, 2014; Wolf and Ellegren, 2017), and disentangling these factors remains challenging in studies of natural populations. Models of the rate, direction and magnitude of gene flow through time tend to rely on summary measures or comparing limited sets of hypothesised scenarios. Additionally, the impact of selection on divergence can sometimes be clearly identified (Jiggins, 2008; Le Rouzic *et al.*, 2011; Forbes *et al.*, 2017; Le Pennec *et al.*, 2017), but it is frequently challenging to characterise selection pressures- increasingly so the further selection is traced back through history. Thus, understanding the role of ecological differentiation, isolation and genomic differentiation in response to specific evolutionary processes is difficult to reconstruct (Langerhans, 2017). Alongside the development of models which can co-estimate demography and selection, the ability to directly observe these processes during experiments designed to track such interactions will provide powerful data to apply to natural systems where direct observation during the evolution of RI is unavailable.

ES studies have tested the impact of either natural or sexual selection, but evolution of RI may require both and so their relative contribution should be studied (Butlin *et al.*, 2012; Safran *et al.*, 2013). No ES study has done this, although one study manipulated natural selection and then tested for RI that could have arisen via sexual selection (Castillo *et al.*, 2015). Strong prezygotic RI was observed but it was independent of local adaptation. Additionally, no ES study has manipulated multiple axes of natural selection to test patterns of speciation under strong uni-dimensional vs multifarious selection, despite this being a long standing speciation question (Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009; see "How can selection overcome gene flow?").

Genetic drift may generate RI but Fry found little ES evidence (Fry, 2009). In the past 10 years, two further studies have manipulated population size to assess the contribution of drift. One study created 1000 bottlenecked, inbred "founder" populations of *D. yakuba*, and although weak RI was occasionally produced, extinction was overwhelmingly the most common outcome (Matute, 2013). Furthermore, when population size constraints were lifted (founder-flush), RI was diminished, suggesting that inbreeding effects, not drift alone, were responsible (Matute, 2013). Another study used a bottleneck treatment combined with divergent selection, but found it did not affect RI (Castillo *et al.*, 2015). Overall, ES studies indicate that drift is not a strong evolutionary force promoting speciation.

While generally studied separately, selection and drift interact in complex ways. Strong selection reduces effective population size, which can increase the role of drift. In turn, genetic drift may restrict genetic diversity, diminishing the effect of selection. Since Fry's review, one ES study has addressed the joint influence of selection and drift. Using an experimental niche shift to produce asymmetric strengths of selection and drift between ancestral and derived populations of the flour beetle, *Tribolium castaneum*, both premating and postzygotic RI evolved (Falk *et al.*, 2012). Due to strong selection and therefore reduced population size during the niche shift, RI likely arose via fixation of deleterious alleles as a consequence of drift. However, only one line of each of the ancestral and derived population was generated and we found no other similar studies, limiting understanding of joint selection and drift effects.

Evolution of different types of reproductive barriers

Previous ES studies focused on premating barriers using patterns of assortative mating to measure RI (Rice and Hostert, 1993). Although this remains true for ES studies post-Fry (Matute, 2010a; Matute, 2010b; Sharon *et al.*, 2010; Matute, 2013; Castillo *et al.*, 2015; Najarro *et al.*, 2015; Debelle, Ritchie and Snook, 2016; Comeault and Matute, 2018), some have included post-mating, prezygotic (Matute, 2010b; Comeault *et al.*, 2016; Syed *et al.*, 2017) and post-zygotic (Kwan and Rundle, 2010; Bérénos, Schmid-Hempel and Wegner, 2012; Ghosh and Joshi, 2012; Castillo *et al.*, 2015), forms of RI. However, more ES studies comparing the speed of evolution, the traits targeted, and relative magnitude of extrinsic and intrinsic RI are necessary to understand mechanisms by which RI evolves. Fry (Fry, 2009) suggested that ES has been underutilised to test the origin of Dobzhansky-Muller-incompatibilities (DMIs; Presgraves, 2010; Seehausen *et al.*, 2014). Some recent ES studies, where postzygotic RI has been identified, have employed analyses such as microarray-based mapping to identify candidate DMIs (Anderson *et al.*, 2010; Dettman, Anderson and Kohn, 2010; Ono, Gerstein and Otto, 2017). However, characterising DMIs and distinguishing these from signatures of extrinsic post-zygotic RI (e.g. low hybrid fitness in a given environment) requires additional experiments, including exploring the consequences of DMIs segregating within a population via synthetic engineering (Moreno, 2012).

Feasibility of speciation-with-gene-flow

Testing for speciation under sympatric and parapatric conditions was frequent in earlier ES studies (Rice and Hostert, 1993; Fry, 2009), and strongly contributed to understanding the importance of multiple-effect traits (Smadja and Butlin, 2011) in overcoming gene flow (Rice and Hostert, 1993; Fry, 2009). While early ES efforts showed conditions for speciation-with-gene-flow, Fry noted models of speciation-with-gene-flow as a "neglected area" (Fry, 2009). Over the past decade, a fundamental shift in speciation research is the acceptance that gene flow frequently occurs at some point before the completion of RI (Abbott *et al.*, 2013; Seehausen *et al.*, 2014) but ES studies incorporating varying levels of gene flow have not been published in the intervening years. Gene flow in the context of hybrid speciation has been tested recently using ES, expanding upon similar work in yeast species (Greig *et al.*, 2002). The number of hybridising *Drosophila* species, and their genetic divergence, affected RI between parental and hybrid lineages. Higher RI occurred when hybrids were derived from three, rather than two species, and when parental species had intermediate levels of divergence (Comeault and Matute, 2018).

Feasibility of reinforcement

Gene flow during cases of secondary contact after initial divergence in allopatry can generate reinforcement. While initially controversial, evidence for reinforcement has accumulated (Lemmon and Lemmon, 2010; Yukilevich, 2012; Smadja *et al.*, 2015). Previous ES reinforcement studies were "destroy all the hybrids" experiments (Fry, 2009) which removes all gene flow between populations and thus tested for increasing isolation between already reproductively isolated species. Post-Fry, Matute addressed this criticism and manipulated amounts of migration and hybridisation (and therefore effective gene flow) between sister species of *Drosophila* (Matute, 2010a; Matute, 2010b). He found premating and postmating prezygotic isolation increased but only when the numbers of migrants were low and selection against hybrids strong. Reinforcement between nascent species could also have indirect effects that generate RI between conspecific populations, known as cascading reinforcement. Using ES, conditions for cascading reinforcement were demonstrated in *Drosophila* (using a "destroy all the hybrids" approach Comeault *et al.*, 2016). Although these ES studies demonstrate that reinforcement can occur, the mechanism by which reinforcement is generated has yet to be explored; linkage of genes for local adaptation with those for assortative mating (Sætre and Sæther, 2010), or via multiple-effect traits conferring local adaptation and assortative mating through pleiotropy (Hopkins and Rausher, 2012). No study has examined the genomics of ES reinforcement, which could test how linkage disequilibrium is generated.

Coevolution

Antagonistic coevolution between species (e.g. hosts and parasites) can potentially drive RI (Marquis *et al.*, 2016) but Fry did not mention any ES study examining this process. Subsequently the use of EE for testing coevolution has been emphasised, but outside of the speciation context (Schulte *et al.*, 2010). We identified one ES study that found higher postmating RI between *T. castaneum* populations that had co-evolved with the parasite *Nosema whitei* than between the non-parasitised controls (Bérénos, Schmid-Hempel and Wegner, 2012). Another ES study tested populations of *D. melanogaster* adapting to different diets in the presence of commensal organisms that may generate RI, and found premating isolation evolved in as little as one generation (Sharon *et al.*, 2010; Najarro *et al.*, 2015). RI was attributed to the mere presence of different microbiota and did not vary

significantly over time, thus it is difficult to conclude these effects were evolutionary, rather than plastic. Attempts to replicate these results have been mixed (Markov *et al.*, 2016; Belkina *et al.*, 2018). Overall, despite coevolution being a potential powerful driver of speciation, ES studies have not tested this.

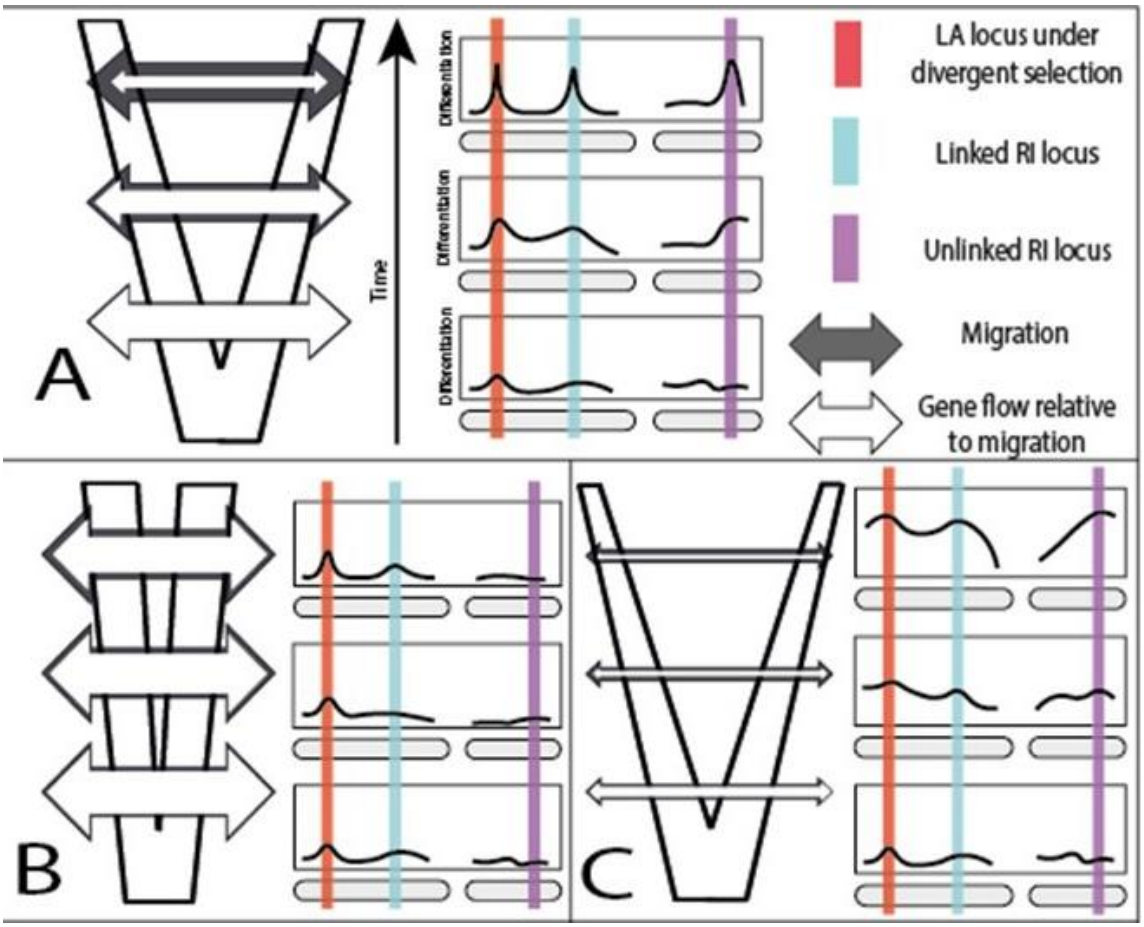

Figure I: Illustrations of divergence and genome scans. Adapted, with permission, from (Ravinet *et al.*, 2017)

Box 2: The importance of gene flow in experimental speciation genomics.

As barrier loci can only be detected when populations are or have recently been exchanging genes (Ravinet *et al.*, 2017), the degree of gene flow between diverging populations in an experimental speciation study using evolve and re-sequence is crucial for genomic analysis. Without gene flow (divergence in allopatry), soft sweeps are predicted to produce large blocks of genomic differentiation around differentially selected alleles. This makes barrier loci hard to pinpoint, a problem which is likely to be particularly pronounced since experimental speciation studies must often use much stronger selection than would be found in nature to generate reproductive isolation within the experimental timeframe. Furthermore, experimental populations are more susceptible to the effects of drift due to their typically small population size. Without gene flow, large genomic regions may drift to differentiation.

(Box 2 continued) As such, gene flow is necessary to detect barrier loci, as it homogenises background genomes, counteracts the effects of selective sweeps and drift, and allows regions of differentiation to be identified. However, too much gene flow will swamp selection and obstruct population divergence. Guidelines on the design of E&R studies focus heavily detecting signatures of selection in allopatric populations (Baldwin-Brown, Long and Thornton, 2014; Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015). When designing future E&R speciation experiments, it will be important to consider these in the context of gene flow, distinguishing the detection of regions under selection from that of barrier regions. Figure I presents three hypothetical illustrations of the consequences stemming from different levels of gene flow, as a guide to considering the consequences of experimental population size and migration levels. In Panel A, populations have diverged, gene flow relative to migration has decreased substantially with time, and the genomic signatures of all 3 barrier loci are clear, allowing identification of markers and further investigation. In Panel B, gene flow is problematically high, selection struggles to overcome gene flow and there is little phenotypic or genomic divergence. In panel C, populations with low levels of gene flow have diverged in near-allopatry, but the identification of barrier loci is difficult because populations have lost genetic variation and the background genomes are strongly differentiated due to drift and linkage.

That was then, this is now

ES continues to be underutilised even after Fry's promotion of its use. We provide ideas for future research drawing on his suggestions. Perhaps more importantly, since Fry's review, two major developments in speciation research have occurred for which ES is highly suited but for which ES has lagged behind. First, speciation-with-gene-flow is now thought to be a dominant mode of speciation, but ES studies have manipulated gene flow in only very specific conditions: hybrid speciation and reinforcement. Second, Fry's review (Fry, 2009) was published on the cusp of the genomic revolution. Subsequent EE studies addressing other evolutionary problems have adopted genome sequencing, including evolve and re-sequence (E&R; Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015) which allows tracking of genetic changes during evolution, revolutionising EE studies (Bruger and Marx, 2018). However, we found surprisingly few new ES studies testing for RI and none incorporated tests of speciation theory using genomics. Given the importance of gene flow during speciation, ES design should include this, as expanded upon in Boxes 2 and 3, and genomic approaches must be used to test fundamental and increasingly sophisticated speciation genomics theory (Box 1). This combination will dramatically increase the ability to directly test how RI is either initiated between individuals within a population or intensified between partially reproductively isolated populations and help fulfil the promise of ES as a powerful approach to understanding speciation. To facilitate this aim, we highlight how ES combined with genomics can address speciation research developments in the past 10 years. Our list, below, is not exhaustive but is designed to inspire and stimulate ES speciation research.

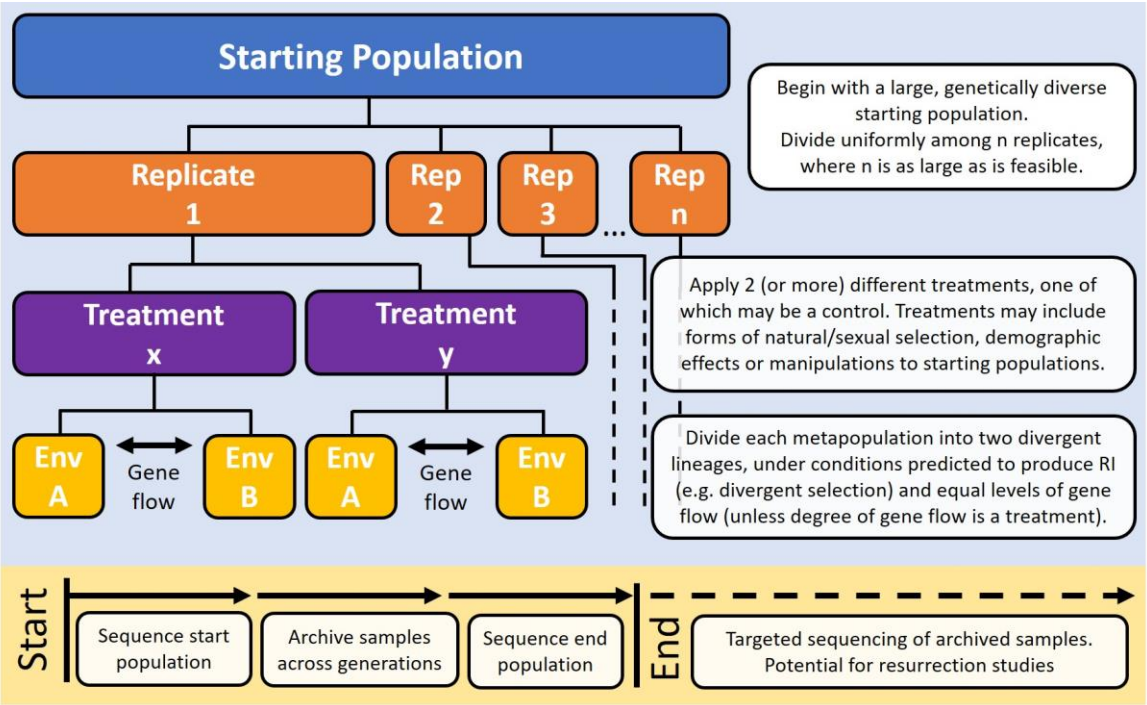

Figure II: A blueprint for ES experimental setup

Box 3: A blueprint for Experimental Speciation design.

Experimental speciation (ES), in combination with genomics, provides the ability to jointly infer phenotypic responses to, and genomic signals of, selection, and should be a high priority for speciation research. We present a 'blueprint' for the design of future ES studies investigating the impact of a process or condition on the evolution of reproductive isolation (RI) in the face of gene flow, Figure II. We particularly focus on gene flow and selection manipulations, and the use of evolve and re-sequence (E&R). In this design, the pair of populations serves as the unit of replication; all measures of divergence (e.g. RI, F_{ST}) describe the paired metapopulation. This differs from designs in which experimental lines radiate from a single ancestral population, which typically involve no gene flow. Demography and migration rate, and the strength of natural and/or sexual selection can be controlled or manipulated. Subsequent consequences on the initiation or elevation of RI can be estimated directly and assessed across different types of reproductive barriers. The time course nature of ES allows both phenotypes that contribute to local adaptation (Blanquart *et al.*, 2013), assortative mating (Belkina *et al.*, 2018) or hybrid viability (Kwan and Rundle, 2010; Ghosh and Joshi, 2012; Castillo *et al.*, 2015) to be assayed from the outset. By employing E&R, effective gene flow and consequences for genomic architecture can be determined.

By archiving populations throughout the experiment, a researcher can build a valuable cache of DNA data that can be analysed post-E&R with "evolutionary hindsight". Having identified candidate barrier loci, the trajectory of allele frequencies of these selected loci can then be examined in detail across the course of experiment by targeted sequencing of archived populations at selected time points.

(Box 3 continued) This can pinpoint how and when changes relating to RI arise and spread in populations. E&R is a potent way to identify genetic signatures of RI but the power to detect these signatures is affected by demography (population size and number of founding haplotypes), strength of selection, and number of replicate populations (as is the success of ES generally; Kawecki *et al.*, 2012; Baldwin-Brown, Long and Thornton, 2014; Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015). While these constraints need to be kept in mind, so should the limitations of detecting signatures of selection in non-ES speciation studies (Noor and Bennett, 2009; Cruickshank and Hahn, 2014; Westram and Ravinet, 2017).

Furthermore, if individuals can be "resurrected" (e.g. yeast, rotifers, Daphnia), a suite of genomic, metabolomic, transcriptomic or fitness-related assays could be performed post-EE at time-points of interest. Replication within each treatment tests for parallel evolution and identifies strong (consistent) candidate barrier loci arising due to selection. Replicates responding similarly allows distinguishing a selective response from other evolutionary processes such as mutation and drift, the latter of which are predicted to affect replicates differently.

A selection of new challenges that experimental speciation can address

What genomic conditions promote speciation?

Variation in mutation rate, recombination rate, and gene density, are all predicted to impact progression towards RI (Ortiz-Barrientos and James, 2017; Ravinet *et al.*, 2017; Westram and Ravinet, 2017). These genome properties can only be assessed *post hoc* in natural populations, making it difficult to disentangle current genome properties as causes or consequences of the speciation process. For instance, suppressed recombination among genes inside chromosomal inversions can generate the linkage disequilibrium required for promoting divergence and speciation. In many species, inversions have been found containing genes important for speciation. However, in natural populations it is difficult to infer whether an ancestral inversion containing barrier loci facilitated speciation or arose after several loci were already in linkage disequilibrium. Furthermore, these properties can shape the genomic landscape independently of the evolution of RI, complicating the identification of barrier loci (Ravinet *et al.*, 2017; Wolf and Ellegren, 2017). In an ES context, these genomic features can be characterised prior to applying EE and their behaviour tracked across time via E&R. Moreover, manipulating genomic properties of starting populations is possible, allowing direct tests of their effects on the evolution of RI in the absence of confounding differences.

Taking recombination rate as an example, low recombination increases linkage around a barrier locus. Clusters of barrier loci are more likely to evolve in low recombination regions, potentially but not necessarily producing coupling (Butlin and Smadja, 2018). Reduced recombination regions could therefore evolve because they enhance clustering (Yeaman, 2013). For example, inversions that reduce recombination between barrier loci are expected to be promoted by divergent selection in the face of gene flow (Faria *et al.*, 2019). Conversely, high recombination can counteract the Hill-Robertson effect, increasing the likelihood of bringing together otherwise competing beneficial alleles in a single individual. So high recombination might speed up local adaptation and divergence during speciation, but could also slow the build-up of RI by uncoupling barrier loci in the genome. The overall effect of recombination rate on RI could be examined by experimentally evolving populations with different patterns of genome-wide recombination rates, known to vary between populations (Dumont and Payseur, 2011; Kawakami *et al.*, 2014), using genetic mapping to show the differences between

populations. If a facultatively sexually reproducing organism is used, then manipulations in recombination rate could be achieved by varying the proportion of time during selection spent in the asexual and sexual phases (Becks and Agrawal, 2012). Alternatively, artificially created inversions via CRISPR/Cas9 (Zhang *et al.*, 2017) might be propagated within a population to explore their effects. We use recombination rate as an example, but these approaches could be applied similarly to genomic features such as mutation rate, gene density or genetic diversity.

How does gene flow impact speciation?

Gene flow is thought to be involved in most cases of speciation at some point before completion of RI (Seehausen *et al.*, 2014). However, its role in both opposing and facilitating speciation is theoretically complex. Gene flow has similar consequences to speciation as recombination. Gene flow opposes divergence under selection, but also makes recombination possible between gene combinations in diverging populations. The latter can promote local adaptation and potentially rescue diverging populations with small founding sizes (Frankham, 2015). Gene flow also impacts the landscape of genomic divergence. In the presence of gene flow and recombination, strength of selection and linkage are expected to influence the establishment of barrier loci, and are predicted to lead to clustered genetic architecture (Rafajlović *et al.*, 2016). In natural populations, correctly inferring gene flow is challenging given uncertainty about demographic history. For instance, modern-day genomic patterns may be due to past gene flow, varying recombination rates and/or bottlenecks (Cruickshank and Hahn, 2014). In contrast, using ES allows gene flow to be either controlled or manipulated throughout an experiment and this can be confirmed directly via sequencing. Gene flow can be manipulated, singly or in combination with other factors of interest, to test conditions under which speciation-with-gene-flow is feasible. Moreover, the phenotypic and genomic patterns produced are directly determined and can then be applied to understanding these patterns in natural systems.

Experiments manipulating the amount of gene flow, with and without recombination, can be done by varying the proportion of migrants between diverging populations at the start of each generation. This would allow testing predictions about how gene flow might oppose RI but facilitate local adaptation, and about the predicted clustering of loci within the genome. For instance, Fry emphasised speciation-with-gene-flow in certain conditions (e.g. finite stepping stone; Felsenstein, 1975; Slatkin and Maruyama, 1975; or Bush's sympatric speciation model; Bush, 1975) which have not yet been tested. This basic setup could be expanded to include how sexual selection impacts speciation-with-gene-flow, to test how it may either enhance or impede the evolution of RI depending on factors such as geography, and mechanisms of assortative mating (Servedio, 2016).

ES is probably best placed to examine the role of gene flow early in speciation. However, it could also be used to test two hypotheses for more divergent populations: reinforcement and the Genome Wide Congealing hypothesis (Feder *et al.*, 2014). ES has demonstrated reinforcement but how linkage disequilibrium is generated to promote reinforcement remains unresolved. Sequencing starting populations, identifying markers for barrier loci, and then employing targeted sequencing of the markers on archived ES samples allows reconstruction of the genomic architecture of populations as reinforcement occurs, testing mechanisms of linkage. This approach also addresses the importance of tight linkage between loci and the likelihood of speciation depending on the basis of assortative mating (Kopp *et al.*, 2017). Speciation-with-gene-flow is theorised to be more feasible when assortment results from matching traits, whereas assortative mating arising from preference/trait

mechanisms requires maintenance of linkage disequilibrium between a larger set of loci, thereby decreasing its likelihood in the face of gene flow.

The Genome Wide Congealing hypothesis posits a tipping point of linkage disequilibrium and adaptive divergence. Crossing this threshold transitions from a number of weakly selected barrier loci accumulating between diverging populations, to RI at specific genes, to a switch of RI across the whole genome (Feder *et al.*, 2014). Whether this threshold exists, and at what point during speciation this theoretical tipping point is reached, depends on how many loci are targets of selection, how strong selection on each locus is, and the genome-wide recombination landscape. ES could empirically test the impact of these factors by taking divergently adapted but not very isolated populations and then manipulating conditions and/or genome properties to test for a tipping point from weak to strong RI.

How can selection overcome gene flow?

Fry reviewed ES studies testing whether selection on multiple-effect traits could overcome gene flow to generate RI (Rice and Salt, 1988, 1990; Fry, 2009). However, many other facets of selection remain unexplored which, while being relatively minor in allopatry, can have major consequences in the presence of gene flow. One example is the dimensionality of selection- how are the components of RI affected by whether a finite quantity of selection is spread over many, or concentrated onto few, traits and/or loci? To what extent is speciation promoted when selection is strong on a single trait compared to multifarious selection? Strong divergent selection, concentrated on a single trait, may overcome gene flow more successfully, leading to greater and more rapid local adaptation, but with lower effects overall on RI and genomic differentiation. In contrast, multifarious selection may accelerate the build-up of RI (Fry, 2009; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009) by impacting linked barrier loci, impacting multiple-effect traits, or producing a snowball effect (Presgraves, 2010) of DMIs (Orr, 1995; Matute *et al.*, 2010; Moyle and Nakazato, 2010; Wang, White and Payseur, 2015). If selection is spread too thinly across many dimensions, however, then it may fail to overcome gene flow (Sousa *et al.*, 2013). Amount of gene flow is also critical in whether uni- vs multi-dimensional selection facilitates complete speciation (Nosil, Harmon and Seehausen, 2009). No ES study has addressed this speciation theory. While not suggested in an ES framework, Figure 3 of Nosil *et al* (Nosil, Harmon and Seehausen, 2009) provides an excellent guide for ES researchers for testing the contribution of uni- and multi-dimensional selection on the evolution of local adaptation and RI.

Concluding remarks

Despite early ES success, the approach has lain relatively fallow in addressing unresolved speciation questions. This is particularly true when incorporating genomic techniques. It is the combination of ES with high-throughput genomics that can provide a step-change in understanding the origin of RI by directly testing competing hypotheses on processes suggested to impact speciation. Such tests are challenging in natural populations. While ES is typically used to reveal the evolution of early RI, its use on partially reproductively isolated taxa can test how existing patterns of RI and the underlying genomic architecture impacts progression to more complete speciation. ES combined with E&R can both disentangle and test confounding demographic and genetic processes, and elucidate the conditions under which speciation is impeded or accelerated. As it is these signals that get erased or over-written during the speciation process in natural populations, such experimental insights can be used to help interpret patterns of divergence in natural populations whose selection and demographic history are unknown. In this way, ES, while perhaps over-simplifying real-world conditions, is a

powerful tool complementing forward (static) speciation studies. As ES studies accumulate, questions about the role of certain types of genes and other types of phenotypic variation, such as gene expression, in speciation can be addressed. All experiments risk failure but given how time-consuming ES is, researchers may be hesitant to adopt this approach for fear RI will not be generated. Rare events can still be very important (Templeton, 2008) so modelling approaches enabling the testing of many more variables over many more replicates than feasible experimentally would be a helpful complement to ES. Furthermore, an additional benefit of taking on the ES challenge is that, even if RI does not evolve, the approach can address other fundamental questions (e.g., how gene flow and recombination impacts the genomic architecture of local adaptation), themselves outstanding evolutionary problems. Unlike our update of ES in the past decade, we anticipate that the next decadal ES review will attest to the power of this approach and its application in interpreting divergence in natural populations.

Table 1: ES and studies using natural populations are highly complementary. Several advantages (bold) and limitations (italicised, shaded) of each approach have been matched to illustrate their complementary nature

Laboratory-based ES	Comparative methods with natural populations
Rare (but important) serendipitous events are likely to be missed unless the experiment is very large	**Better represents the importance of a given process, rather than just its occurrence**
Starting population characteristics and genome are defined or quantified a priori by the researcher	*In most cases, it is challenging to reconstruct ancestral populations and their genomes*
Typically reliant upon standing variation alone	**Greater potential for *de novo* mutation or introgression from other populations to play a role**
Environment is controlled and can be kept constant or manipulated in a controlled manner, throughout	*Often difficult to determine ancestral environment required to delineate the role of geography in restricting gene flow*
Many initial effects may be due to lab adaptation. If lab adaptation has occurred pre-EE, genetic diversity will be lower	**Populations are typically close to equilibrium in the wild**
Evolutionary responses are replicated over a series of lines to robustly link conditions to responses	*No true replication. Lack of parallelism may create uncertainty that a phenotypic change is a direct response to a given variable*
Evolution of traits is limited to what can be performed in culture conditions. Low niche dimensionality means only simple contrasts can be made	**A much wider range of traits can be selected upon or arise**
Gene flow can be more accurately and reliably determined from highly controlled migration levels, and measures of local adaptation & RI	*Difficult to determine level of ongoing gene flow*
Limited to a subset of organisms suitable for EE	**Can study any diverging populations**
Easy to separate intrinsic and extrinsic forms of RI	*Difficult to disentangle intrinsic from extrinsic RI*
Laboratory settings may exclude many of the ecological aspects that separate species	**Can assess the full range of isolating mechanisms found in the wild**
Phenotypic and genomic data can be collected with high temporal resolution providing estimates of phenotypic change and evolutionary hindsight of underlying genomic changes	*Even if ancestral genomes can be reconstructed, phenotype data is typically only a single 'snap-shot', so cannot be matched to genomic data*
Experiments can only cover short timescales and subsets of the speciation process	**Very long timescales of divergence can be studied (although histories must be inferred)**

Chapter 5: Experimental evolution of local adaptation under unidimensional and multidimensional selection

Abstract

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.

Introduction

When faced with selection pressures that vary spatially, populations may adapt to local conditions (Kawecki and Ebert, 2004). 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 (Savolainen, Lascoux and Merilä, 2013). 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 (Rundle and Nosil, 2005; Nosil, 2012). 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 (Tigano and Friesen, 2016; Tusso *et al.*, 2021), and genomic conditions such as the amount and distribution of standing genetic variation (Barrett and Schluter, 2008; Lai *et al.*, 2019) and the genomic architecture (Yeaman, 2013; Morales *et al.*, 2019).

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 (Rice and Hostert, 1993; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009). 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 (Rice and Hostert, 1993; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009; Butlin *et al.*, 2012). However, to date, no study has tested how local adaptation builds under different dimensionalities of selection (White, Snook and Eyres, 2020).

It is important to distinguish the dimensionality of divergent selection from the overall dimensionality of selection (sometimes referred to as 'niche dimensionality'; Hutchinson, 1957; Nosil, 2008a; Chevin, Decorzent and Lenormand, 2014; Ingram, Costa-Pereira and Araújo, 2018). Overall dimensionality reflects the total number of selection pressures impacting a population, many of which will act via stabilising selection (Roughgarden, 1972; Pfrender, 2012; Chevin, Decorzent and Lenormand, 2014; Ingram, Costa-Pereira and Araújo, 2018). 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 variable, 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 (Kassen, 2002; Gray and Goddard, 2012).

However, the dimensionality of divergent selection considers only selection pressures which vary between environments. Using a framework such as Fisher's Geometric Model (Fisher, 1930), 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 (Arnold *et al.*, 2008; MacPherson, Hohenlohe and Nuismer, 2015; White and Butlin, 2021).

Predictions of how dimensionality might impact local adaptation and speciation, such as Nosil *et al*.'s (2009) '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 (White and Butlin, 2021). 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 (Nosil and Harmon, 2009; MacPherson, Hohenlohe and Nuismer, 2015). Moreover, more selection pressures might produce divergence in more orthogonal traits (Steppan, Phillips and Houle, 2002; Arnold *et al.*, 2008). 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 (Barrett and Schluter, 2008; Flaxman *et al.*, 2014).

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 (White and Butlin, 2021). 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 (Yeaman, 2015). Greater total genetic variance, due to increased mutational input, may allow faster and stronger local adaptation (Yeaman, 2015; Höllinger, Pennings and Hermisson, 2019). 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 (Nosil, Harmon and Seehausen, 2009). 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; Comeron, Williford and Kliman, 2008) 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 (Jiggins, 2008; Le Rouzic *et al.*, 2011; Forbes *et al.*, 2017; Le Pennec *et al.*, 2017). 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 (Nosil and Sandoval, 2008; Egea-serrano *et al.*, 2014; Aguirre-Liguori *et al.*, 2019). Comparison of these studies indicates that multidimensionality is associated with stronger local adaptation. A meta-analysis of 35 reciprocal transplant studies (MacPherson, Hohenlohe and Nuismer, 2015) identified that the dimensionality of environmental divergence (maximum likelihood estimate from available data, as defined in Hohenlohe and Arnold 2010) accounted for a larger proportion of variance in local adaptation (20%) than environmental variables alone (4%; Hereford, 2009).

Laboratory experiments have been proposed as one of the most effective methods available to assess the impact of dimensionality on local adaptation (Kawecki and Ebert, 2004). Using experimental evolution, environmental selection pressures and levels of ongoing gene flow can be manipulated and controlled (Fry, 2009). However, a review identified no single experimental evolution study that has varied the dimensionality of selection (White, Snook and Eyres, 2020). Although comparisons between experiments have been made (Rice and Hostert, 1993; Nosil and Harmon, 2009), only 5 studies have imposed divergent selection along more than one axis (Kilias, Alahiotis and Pelecanos, 1980; Rice, 1985; Rice and Salt, 1988, 1990; Rundle, 2003). Although three of these produced strong reproductive isolation, it has been argued that this was due to selection on multiple-effect traits (traits that underlie more than one component of reproductive isolation; Smadja and Butlin, 2011) rather than multidimensional divergent selection (Fry, 2009; White and Butlin, 2021).

In this study, we used experimental evolution to test how the dimensionality of divergent environmental selection affected the speed and magnitude of evolution of local adaptation. We exposed populations of the monogonont rotifer, *Brachionus plicatilis*, to unidimensional and multidimensional divergent selection pressures whilst keeping total selection equal (Figure 1). Populations were paired and connected via gene flow to form metapopulations with 'home' and 'away' environments for each subpopulation. We tested the strength of local adaptation in each metapopulation at regular intervals over the course of the experiment, defined by differences in fitness when exposed to home vs. away environments (Kawecki and Ebert, 2004; Blanquart *et al.*, 2013). Our planned analyses sought to answer three questions: 1) do the speed and magnitude of local adaptation vary by dimensionality, 2) if so, are these patterns specific to dimensionality, or could

they be explained by the individual stressors used, and 3) what underlying patterns of fitness in the home and away environments are responsible for changes in local adaptation?

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

Materials and Methods

Source and maintenance of rotifer populations

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 (Wallace, Snell and Smith, 2015). 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 (Fontaneto and De Smet, 2015). *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 (Mills *et al.*, 2017), sequencing of mitochondrial cytochrome c oxidase subunit 1 (CO1) was performed to confirm species identity as *B. plicatilis sensu stricto* (Gomez *et al.*, 2002). 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 (2018). 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.

Unidimensional vs multidimensional divergent selection

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, the addition of HCl to form an acidic shock media was sufficiently small as to not impact salinity to the degree that fitness would be affected.

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

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

At day 7 (24 hours post-shock), population density was measured, and demes were passaged to form new cultures. Following passage, exactly 24 of the approximately 2,400 rotifers were reciprocally transferred between demes (1% migration). Migration did not use filtration; hence the microbiota was also shared between demes. A 1% migration rate was selected based on experimental evolution guidance (Baldwin-Brown, Long and Thornton, 2014; Kofler and Schlötterer, 2014) to achieve homogenisation of neutral genomic regions ($Nm > 1$) without impeding response to moderate selection pressures per locus.

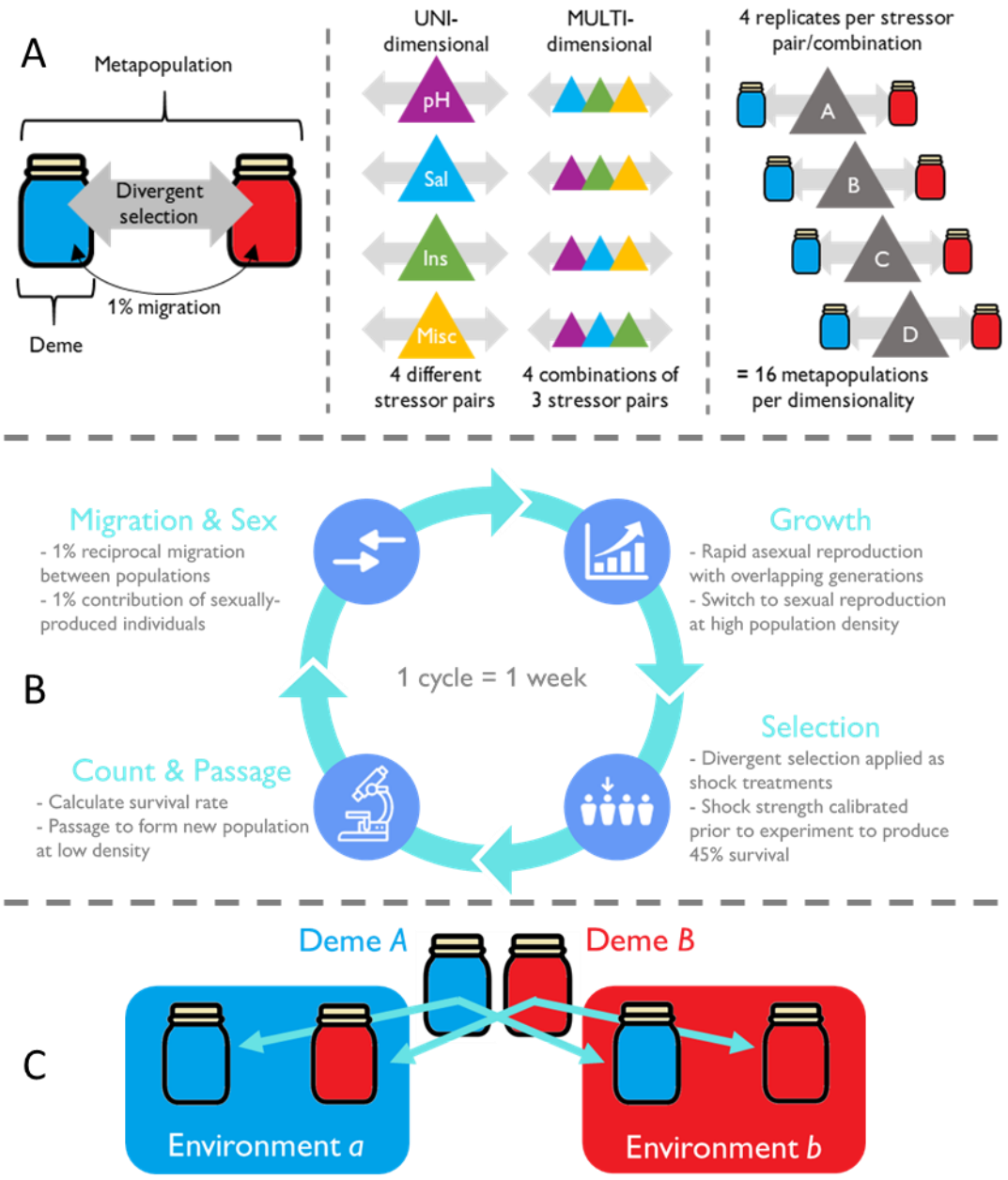

Figure 1: Experimental design.

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.

With this passaging routine, there is no contribution from sexual reproduction. To include the effects of recombination arising from sexual reproduction, the remaining non-passaged culture was retained for three more days to allow for additional sexual reproduction. On day 10, diapausing embryos were collected from culture sediment and incubated in 1.2ml 50 g/L artificial seawater at 4°C in the dark for

a 2-week dormancy period, after which they were hatched via incubation in 6 g/L artificial seawater at 25°C under constant illumination. 24 hatchlings per deme were transferred to tubes containing 30ml 12g/L seawater for a further 4 days, then added to the experimental demes coinciding with weekly passage, to form a 1% sexually produced contribution of new clonal genotypes.

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 multidimensional 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.

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 (Blanquart *et al.*, 2013). 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 ('R: A language and environment for statistical computing', 2020). 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.

1. 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 (Fox and Weisberg, 2019). 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.

2. 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 (Lenth, 2021). 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.

Finally, for completeness, we repeated this statistical analysis using a 'local-foreign' (LF) measure of local adaptation and checked for any asymmetries between deme-level LF values which may reflect asymmetric gene flow (Supplementary Information).

3. What components of home vs away fitness are responsible for any observed differences?

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

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: 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.

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 = 3.04×10^{-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.

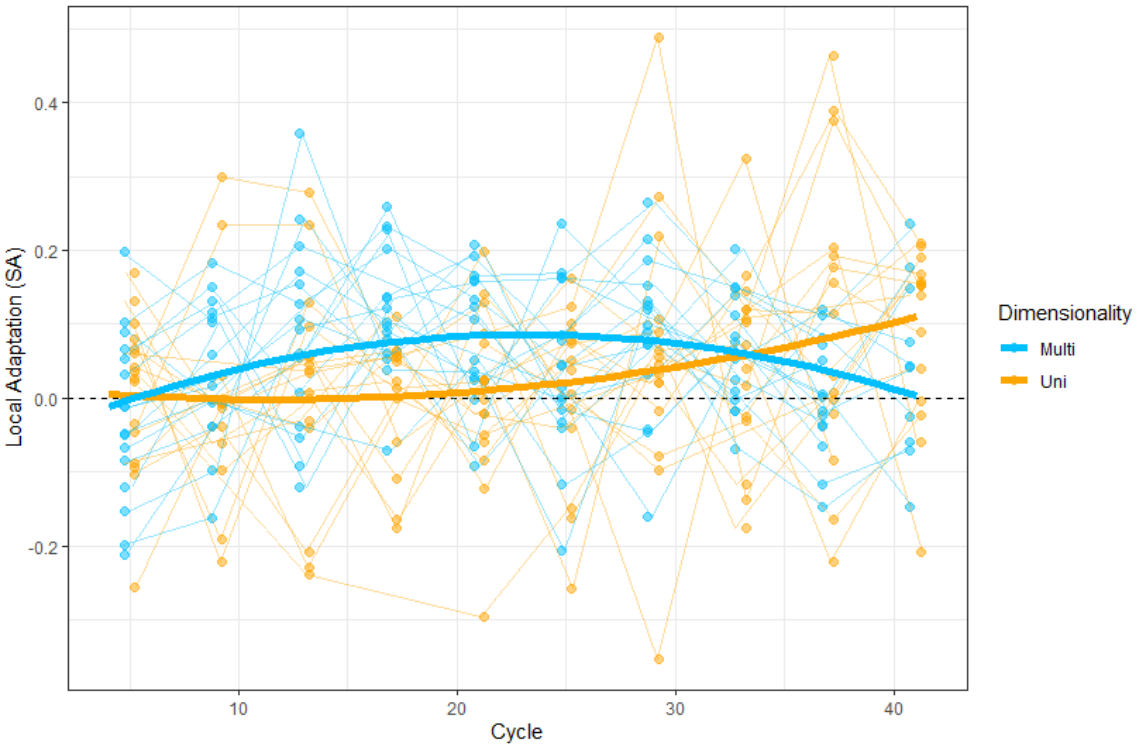

Figure 2: Local adaptation under experimental divergent selection with gene flow.

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.

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).

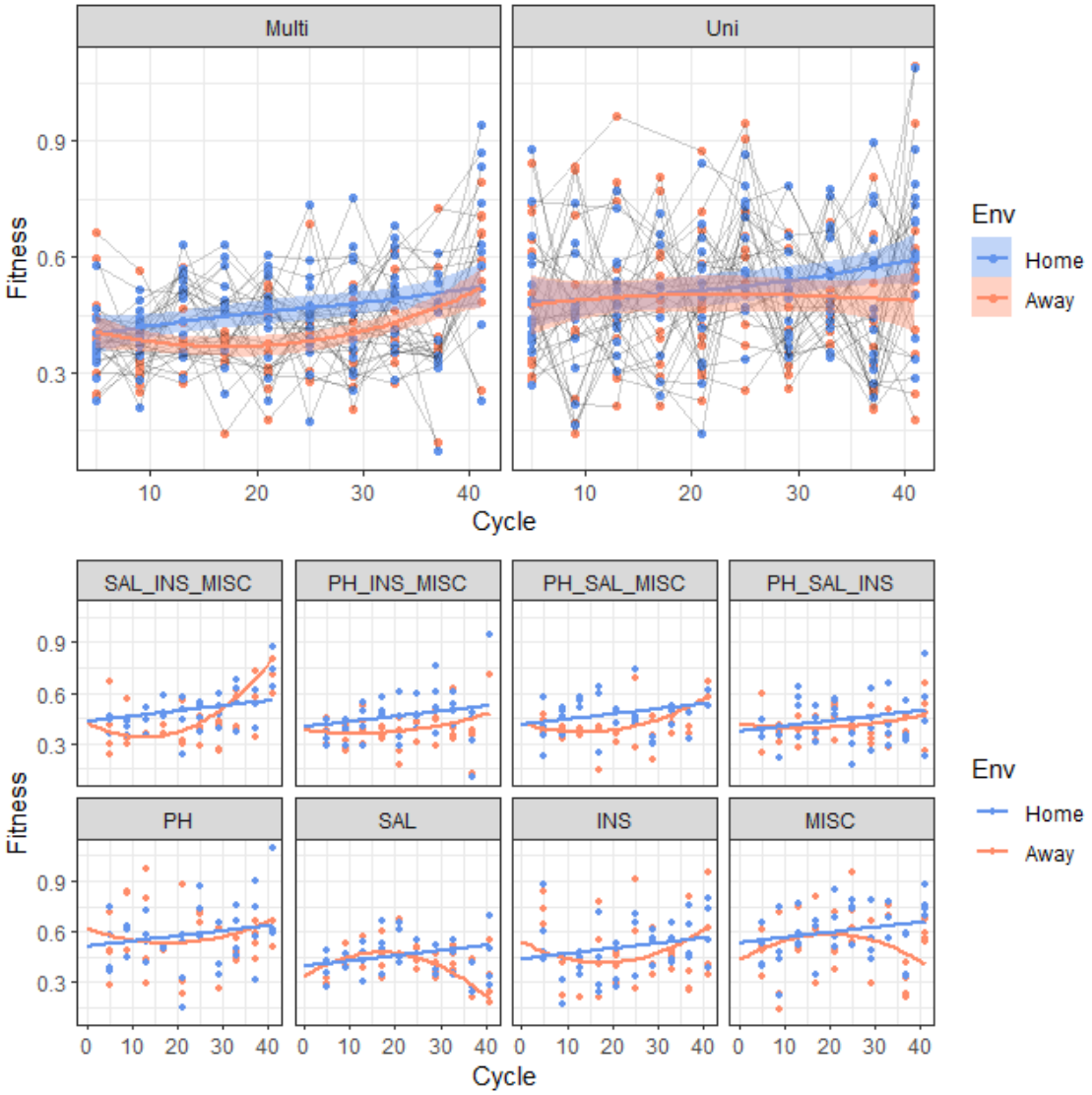

Figure 3: Home vs away fitness of metapopulations.

Top panels: 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.

Bottom panels: 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.

Asymmetric adaptation within metapopulations did not impact the observed patterns (see Supplementary Information; 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, through 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).

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 (Fry, 2009; White, Snook and Eyres, 2020; White and Butlin, 2021). 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 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 that the migration rate was low and hence gene flow from the other deme was not expected to be a strong opposing force (Tigano and Friesen, 2016), we suggest that the main determinant of local adaptation was the fitness of these genotypes in the away environment. With respect to their performance under away conditions, three possible genotype classes might compete. A genotype class with positive fitness effects in an away environment suggests a generalist genotype, whereas a genotype class with negative away fitness effects suggests a specialist in the home environment with antagonistic pleiotropy. The third genotype class represents conditional neutrality, where the genotype is a specialist in the home environment but there are no observed costs in the away environment (Bono *et al.*, 2017). Interpreting patterns of local adaptation in our experiment requires consideration of the away fitness of genotypes that were available as standing variation at the start of the experiment and those that were generated by mutation and recombination as the experiment progressed.

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

The initial response to divergent selection is likely to depend on standing genetic variation, in this case the diverse set of 500 clones used to initiate the experimental metapopulations (Arnold *et al.*, 2008). Our results imply that this included clones that increased fitness under all experimental treatments. They further imply that clones with high fitness in response to one stressor also tended to have increased fitness in response to at least one other stressor or to be neutral in the away environment and, hence, they behaved as generalists in the context of the unidimensional treatments (Kassen, 2002). However, this generalist behaviour appears to be limited: clones with positive fitness effects

on average across the three stressors used in a multidimensional treatment were neutral or antagonistic with respect to average fitness across the three different stressors used in the away environment, i.e. they exhibited conditional neutrality (Anderson *et al.*, 2013; Bono *et al.*, 2017) 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 (Steppan, Phillips and Houle, 2002; Arnold *et al.*, 2008). 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 (Sheppard *et al.*, 2014; Bisschop *et al.*, 2019). 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 (Fisher, 1930). 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 (Bolnick *et al.*, 2018; Thompson, Osmond and Schluter, 2019). 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; Steppan, Phillips and Houle, 2002; McGuigan, Chenoweth and Blows, 2005; Svensson *et al.*, 2021). 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 (Bisschop *et al.*, 2020). 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.* 2018). 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 (Beldade, Koops and Brakefield, 2002)?

Limitations

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 (Bell, 1982) but it may

influence the dynamics of local adaptation. Sex and recombination both create and decompose locally adapted genotypes (Barton and De Cara, 2009; Smadja and Butlin, 2011; Gray and Goddard, 2012). 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 (Hurst and Peck, 1996).

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 (Gray and Goddard, 2012). 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 (Gunderson, Armstrong and Stillman, 2016; Orr *et al.*, 2020). 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.

Broader implications

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.* (2009) 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 (Nosil and Harmon, 2009). 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 (Butlin and Smadja, 2018). 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.

Chapter 6: Dimensionality and genomic differentiation: an evolve and resequence experiment

Abstract

The number of divergent selection pressures imposed on diverging populations is thought to be an important determinant of patterns of divergence, including local adaptation and genomic differentiation. Exposure to more divergent selection pressures, or 'multidimensional' divergent selection, is predicted to produce divergence in more adaptive loci, which may have consequences for broad genomic patterns of differentiation. However, to date, there has been no empirical test of these hypotheses. In this study, we experimentally evolve populations of the monogonot rotifer, *Brachionus plicatilis*, under either unidimensional or multidimensional divergent selection. Using an evolve and resequence approach with 'pool-seq', we compare patterns of genomic differentiation under different dimensionality treatments. We find that there is no broad effect of dimensionality on the number of significantly differentiated outlier single nucleotide polymorphisms, their clustering across the genome, or their strength of differentiation. However, we find that under unidimensional divergent selection, there is a greater ratio of outliers identified between ancestral and evolved populations to outliers identified within evolved metapopulations. These results indicate that increased availability of alleles for local adaptation does not necessitate a broader genomic response, and that unidimensionality produces stronger selection for generalist alleles than multidimensionality.

Introduction

How the dimensionality of divergent selection affects patterns of evolutionary divergence is unclear (Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009; White and Butlin, 2021). Informally, the dimensionality of divergent selection can be defined as the number of spatially heterogenous environmental conditions that impose selection on a population (Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009; Chevin, Decorzent and Lenormand, 2014; White and Butlin, 2021). In response to spatially heterogeneous selection pressures, populations adapt to increase fitness under their local subset of conditions, thereby becoming locally adapted (Kawecki and Ebert, 2004; Nosil, 2012; Savolainen, Lascoux and Merilä, 2013). Understanding how various characteristics of divergent selection, such as its dimensionality, impact the evolution of local adaptation is a major question in evolutionary research (Butlin *et al.*, 2012). In particular, where divergent selection leads to local adaptation, the dimensionality has been predicted to have widespread consequences for genome divergence, potential ecological speciation and the generation of biological diversity (Nosil, Harmon and Seehausen, 2009; White and Butlin, 2021). However, empirical tests of these predictions, either in laboratory or natural populations, remain scarce (Fry, 2009; White, Snook and Eyres, 2020; White and Butlin, 2021).

The ways in which the dimensionality of divergent selection might impact local adaptation and divergence of the genome are wide-ranging and depend on many potential covariates (White and Butlin, 2021). A key factor is whether the overall strength of divergent selection scales with

dimensionality; does each additional selection pressure add to the overall quantity of selection, or dilute it across more selection pressures (Nosil, Harmon and Seehausen, 2009)? Under the first, 'additive' scenario, models are relatively unequivocal in concluding that the speed and magnitude of local adaptation should increase with dimensionality (Nosil and Harmon, 2009; MacPherson, Hohenlohe and Nuismer, 2015). However, if multidimensionality dilutes divergent selection over traits and so over many adaptive alleles, expectations can vary considerably. Over short periods of time, spreading divergent selection over many loci might allow for more standing additive genetic variation to contribute to adaptation, enabling a rapid adaptive response to selection. In the longer term, this would also provide greater overall mutational variance, further speeding adaptation (Yeaman, 2015). However, a fixed quantity of divergent selection over many loci might spread selection thinly across the genome, to the extent that per-locus divergent selection may, at least initially, be overcome by the homogenising force of gene flow (Nosil, Harmon and Seehausen, 2009; Flaxman, Feder and Nosil, 2012; Feder *et al.*, 2014).

Local adaptation relies upon the maintenance of polymorphism at loci with locally adaptive alleles, which may produce divergence in other regions of the genome that are in linkage disequilibrium with locally-adaptive alleles (Savolainen, Lascoux and Merilä, 2013; Feder *et al.*, 2014; Tittes and Kane, 2014; Tigano and Friesen, 2016). However, divergent selection to maintain polymorphism between populations is opposed by the homogenising force of gene flow. The extent to which gene flow homogenises genomic regions between locally adapted populations depends on how strongly divergent selection acts on linked adaptive alleles and on how strong linkage disequilibrium is between the focal region and the locally adaptive alleles (Ellegren *et al.*, 2012; Burri *et al.*, 2015; Ravinet *et al.*, 2017). Therefore, where divergent selection and gene flow both impact spatially heterogeneous populations, there is an expectation that small regions of the genome will display differentiation, against a backdrop of widespread homogeneity (Nosil, Funk and Ortiz-Barrientos, 2009; Ravinet *et al.*, 2017; Westram and Ravinet, 2017). However, it remains challenging to infer the historical effects of evolutionary processes and demographic scenarios from contemporary genome data (Abbott *et al.*, 2013; Ravinet *et al.*, 2017; Wolf and Ellegren, 2017). Similar genomic patterns might be observed from different evolutionary processes, and ancestral signals might become 'over-written' by more recent events (Cruickshank and Hahn, 2014; Ortiz-Barrientos and James, 2017; Ravinet *et al.*, 2017).

Whether local adaptation arises under one or more dimensions of selection, the same core processes operate: new mutations, or new combinations of alleles, will be positively selected if they increase fitness under (a set of) local selection pressures (Kawecki and Ebert, 2004). Gene flow opposes divergent selection and increases the range of environments encountered by alleles. Certain alleles may increase fitness in all environments connected by gene flow, and so might be termed 'generalist' alleles. Other 'specialist' alleles may only increase fitness in one environment (often more so than a generalist allele), having either neutral (conditional neutrality) or negative (antagonistic) fitness effects in others (Kassen, 2002; Gray and Goddard, 2012; Savolainen, Lascoux and Merilä, 2013; Bono *et al.*, 2017). Whether adaptation is driven by specialist or generalist alleles depends in part upon gene flow: whether an allele mostly encounters selection in a single environment (specialist) or across multiple environments (generalist). Selection for purely generalist alleles will not lead to local adaptation, instead producing 'global adaptation' (Bisschop *et al.*, 2020). Gene flow thus is antagonistic to divergent selection by increasing the fitness of generalist alleles relative to specialist alleles, and by breaking down associations between specialist alleles and the environments in which they increase fitness.

In defining the dimensionality of divergent selection formally, one might stipulate that each additional selection pressure exerts an independent and orthogonal effect on fitness, as various simulation studies have assumed (Nosil and Harmon, 2009; Chevin, Decorzent and Lenormand, 2014). The reality is more complex, as selection pressures are rarely independent of one another (Orsini, Spanier and De Meester, 2012; Pfrender, 2012; Langerhans, 2017), covarying spatially and temporarily, and might have interactive effects on fitness whether experienced simultaneously or successively (Gunderson, Armstrong and Stillman, 2016). Furthermore, even when selection pressures are fully independent, there may be pleiotropic alleles which affect fitness across multiple selection pressures, which may be classed as either generalist or specialist antagonistic alleles as defined above. The dimensionality of selection has clear consequences for the types of alleles that are likely to spread; with more selection pressures there is more opportunity for the pleiotropic effects of an allele to be made manifest (Nichols *et al.*, 2011).

The interpretation of genomic data from natural populations requires overcoming many common obstacles. Isolated populations diverge due to genetic drift, regardless of selection, so divergence due to drift is the baseline against which peaks of differentiation – that may indicate barriers to gene flow – are measured. However, without knowing population sizes over time, baseline estimates can vary considerably, leading researchers to over/underestimate the contribution of genetic drift, for instance. An approach which overcomes many of these challenges is experimental evolution using evolve and resequence (Fry, 2009; Kawecki *et al.*, 2012; Baldwin-Brown, Long and Thornton, 2014; Schlötterer *et al.*, 2015). Experimental evolution allows direct control, or else accurate quantification, of a study population's demographic characteristics such as population size and migration rate (Fry, 2009; White, Snook and Eyres, 2020). Furthermore, the pairing of experimental evolution with evolve and resequence allows detection of loci under selection, both through comparison of different evolved treatment groups and of evolved with ancestral populations (Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015; Barghi and Schlötterer, 2020). In doing so, it sidesteps many of the challenges encountered by studies of natural populations (Ortiz-Barrientos and James, 2017; Ravinet *et al.*, 2017) and allows the connection of phenotype with genotype (e.g Michalak *et al.*, 2019; Tusso *et al.*, 2021).

In this study, we used evolve and resequence data from a previous evolution experiment (Chapter 5) to test for an effect of the dimensionality of divergent selection on patterns of genomic differentiation (Kofler and Schlötterer, 2014; Schlötterer *et al.*, 2015). The evolution experiment evolved populations of the monogonont rotifer, *Brachionus plicatilis* under two broad treatments; unidimensional divergent selection and multidimensional divergent selection. This study sought to test the broad hypothesis that patterns of genomic differentiation would vary by dimensionality treatment. We predicted that unidimensional divergent selection, concentrated strongly onto few loci, would produce a pattern of strong divergence at few sites, whereas multidimensional divergent selection, diluted over many loci, would generate divergence at more genomic regions but with comparatively lower divergence per-site. Furthermore, previous analysis of local adaptation data from the evolution experiment identified contrasting temporal dynamics between treatment, with slow but strong local adaptation under unidimensional divergent selection, and weak but transient local adaptation under multidimensional divergent selection. This interpretation would mean that in the unidimensional treatment there would be greater differentiation between evolved populations (where specialist alleles dominant), relative to the differentiation between ancestral and evolved populations, than multidimensional divergent selection (where generalist alleles dominate).

To test these predictions, we considered differences between unidimensional and multidimensional divergent selection treatments for three measures; 1) the number of differentiated single nucleotide polymorphism (SNP) outliers at a range of significance thresholds, 2) the distribution of outliers across scaffolds of the *B. plicatilis* genome, and 3) the distribution of XtX (an measure of differentiation analogous to F_{ST}, see 'Methods'). To test whether the relative contribution of specialist vs generalist alleles varied by dimensionality, we calculated the ratio between the number of outliers identified between ancestral and evolved populations and the number identified between evolved paired populations.

Methods

Full materials and methods relating to source of rotifer populations and experimental design are given in Chapter 5, hence here I present a less-detailed summary.

Source and of rotifer populations

B. plicatilis is an aquatic animal that exhibits facultative sexual reproduction; at low population density reproduction is asexual and mitotic, whilst at high population density reproduction switches to being sexual and meiotic. The product of sexual reproduction is a diapausing embryo, also known as a resting egg, which exhibits developmental dormancy until presented with optimal hatching conditions. *B. plicatilis* populations used in this experiment were derived from 54 diapausing embryos collected from the sediment of two (27 from each) brackish ponds in the Juncar-Segura basin, Albacete province, Spain. Further details of clones and collection method are available in García-Roger and Ortells (2018). These 54 embryos were hatched to form clonal cultures, which were combined for one generation and produced diapausing embryos via sexual reproduction. Clonal cultures were established from 500 of these genetically diverse diapausing embryos, which were then combined and samples from the mixed population were used to form experimental starting populations.

Experimental design

Two experimental treatments were used in this experiment: unidimensional and multidimensional forms of divergent selection. Within each experimental treatment, four different stressor pairs were used, individually for unidimensional divergent selection and in four different combinations of three for multidimensional divergent selection. Divergent selection was applied across two demes that formed a metapopulation, linked by 1% reciprocal migration per sexual generation. Unidimensional forms of divergent selection were delivered using different shock media per deme. For multidimensional selection, each deme was divided equally into three cultures, each of which was exposed to a different shock medium, and then remixed. There were 4 metapopulations per stressor pair, hence each dimensionality treatment was replicated 16 times (four stressor pairs/combination x four metapopulations). Experimental demes were cultured on a weekly cycle. Stressors were delivered as shock treatments following six days of exponential growth. Each individual stressor was calibrated prior to experimental evolution using the same starting population to produce 50% survival over 24 hours. Surviving rotifers were passaged to form a new deme at 6 rotifers ml^{-1} in 400ml culture media, with 1% of each deme being new clonal genotypes having hatched from sexually produced resting eggs.

Sample collection & extraction

Populations were grown to high density then passed through a 100µm filter to collect the rotifers whilst removing algal and bacterial contaminants. Liquid nitrogen was then poured over each filter, freezing the filtered rotifers and allowing them to be scraped into 70% ethanol tubes for preservation.

For DNA extraction, sample tubes were spun down in a centrifuge and the ethanol poured off. A lysis solution of 500µl CTAB buffer, 40µl proteinase K and 1µl β-mercaptoethanol was added to each sample, tubes were vortexed, then incubated at 55˚C with rotation. After 1 hour, 4µl RNase A was added to each sample before they were returned to incubation at 55˚C with rotation for a further hour. Tubes were then removed, and 500 µl of chloroform:isoamyl alcohol (24:1) was added and mixed by inversion for 10 minutes. The contents were then poured into gel lock columns and spun at 14000 rpm for 5 minutes, before the upper phase was poured into new tubes. 500 µl of isopropanol was added, mixed by inversion, and the tubes allowed to incubate for 5 minutes at room temperature. Samples were then spun at 14000 rpm for 40 minutes at 4˚C to precipitate DNA. The liquid phase was poured off, then 1000 µl of 70% ethanol was added, mixed by inversion, and the tubes were spun again at 14000 rpm at room temperature for 5 minutes. The liquid was again poured off, and a further 500 µl of 70% ethanol added, mixed and spun at 14000 rpm at room temperature for 5 minutes. The ethanol was then poured off and the DNA pellet left to air dry. Finally, 60 µl of TE buffer was added to each tube and the DNA pellets allowed to dissolve.

Sequencing and mapping

Genomic fragment libraries were built using the NEBNext Ultra II FS Kit (Illumina) with 300 bp inserts and were sequenced using an Illumina NovaSeq with S4 chemistry at the Centre for Genomic Research, Liverpool, UK. All singleton reads were discarded. Remaining paired-end reads were trimmed for adapter sequences (Illumina) using Cutadapt v1.2.1 (Martin, 2011), trimming where the 3' end of any sequence matched the adapter for 3bp or more. Reads were then passed through a second trimming step using Sickle v1.2 for a minimum window quality score of 20 and minimum read length of 15bp. Trimmed FASTQ files were mapped to the *B. plicatilis* reference genome [716 scaffolds, 5950 contigs, 106.939 Mb (Han *et al.*, 2019)] using the Burrows-Wheeler aligner v0.7.17 (Li and Durbin, 2009). BAM files were processed with SAMtools v1.7 (Li *et al.*, 2009). For each BAM file we applied a filter removing reads with a base quality score lower than 20, and then compiled a single pileup file consisting of all 64 end-experiment demes and two samples of the ancestral population. We used the software package 'PoPoolation2' v1.013 (Kofler, Pandey and Schlötterer, 2011) to convert the pileup files into the flexible 'synchronised' format, using the java script 'mpileup2sync.jar'. We created a single master synchronised file for outlier detection and a synchronised file per metapopulation for the calculation of differentiation statistics (see 'Outlier detection' below). These pileup files were passed to the R software package 'poolfstat' (Hivert *et al.*, 2018) which was used to produce files compatible for analysis with the software package 'BayPass' v2.2 (Gautier, 2015).

Outlier detection

We used the Bayesian outlier detection software, BayPass (Gautier, 2015) to identify outliers that were statistically associated with one or another stressor within a stressor pair, after allowing for underlying covariance among pools. BayPass is an extension of the outlier detection model used in BayEnv (Coop *et al.*, 2010) developed to identify genetic markers subject to selection or which are associated with user-specified covariates from pool-seq data. BayPass allows computation of the C_2 statistic (Olazcuaga *et al.*, 2020) which measures a contrast between two groups of pools representing

a binary environmental/trait difference whilst accounting for the underlying population structure. To calculate the association of SNP allele counts with each stressor within a stressor pair, we used a binary variable for each stressor (e.g. for "Salinity"; 1 = "High Salinity", -1 = "Low Salinity"). This was performed both for all populations for a given stressor pair (a 4 vs 4 comparison) and within each metapopulation (a 1 vs 1 comparison) using the core model. We identified outliers as SNPs with C_2 values which were statistically significant using the assumption of a χ^2 distribution with a single degree of freedom (Olazcuaga *et al.*, 2020). To account for the predicted pattern of few strongly divergent SNPs vs many weakly divergent SNPs between treatments, we used three different significance thresholds ($p < 10^{-3}$, $p < 10^{-4}$ and $p < 10^{-5}$) when determining what would constitute an outlier.

To reduce computation time, we adopted a sub-sampling strategy in which each dataset was split into *k* pseudo-replicates by sampling one in every *k* SNPs from the ordered map to produce *k* subsets of SNPs (Frachon *et al.*, 2018; Gautier *et al.*, 2018). *k* varied by comparison (see results).

Tests of predictions

Loci were identified as outliers based on the significance of C_2 values within stressor pair (4 vs 4) contrasts. In computing contrasts, BayPass also returns estimates of the XtX statistic of population differentiation for each SNP- analogous to F_{ST} but standardised for the effects of unequal sample variance and covariance among populations, as can be common for pool-seq studies (Günther and Coop, 2013). It also returns the XtXst statistic, developed specifically for use with BayEnv, which standardises XtX by subtracting the mean and dividing by the standard deviation (Olazcuaga *et al.*, 2020), and its associated p-value for significant differentiation among populations.

Our analysis was structured to test three predictions regarding how outlier distributions might differ between dimensionality treatments and across stressor pairs. We tested for differences between; 1) the number of outliers, 2) the clustering of outliers across the genome, and 3) the strength of their differentiation.

For our first test, we used the master synchronised file of all pools to calculate the total number of significantly differentiated outliers both within each stressor pair and within each metapopulation contrast. To test if the number of outliers varied between dimensionality treatments, we performed a Mann-Whitney U test (N = 4 for stressor pair contrasts, N = 16 for metapopulation contrasts). Furthermore, to test for associations between the total number of outliers and stressor pairs, we performed a Kruskal-Wallis test using the outliers identified in metapopulation contrasts (N = 16). We repeated these tests for outliers identified at each of the three significance thresholds ($p < 10^{-3}$, $p < 10^{-4}$ and $p < 10^{-5}$).

For our second test, using the outliers identified above within each stressor pair contrast and each metapopulation contrast, we grouped outliers by genomic scaffold and calculated the outlier density per scaffold density (number of outliers divided by the number of SNPs) per scaffold. This included scaffolds with no outliers. We calculated the mean and standard deviation of the per-scaffold outlier densities, and for each comparison, we divided the standard deviation by the mean to obtain the standardised coefficient of variation. As above, used Mann-Whitney U and Kruskal-Wallis tests to test whether the coefficient of variation was associated with dimensionality treatment or stressor pair respectively. Again, we used the tree significance thresholds in separate analyses.

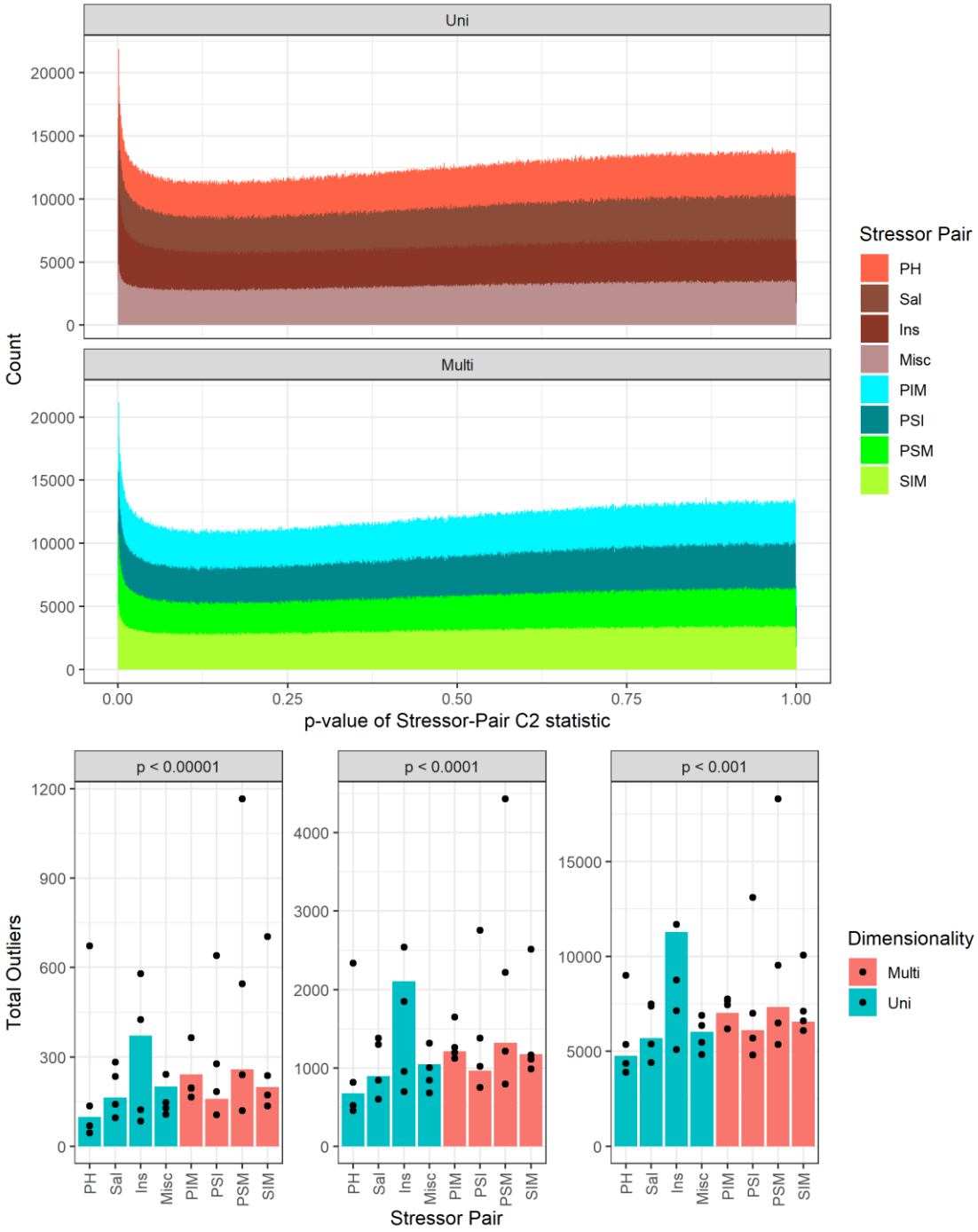

Figure 1: Distribution of per SNP p-values.

Top panels show the distribution of p-values for all SNPS identified in stressor pair contrasts, split between dimensionalities. Bottom panels show the number of outliers identified for different stressor pairs as coloured bars, and for each metapopulation as black dots, grouped by the three outlier significance thresholds (horizontal panels).

For our third test, we used BayPass to calculate the XtX values of outlier loci when calculated within metapopulations (not including pools outside each metapopulation within the analysis). We took the 90th, 95th and 99th percentiles of the XtX distribution per metapopulation and compared these points

between dimensionality treatments and stressor pairs similarly to tests 1 and 2. We performed a two-sample t-test to compare percentiles between dimensionality treatments and a one-way ANOVA to test for an association with stressor pair.

Finally, to test for different contributions of specialist vs generalist alleles, we introduced the ancestral population into metapopulation contrasts, and ran BayPass twice, firstly calculating contrasts between the ancestral population and both evolved demes, then secondly calculating contrasts between the two evolved demes within the metapopulation. We took the ratio of the number of outliers identified in each contrast, again using the three significance thresholds, and performed ANOVA and t-tests on these ratios as described above for test 3.

Results

Mapping quality and outlier detection

Genomic DNA was sequenced using pool-seq from pools estimated to contain at least 100 individuals, although this may have varied. The average number of properly paired reads per sample was 42,232,562 and the average percentage of mapped and properly paired reads was 97.8%. The synchronised master file comprised 98,207,697 bp (91.8% mapped coverage of the reference genome), of which there were 3,153,000 SNPs. The average sequencing depth averaged across all pools was 60.13.

During outlier detection, for each stressor pair comparison, BayPass identified an average of 6848 outlier SNPS at significance level $p < 10^{-3}$, 1177 outlier SNPs at $p < 10^{-4}$, and 213 outlier SNPs at $p < 10^{-5}$ per stressor pair comparison (Figure 1). Stressor pair comparisons were run using 100 pseudo-replicates (k = 100) whilst metapopulation comparisons were run using 30 pseudo-replicates (k = 30).

Total number of outliers

We find no significant differences in the number of outliers identified by BayPass between unidimensional and multidimensional divergent selection treatments, regardless of whether outliers were identified per stressor pair or per metapopulation (Figure 1).

There was no significant effect of dimensionality using stressor pair outliers at any outlier significance threshold (for $p < 10^{-3}$ – W = 12, p = 0.343; for $p < 10^{-4}$ – W = 11, p = 0.486; for $p < 10^{-5}$ – W = 9, p = 0.886). Furthermore, we identified no effect when using the number of outliers identified from metapopulation contrasts rather than stressor pair contrasts. There remained no effect of overall dimensionality treatment at any outlier significance threshold (for $p < 10^{-3}$ – W = 170, p = 0.119; for $p < 10^{-4}$ – W = 176, p = 0.073; for $p < 10^{-5}$ – W = 174, p = 0.087), nor was there any association with stressor pair (for $p < 10^{-3}$ – $\chi^2_7 = 6.32$, p = 0.503; for $p < 10^{-4}$ – $\chi^2_7 = 5.20$, p = 0.636; for $p < 10^{-5}$ – $\chi^2_7 = 4.10$, p = 0.768).

Clustering within genome

We found no effect of dimensionality treatment or stressor pair on the coefficient of variation of outlier density per scaffold at any outlier significance threshold (Figure 2). There was neither an effect of dimensionality treatment using stressor pair outliers (for $p < 10^{-3}$ – W = 95, p = 0.224; for $p < 10^{-4}$ – W = 125, p = 0.926; for $p < 10^{-5}$ – W = 103, p = 0.520) nor metapopulation outliers (for $p < 10^{-3}$ – W = 98, p = 0.270; for $p < 10^{-4}$ – W = 105, p = 0.402; for $p < 10^{-5}$ – W = 90, p = 0.160), and there was no

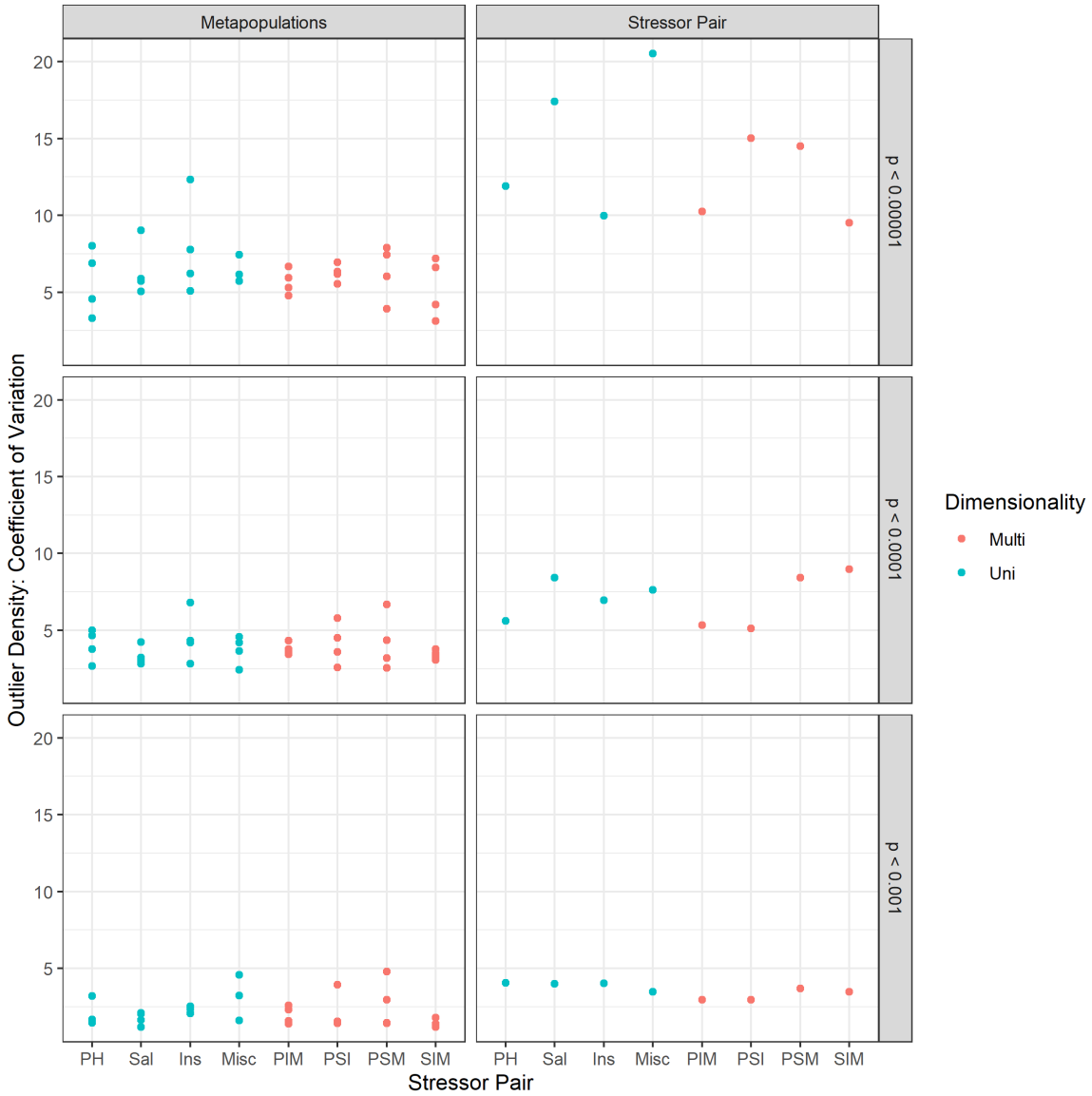

Figure 2: Outlier density coefficients of variation.

Horizontal panels display data from outliers identified through metapopulation or stressor pair contrasts from the same master synchronised file. Vertical panels display data at three different outlier significance thresholds.

association of stressor pair with coefficient of variance (for $p < 10^{-3}$ – $\chi^2_7 = 8.97$, $p = 0.255$; for $p < 10^{-4}$ – $\chi^2_7 = 3.23$, $p = 0.863$; for $p < 10^{-5}$ – $\chi^2_7 = 4.60$, $p = 0.708$).

Differentiation of outliers

No effect of dimensionality treatment on the differentiation of outliers, measured as the XtX statistic, was detected using metapopulation contrasts (Figure 3). There was no significant difference between mean XtX values of dimensionality treatments at the 90th ($t = 1.16$, $df = 30$, $p = 0.255$), 95th ($t = 1.31$, $df = 30$, $p = 0.201$) or 99th ($t = 1.28$, $df = 30$, $p = 0.210$) percentiles. Equally, there was no significant association of XtX value with stressor pair at the 90th ($F = 1.30$, $df = 7, 24$, $p = 0.292$), 95th ($F = 1.50$, $df = 7, 24$, $p = 0.215$) or 99th ($F = 1.75$, $df = 7, 24$, $p = 0.146$) percentiles.

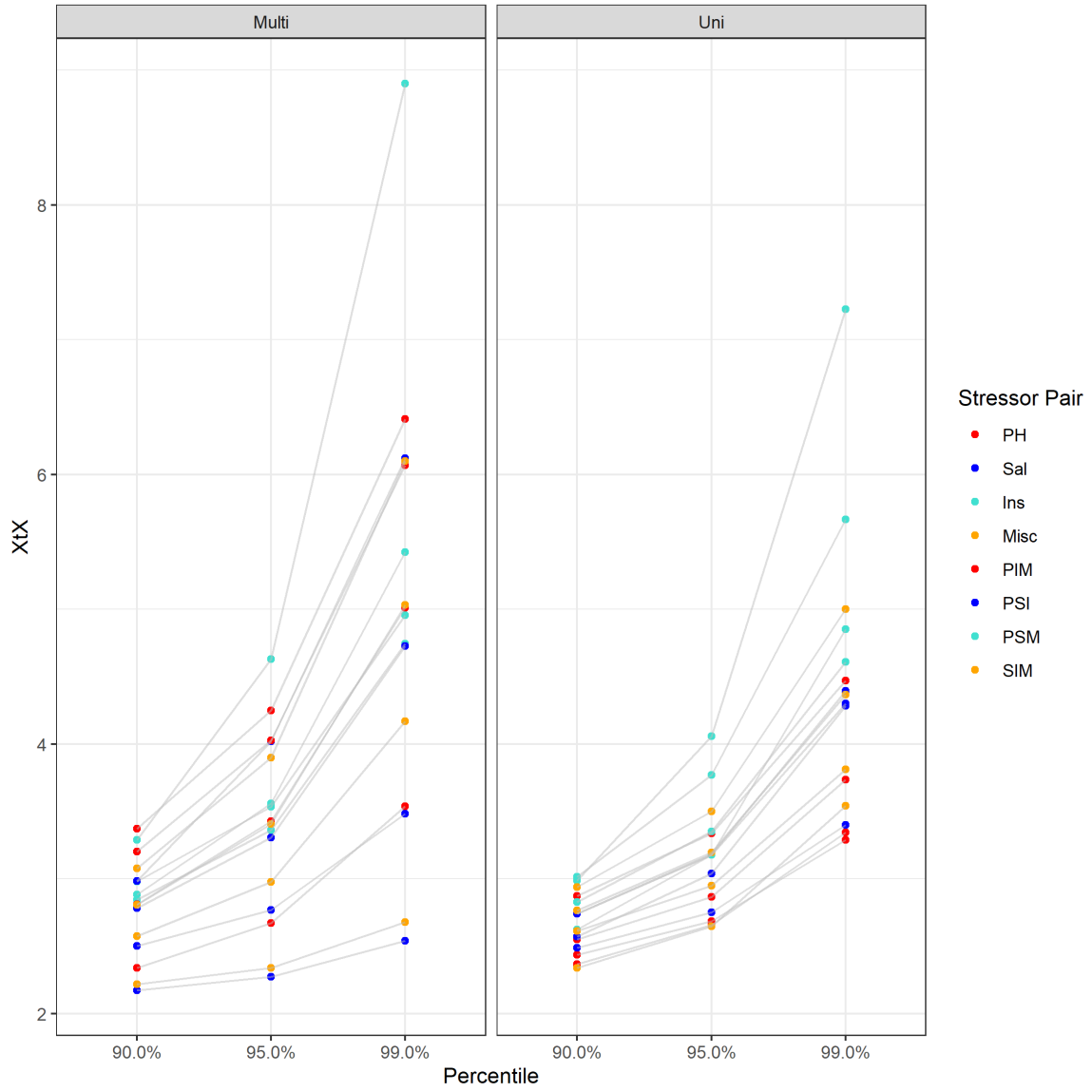

Figure 3: Distribution of XtX values from metapopulation contrasts for unidimensional and multidimensional divergent selection treatments.

Ancestral-Evolved vs Within-Metapopulations

We identified a significant effect of dimensionality treatment on the ratio of outliers identified in ancestral-evolved contrasts to outliers identified in metapopulation contrasts (Figure 4). This ratio was significantly higher under unidimensional divergent selection than multidimensional divergent selection)- i.e., the ratio of generalist alleles to specialist alleles was higher under unidimensional divergent selection. This significant effect was identified for all outlier significance thresholds (for $p < 10^{-3}$ – $t = 2.90$, $df = 30$, $p = 6.91 \times 10^{-4}$; for $p < 10^{-4}$ – $t = 2.87$, $df = 30$, $p = 7.38 \times 10^{-3}$; for $p < 10^{-5}$ – $t = 2.56$, $df = 30$, $p = 0.016$). This ratio did not significantly vary by stressor pair (for $p < 10^{-3}$ – $F = 1.55$, $df = 7, 24$, $p = 0.198$; for $p < 10^{-4}$ – $F = 1.41$, $df = 7, 24$, $p = 0.245$; for $p < 10^{-5}$ – $F = 1.45$, $df = 7, 24$, $p = 0.232$).

Discussion

We found no effect of dimensionality treatment on any measure of genomic differentiation between evolved demes, although we did identify an effect on the ratio of outliers between ancestral-evolved and metapopulation contrasts. First, we interpret these results before proceeding to discuss some limitations to our method which may also account for the lack of detected effect.

Patterns of differentiation do not vary by dimensionality

That we found no effect of dimensionality on patterns of differentiation indicates that dimensionality may not impact genomic differentiation as previously hypothesised. The dilution of selection over many loci with multidimensionality does not necessitate a dilution of the genomic response to selection (Yeaman and Whitlock, 2011; Rafajlović *et al.*, 2016). In this study, the same four unidimensional divergent selection pressures were used in combination to create multidimensional divergent stressor pairs, and stressors were applied independently hence there were no interactive effects of stressors. Therefore, we can only conclude that, on average, there were more loci under selection in multidimensional treatments than unidimensional treatments, but that this did not produce different patterns of response. That there were more loci available for selection does not equate to more loci responsible for local adaptation.

Furthermore, it is important to note that the quantification of local adaptation from phenotype data as was done by the previous study noted no local adaptation at the end of the experiment in multidimensional metapopulations. It was only present overall in unidimensional metapopulations. Given that the difference in local adaptation at the end of the experiment, as calculated using phenotype data, was nonetheless statistically significant, we had expected to find different numbers of SNP outliers between dimensionalities. However, in attempting to quantify how the distribution of outliers may vary across the genome, we may have made an unsubstantiated assumption. We assumed that local adaptation might be sustained by few, strongly differentiated loci under unidimensional divergent selection, but many, weakly differentiated loci under multidimensional divergent selection. However, as seen in Chapter 5, there was no local adaptation remaining overall in multidimensional metapopulations. Whatever local adaptation had been present previously had been undone by gene flow. Therefore, the hypothesis that local adaptation is underlain by different distributions of allele sizes is flawed from the outset. However, in this case, the 'amended' hypothesis that more outliers would be identified under unidimensional divergent selection was equally incorrect.

Ancestral-evolved vs within-metapopulation

This additional test was performed to test for differences between the ratio of generalist to specialist alleles. A generalist allele responds to selection and may be identified as an outlier when comparing ancestral with evolved populations, but only differs a little, if at all, between the demes within a metapopulation. The significant difference between dimensionality treatments indicates that the selection of generalist alleles is more likely under unidimensional divergent selection than under multidimensional divergent selection. This follows the logic that it would be considerably easier for an allele to increase fitness under two stressors than many stressors (e.g., six in this study), and hence there will be more generalist alleles available in low-dimensionality environments. Although this runs somewhat contrary to our predictions based on the interpretation of Chapter 5, it remains coherent because the number of alleles impacted by selection need not covary strongly with local adaptation.

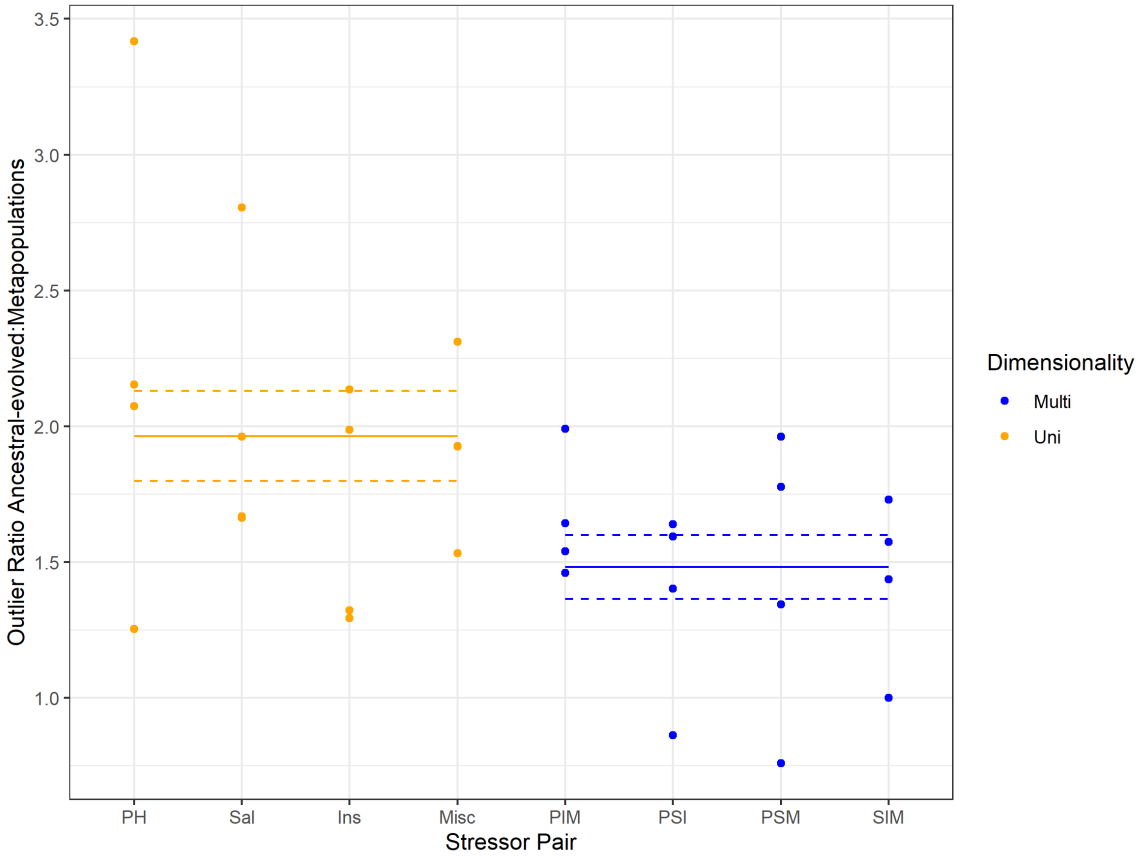

Figure 4: The ratio of outliers identified in ancestral-evolved contrasts to metapopulation contrasts (plotted data only at outlier significance threshold of $p < 10^{-3}$). Solid horizontal lines show the mean per dimensionality treatment, dashed horizontal lines represent one standard error above and below the mean.

There may be stronger local adaptation under unidimensional divergent selection (either due to more loci impacted or stronger effects of alleles) but adaptation may still comprise many generalist alleles contributing to increased fitness. Indeed, this increased abundance of generalist alleles under unidimensional divergent selection also provides an explanation to the finding in Chapter 5 that unidimensional divergent selection produced higher 'home' fitness throughout the experiment.

Methodological constraints

Although there may be no true effect of dimensionality on patterns of genomic differentiation, an alternate explanation would be that our method was under-powered in its ability to detect these variable patterns. This is likely due to a lack of statistical power to identify differences between treatments and stressor pairs.

Firstly, the power of BayPass as a tool to detect significantly differentiated SNPs is highly dependent on sequencing depth and quality. This would have been a relatively minor effect for stressor pair comparisons, where contrasts were made effectively between eight separate sequencing pools. However, this may have affected the interpretation of individual metapopulation contrasts which compared only two pools. Nevertheless, in stressor pair contrasts, there may have been some stochasticity in which alleles responded to selection. If functionally equivalent alleles at different loci

were to respond to the same selection pressure(s), BayPass would have aggregated their differentiation and perhaps failed to identify them as outliers.

Secondly, monogonont rotifers are facultative sexual organisms (Carmona, Gómez and Serra, 1995; Aparici, Carmona and Serra, 2001). The reduced rate of sexual reproduction compared with the contribution of asexual reproduction will have reduced the effective population size and reduced the available genetic variation for selection to act on. This may have been exacerbated as a consequence of the two-cycle delay between mating (recombination) and the new genotype entering the population as a result of the required dormancy period for resting eggs. Genetic variation may also have been reduced due to laboratory adaptation- as discussed in Chapter 5, there was a sharp increase in asexual growth rate at the start of experimental evolution. This may have reduced diversity at loci that were in linkage disequilibrium with genes for increased sexual reproduction. Furthermore, the clonal structure of genotypes may have made it difficult to identify the targets of strong and weak selection; if there is little allele differentiation within demes (for instance if alleles are differentially fixed) the only factor in influencing XtX would be the sequencing coverage and resulting allele counts.

Finally, it could be argued that the use of 1% migration between demes in a metapopulation was too high to expect patterns of differentiation to differ between dimensionalities. Certainly, it was not too high to prevent significant differentiation of individual loci under selection, as we see from Figure 1, nor was it too high to prevent local adaptation in either treatment at points throughout experimental evolution (Chapter 5). However, within the scope of the planned analyses, using a wide range of SNPs may not have been highly powered enough to detect differences in outliers spread across the genome.

Future work

Further studies using evolve and resequence might learn from this attempt to identify patterns of local adaptations by taking more regular samples of genomic DNA for sequencing, rather than just at the start and end of experimental evolution. In hindsight, it would have been valuable to sequence population genomes at the halfway stage of the experiment. This perhaps applies particularly for experiments attempting to identify reproductive isolation loci in populations connected by gene flow, as there is always the risk that these differences might be lost over time.

Conclusions

There is an inherent strangeness in our approach to this analysis. We are not, as is usual, attempting to identify any individual signature of selection. Instead, we attempted to contrast the overall patterns of differentiation, with no focus on how reliably or accurately signatures of selection could be identified within stressor groups. Certainly, our analysis could have extended to identifying and characterising signatures of selection between stressor pairs, however, this would have been to extend our analysis beyond the set of questions we sought to ask. We conclude that the dimensionality of divergent selection does not produce widespread differences in the patterns of genomic differentiation but does affect the balance of generalist vs specialist alleles impacted during local adaptation.

Chapter 7: Discussion

This thesis has examined how the dimensionality of divergent - and overall - selection can impact the evolution of local adaptation and extrinsic isolation. Dimensionality is a property of all environments, with a wide range of potential consequences for local adaptation and ecological speciation, yet there has been comparatively little empirical research into its effects. As I argue in Chapter 2 (White and Butlin, 2021), this under-study of such a fundamental aspect of evolutionary theory likely stems from a high degree of confidence in premature conclusions within classic reviews of speciation (Rice and Hostert, 1993; Nosil and Harmon, 2009).

To empirically address the diversity of ways dimensionality impacts these barriers to reproduction, I have used a combination of theoretical, simulation, experimental evolution, and genomics approaches. In concluding this thesis, I begin by returning to some of the key theoretical points presented in Chapter 2 and discuss how studies within this thesis have addressed them. I then draw together these key points and synthesise an integrated summary of how dimensionality impacts the long-term divergence of populations in nature. Finally, I conclude by discussing future directions for research into multidimensionality.

Key theoretical advances

As I outline in Chapter 2, there is broad consensus in the literature that multidimensionality is important for driving the evolution of local adaptation and speciation (Rice and Hostert, 1993; Nosil and Harmon, 2009; Nosil, Harmon and Seehausen, 2009). I argue however, that the concepts and evidence base underlining them are weak and insufficient for firm conclusions to be made. In this section, I revisit three key concepts identified within Chapter 2 which require a deeper understanding, and I discuss how this thesis develops theory relating to them.

Defining dimensionality

The first key point, articulated both in Chapters 2 and 3, is the requirement for a definition of both overall dimensionality and the dimensionality of divergent selection in formal quantitative terms. By verbal argument (Chapter 2), and by simulation (Chapter 3), I show that the dimensionality of divergent selection is a relatively arbitrary concept with respect to its effects on barriers to reproduction. I demonstrate that, instead, it is the ways in which the environment, traits and genome evolution map onto selection pressures that determines the evolution of reproductive barriers.

Divergence between the environments inhabited by any two populations can always be expressed as a unidimensional process, with a single axis describing variation in environmental conditions. Any number of environmental variables may then co-vary with this axis and remain independent from one another across spatial distributions when considering potentially uninhabited niches. This likely represents the most practically useful definition of divergence dimensionality when considering natural populations. For more than two populations, divergence dimensionality becomes difficult to define, unless perhaps populations are distributed along a gradient of environmental change, where multiple selection pressures co-vary with the gradient.

Traits evolve in response to the divergent selection produced by environmental variation. Trait dimensionality can be mapped onto an adaptive landscape in a similar way to environmental dimensionality- indeed this mapping can be directly equivalent if one were to define traits as the

responses to individual selection pressures. Multiple traits can co-vary because of correlated selection stemming from correlated environmental variables, as might be defined by an E-matrix, and because of genetic covariance (G-matrix associations). Where traits diverge between two populations, as with correlated environmental variables, in strict terms there can only be a single orthogonal trait axis separating two populations. This is because traits that share no common genetic basis and would otherwise be able to evolve independently are constrained by the correlated effects of selection (Steppan, Phillips and Houle, 2002; Arnold *et al.*, 2008; De Lisle and Bolnick, 2020). Divergent selection might produce multidimensional divergence in any number of these otherwise orthogonal traits due to correlated selection. This provides a useful way to map multidimensional divergent selection onto genome evolution. An increased number of divergent selection pressures may lead to an increased number of divergent traits, which may be underlain by an increased number of loci. As outlined in Chapter 2, if each selection pressure corresponds to an orthogonal trait, this association is true by definition. If traits are not orthogonal due to some shared genetic basis, then this is relationship is not necessarily linear, as loci may be pleiotropic, but the association will hold overall.

The distinction between the 'true' dimensionality of a system as defined in quantitative terms and the, perhaps more practically useful, definition of the number of divergent selection pressures may seem initially to be a fairly abstract and intangible distinction, with limited utility to *in vitro* systems. However, considering the results of the experimental evolution presented in Chapter 5, it becomes clear that an understanding of the true dimensionality of an environment and the selection pressures it imposes is important for comprehension of patterns of local adaptation and differentiation. Notably, in developing the evolution experiment presented in Chapters 5 and 6, post-experimental evolution follow-up assays were planned which would have determined how population fitness varied when assayed across the range of experimental stressors. Analysis of these assays would have provided valuable insight into to what extent different environments represented divergent, as opposed to more parallel, selection. Unfortunately, these were not possible due to the onset of the Covid-19 pandemic.

Nevertheless, my interpretation of Chapter 5 provides a link between overall multidimensionality of an environment and the behaviour of locally adaptive alleles. I conclude that, for an allele to be selected when local conditions are heterogenous and multiple selection pressures impose directional selection, it must tend to have low antagonistic pleiotropy. This conclusion would apply regardless of the range of selection pressures experienced in the 'away' environment or environments. In contrast, for lower-dimensionality environments, the home environmental niche is narrower; there are fewer stressors against which any antagonistic effects of an allele might be manifest, allowing specialist antagonistic alleles to contribute increasingly to local adaptation. Additionally, as there is lower environmental heterogeneity across the metapopulation, a wider pool of adaptive alleles can become generalist alleles (Welch and Waxman, 2003).

Extrinsic mechanisms

The second key point articulated within this thesis concerns the two main mechanisms by which multidimensional divergent selection might impact extrinsic isolation: by impacting the strength of divergent selection, and by spreading selection across more loci.

Firstly, and most straightforwardly, increasing the dimensionality of divergent selection is likely to drive local adaptation if there is an associated increase in the strength of divergent selection, as I

demonstrate using additive multidimensionality in Chapter 3. When comparing systems, the strength of this association between divergence dimensionality and divergent selection is very much affected by perspective and the comparison that one is making. The introduction of an additional divergent selection pressure to a system is almost certain to increase overall selection to some degree. However, this need not be true if comparing two systems with *n* and *n* + 1 divergent selection pressures. Do multi-stressor ecosystems impose stronger selection, either when measured directionally or divergently, than ecosystems dominated by a single selection pressure? This question lies beyond the scope of this thesis but could be usefully addressed by future research to advance our understanding of the ecological interactions within multistressor communities (Galic *et al.*, 2018).

Secondly, local adaptation to an increased number of selection pressures is associated with a more polygenic genomic response. As described above, this relationship may not be linear, but the association will likely hold in general, as is discussed in Chapter 2. With more loci underpinning local adaptation, adaptation to home environments can occur more rapidly via greater potential for selection on (and recombination among) standing variation in the short-term and greater mutational input in the long-term. However, through both simulation (Chapter 3) and evolve and resequence (Chapter 6), I have shown that more loci available for selection does not necessarily equate to more loci responsible for local adaptation. The dilution of selection across the genome has been proposed elsewhere as a mechanism by which weak per-locus selection leads to divergence being driven by weak-effect alleles which may individually be more vulnerable to the homogenising effect of gene flow (Nosil, Harmon and Seehausen, 2009). In Chapter 3, I show that this is a mischaracterisation of the effects of multidimensional divergent selection. In classical theory, we see that large effect alleles are favoured when a population is far from its optimum, whilst small alleles are favoured when a population is close to its optimum. By spreading selection over more loci, there is little plausible reason why this ought to impact the distribution of per-locus effect sizes (Barton and Keightley, 2002). By increasing the number of loci without impacting the variance of mutational effects, one would expect higher mutational variance, and an increased incidence of large-effect adaptive alleles (Baer, Miyamoto and Denver, 2007; Francioli *et al.*, 2015).

Intrinsic mechanisms

The completion of ecological speciation in the face of gene flow would typically depend on the coupling of multiple barriers, necessitating divergently-selected loci to become associated with one or more loci underlying pre-zygotic isolation (Kirkpatrick and Ravigné, 2002; Smadja and Butlin, 2011). The coupling of multiple barriers might produce a stronger overall barrier to gene flow as large sections of the genome enter linkage disequilibrium, and potentially lead to genome-wide congealing (Barton and De Cara, 2009; Feder *et al.*, 2014; Flaxman *et al.*, 2014; Nosil *et al.*, 2017; Butlin and Smadja, 2018). These can arise as 'by-products' of adaptation such as assortative mating or constitutive Dobzhansky-Muller incompatibility (DMI) loci (Dobzhansky, 1936, 1937; Muller, 1942). In Chapter 2, I identify two other mechanisms of reproductive isolation beyond extrinsic isolation which might relate to aspects of dimensionality.

Firstly, we can consider how adaptive trait dimensionality extends into sexual traits and impacts mating trait dimensionality (Hohenlohe and Arnold, 2010; Nosil and Hohenlohe, 2012). An example cited multiple times in this thesis is the rapid experimental evolution of reproductive isolation in the parasitic louse, *Columbicola columbae*, through divergent selection on body length, which acts as a multiple-effect trait by impacting mating isolation (Villa *et al.*, 2019). In this example, strong divergent

selection on a single trait was sufficient to produce strong reproductive isolation, but notably the researchers had deliberately chosen body size for divergent selection as it represented a strong candidate for being a multiple-effect trait. This raises the question of how often divergently selected traits become multiple-effect traits in natural populations, and how the dimensionality of trait divergence influences the frequency at which they arise.

The importance of multiple-effect traits in generating reproductive isolation remains relatively uncertain (Servedio *et al.*, 2011; Smadja and Butlin, 2011; Thibert-Plante and Gavrilets, 2013). However, as argued by Haller *et al.* (2012), for a multiple-effect trait to be important for speciation, the divergence of the trait itself has to have a large magnitude. By way of illustration, under the dilution mode of multidimensionality presented in Chapter 2, with increased divergence dimensionality each divergent trait becomes individually less divergent. The trade-off between a single/few divergent traits with large magnitude versus many divergent traits of low magnitude in affecting mating isolation is relatively unexplored. This may be due to the estimation of magnitude being almost entirely retrospective and difficult to compare across systems. Nevertheless, in driving the divergence of multiple effect traits, the ability of divergent selection to produce a large magnitude of divergence may be more important than its multidimensionality (Bolnick and Fitzpatrick, 2007).

Secondly, we can consider the effect genomic dimensionality might have on further mechanisms of reproductive isolation through linkage. Constitutive DMIs, unlike transgressive DMIs, are combinations of individually adaptive alleles that come together in hybrids and reduce fitness regardless of environmental factors (Fishman and Willis, 2001; Ono, Gerstein and Otto, 2017). The evolution of these barriers depends either on pleiotropic effects of adaptive alleles or on the loci underlying them entering linkage disequilibrium with locally adaptive alleles. If, with increasingly multidimensional divergent selection, a greater number of loci are impacted, more of the genome will be linked with locally adaptive loci. This may include loci that underlie secondary barriers, such as assortative mating loci or DMIs. The concept of genomic dimensionality describes how different loci form independent units which can evolve independently. To what degree loci constitute independent units of evolution is mediated by linkage disequilibrium; either through physical linkage or epistasis. Understanding how individual loci are affected by the interplay of selection and migration remains a major challenge in evolutionary biology (Barton, 1983; Yeaman and Whitlock, 2011; Flaxman *et al.*, 2014; Yeaman, 2015; Hoban *et al.*, 2016).

A holistic appreciation of multidimensionality

In this section, I draw upon several key findings from this thesis and, by aligning them with a wider body of research, construct a unified theory of multidimensionality in the evolution of local adaptation and speciation.

Throughout this thesis, the establishment and maintenance of local adaptation has been referenced as a key first step in ecological speciation. I have found that the dimensionality of divergent selection impacts the speed and strength of local adaptation in two ways. In Chapter 3, I demonstrated that by impacting more loci, multidimensional divergent selection is able to access a greater quantity of additive genetic variance, both standing and mutational, and rapidly drive local adaptation. I found that having more loci available for selection does not substantially alter the composition of genetic variation- local adaptation is still dependent on few large effect loci, and hence divergence remains robust to homogenisation from gene flow (Yeaman and Whitlock, 2011). Resultantly, there is no

'threat' posed to local adaptation by spreading selection more thinly over the genome; in Chapter 3 I demonstrate that this would only occur if the variance of mutational effects trades-off with the number of loci. Secondly, however, evidence from experimental evolution (Chapter 5) suggests that multidimensionality might alter the behaviour of adaptive alleles away from antagonism and toward conditional neutrality. With gene flow, these conditional neutrality specialist alleles flow between demes, owing to the lack of negative effects in the away environment, and local adaptation collapses. This places great emphasis on the role of gene flow between diverging populations; multidimensional divergent selection might be very effective at generating local adaptation in allopatry, but become increasingly vulnerable to homogenisation when gene flow is present.

In considering the effect of multidimensional divergent selection on broader ecological speciation, at several points in this thesis, I have referenced the two hypotheses that are outlined by Nosil *et al.* (2009) for how the dimensionality of divergent selection might impact speciation. In the 'stronger selection' (unidimensional) hypothesis, selection is concentrated onto few traits/loci but does not produce widespread divergence and may fail to couple reproductive barriers. In the 'weak multifarious selection' (multidimensional) hypotheses, selection is spread over many traits/loci, and may produce strong reproductive isolation via coupling of different barriers, but equally may be to dilute to overcome the homogenising force of gene flow. I propose that these models are easily reconcilable with a more holistic view of niche dimensionality as selection pressures (both biotic and abiotic) themselves evolve in step with diverging populations.

I argue that the key factor differentiating these patterns is time since divergence and the subsequent stage of speciation. Both in my simulation (Chapter 3) and experimental evolution (Chapters 5-6) studies, populations are suddenly exposed to dramatic shifts in their environment, with many selection pressures diverging in an instant. This likely does not happen as a matter of course in nature- it seems implausible that pairs (or higher groupings) of isolated populations should experience simultaneous shifts in ecological conditions across multiple selection pressures. This perhaps has only two exceptions- one being colonisation of two previously empty niches by branches of a source population, the other being an ecological shift in one environmental variable leading to shifts in others which, although not inherently independent, have independent effects on trait/genome evolution. However, progression towards ecological speciation- the establishment of local adaptation allowing the coupling of multiple barriers- happens over long evolutionary time periods in which environments change and may become increasingly multidimensionally divergent. Initial strong divergence in one selection pressure does not prevent other selection pressures diverging sequentially. It may be that over long periods of evolutionary time, environmental divergence becomes sequentially more multidimensional as populations sequentially couple more barriers to reproductive isolation.

A key piece of evidence for this is the study of *Zea mays* subspecies- notably populations that are significantly diverged but where speciation is not yet complete (Aguirre-Liguori *et al.*, 2019). In this example, the response to selection is multidimensional, but only two environmental axes (temperature and phosphorous concentration) differ significantly, and 71% of outlier SNPs were associated with just one axis. Additionally, there is evidence that an initial perturbation along a single axis will produce ripple effects that change other axes, particularly in biotic interactions where evolutionary equilibria become destabilised (Gilman, Nuismer and Jhwueng, 2012; Débarre, Nuismer and Doebeli, 2014).

Future directions- an integrated approach to multidimensionality

In this thesis, the application of complementary methodological approaches has enabled the identification of several factors which influence the evolution and maintenance of barriers to reproduction. My theoretical (Chapter 2) and simulation (Chapter 3) approaches have explored and tested a range of assumptions made regarding dimensionality. By constructing verbal theories and exploring them in a simulated environment, I have shown the importance of overall selection strength and mode of mutational variance in multidimensionality. By contrast, the effects we hypothesise in Chapter 5, namely the antagonistically-pleiotropic/conditionally-neutral behaviour of alleles would not have been identified by the simulations work (Chapter 3) due to the limiting assumptions regarding unrestricted pleiotropy. Within this thesis, I have demonstrated the benefits of combining multiple approaches in approaching outstanding questions in evolutionary biology, as I indeed advocate for in Chapter 4 (White, Snook and Eyres, 2020).

Ideally, future directions will take an increasingly integrated approach to exploring this research area, combining studies on natural populations with experimental evolution and theoretical work. Theoretical work might be easily expanded to consider how the effects of multidimensional divergent selection, even using a dilution mode, might vary given G-matrix constraints or more than two populations. Future experimental work might use a fully factorial design, perhaps mirroring the conditions described in Chapter 2. It is certainly encouraging to see that since the publication of Chapter 4 '*The Past and Future of Experimental Speciation*' (published online Aug 2019) there have been several experimental evolution studies on aspects of local adaptation and/or speciation which have adopted an evolve & resequence approach with gene flow (Bush *et al.*, 2019; Villa *et al.*, 2019; Tusso *et al.*, 2021; Wiberg *et al.*, 2021).

However, these laboratory-based experimental studies need to be combined with studies of natural populations and natural environments where examples of multidimensional divergent selection can be accurately quantified. Divergent selection-based experimental speciation studies almost exclusively examine scenarios of primary divergence, are conducted over short timescales, and are heavily biased towards identifying mechanisms for rapid build-up of reproductive isolation (e.g. Villa et al., 2019). Whilst these findings may be vital at the outset of speciation, experiments are less likely to identify equally important components that function later in the process of divergence. Integrating these approaches will be key to reaching a deeper understanding of multidimensionality.

Conclusion

To summarise, in this thesis, I have deconstructed some prior conclusions regarding how the dimensionality of divergent selection impacts local adaptation and speciation. My simulations have established how key facets of dimensionality interact with evolutionary forces to drive the evolution of local adaptation and extrinsic isolation. I have reviewed the use of experimental evolution for speciation research and provided advice and guidance for its continued use. I have performed the first evolution experiment to vary the dimensionality of divergent selection and combined it with evolve and resequence genomics. I have uncovered new insights into how dimensionality and its many nuances impact the evolution of local adaption, extrinsic isolation and the divergence of populations.

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Appendices

A: Chapter 3 supplementary material

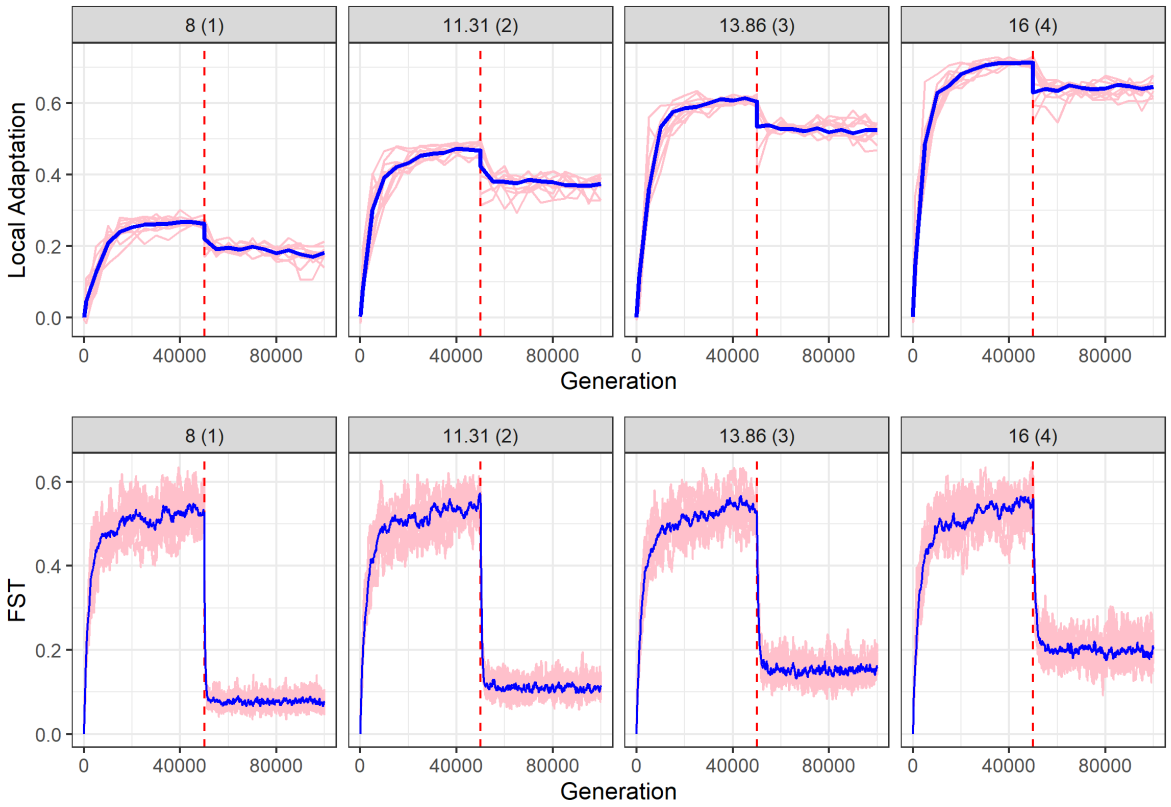

Figure S1. Local adaptation (top panels) and F_{ST} (bottom panels) across four values of *d*, the Euclidean distance between multidimensional trait optima (horizontal panels). Pink lines represent each metapopulation under divergent selection along just one axis. For comparison, the average value of all metapopulations under Additive multidimensionality is shown by the blue line (means of Additive populations in Figure 2). Values in brackets above each panel correspond to the dimensionality of divergent selection under additive multidimensionality with corresponding values of *d*.

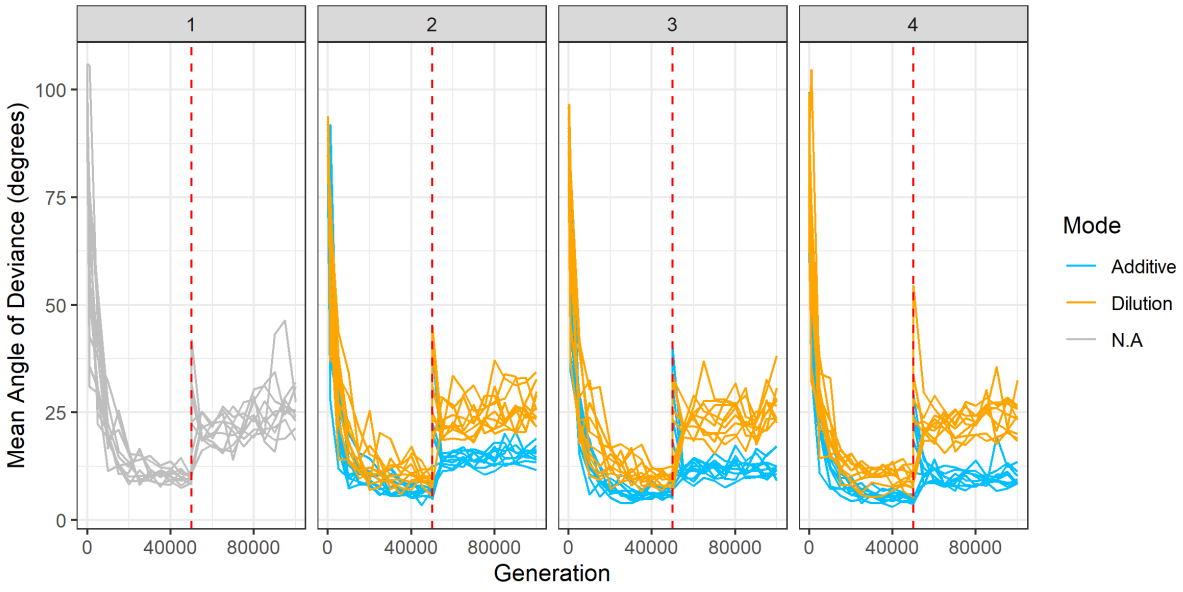

Figure S2: The mean angle of deviance over time, measured for each individual's multidimensional phenotype vector, averaged within each replicate. Red vertical dashed line represents the point of secondary contact.

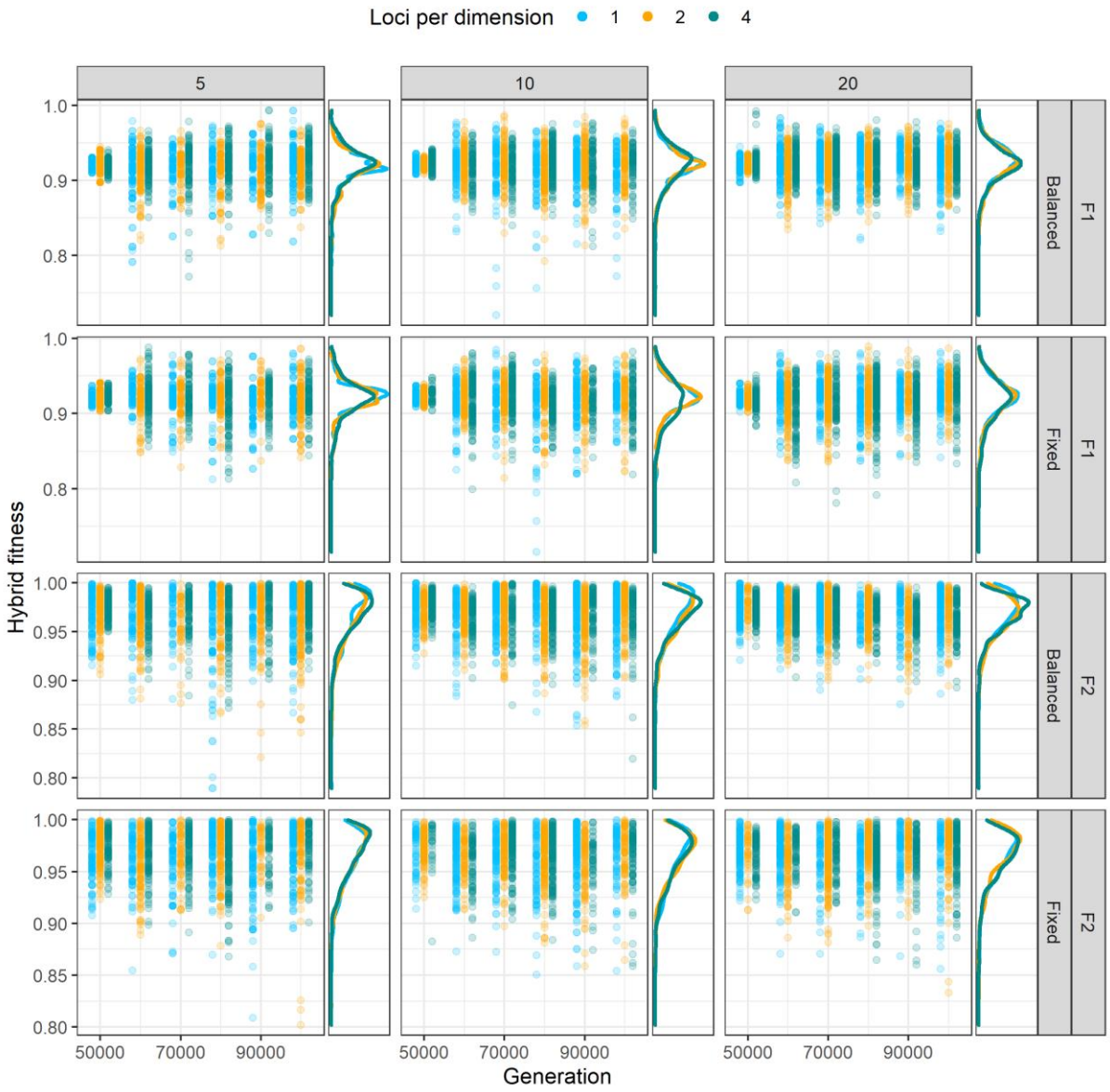

Figure S3: Fitness of F1 and F2 hybrids under different modes of mutational variance (both vertical panels) and variable numbers of quantitative loci (horizontal panels). Distribution of fitness values displayed in side panels (Landis, 2021).

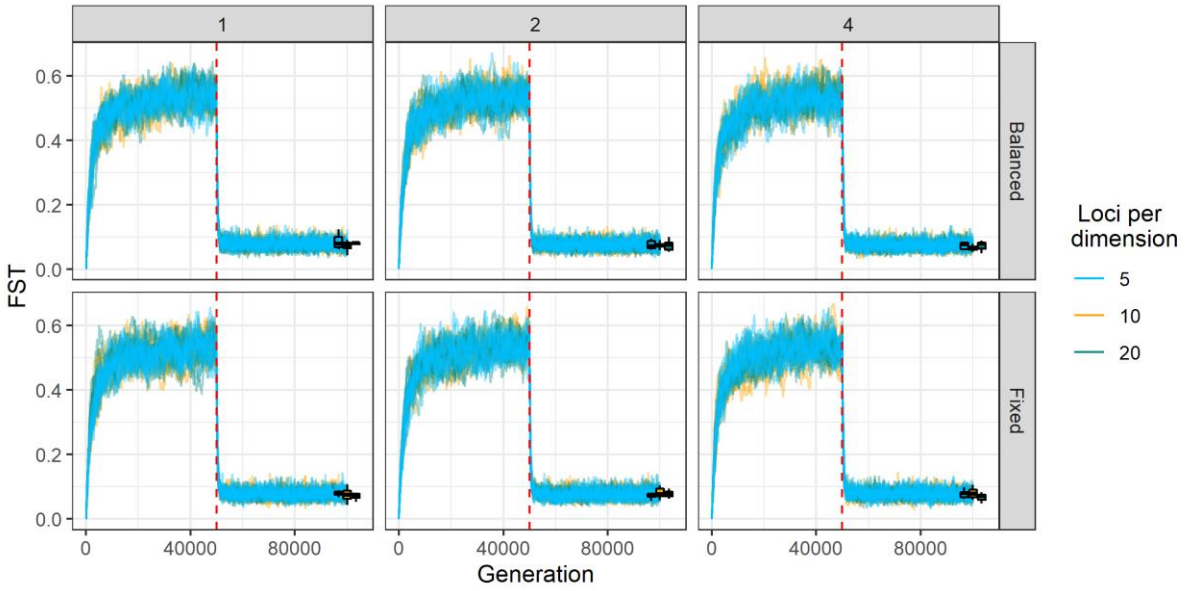

Figure S4: F_{ST} over time for different dimensionalities of divergent selection (horizontal panels) and different modes of mutational variance (vertical panels). Red vertical dashed line represents the point of secondary contact.

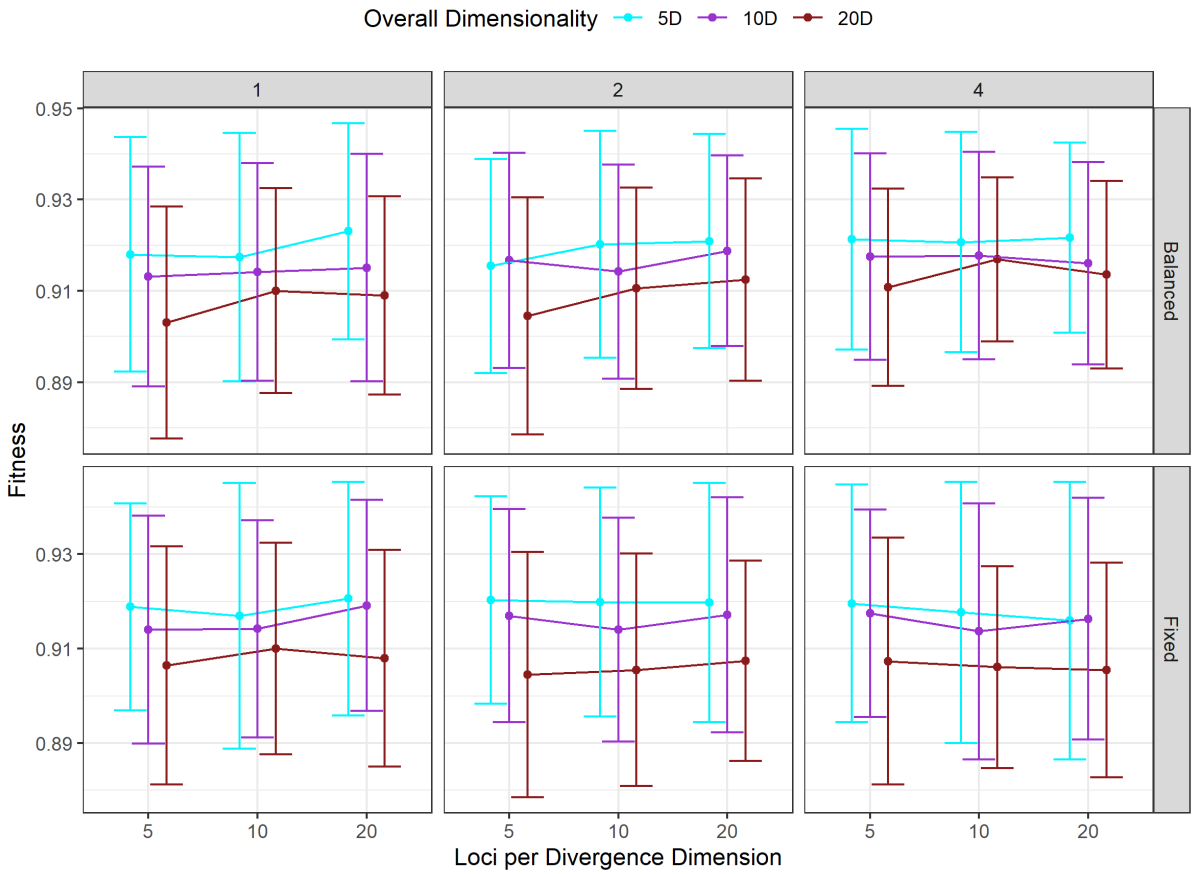

Figure S5: Fitness of F1 hybrids under variable dimensionalities of overall selection for different dimensionalities of divergent selection (horizontal panels) and different modes of mutational variance (vertical panels). Points show mean values for F1 hybrid fitness ± 1 standard deviation.

B: Chapter 5 supplementary material

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.

Methods

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.

Results

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.

Validation of SA results using the Local-Foreign contrast

Methods

Additional response variables were calculated to quantify differences in fitness between environments. The 'local-foreign' (LF) contrast(Kawecki and Ebert, 2004; Blanquart *et al.*, 2013) 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. Using the same notation as in the Main Text, these were calculated as;

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

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

Furthermore, SA measures of local adaptation can be positive because of large asymmetry between the two estimates of LF within a metapopulation, in which case there would be increased gene flow from one deme to another(Kawecki and Ebert, 2004; Blanquart *et al.*, 2013). To test whether LF asymmetry might be driving patterns of SA, we also fitted models for the absolute difference between LF statistics within each metapopulation (LF Asymmetry = |LFa – LFb|).

Results

We repeated the analysis of the effects of cycle and dimensionality using the LF measure of local adaptation. The pattern of results remained consistent with the SA contrast; an initial increase of local adaptation in multidimensional metapopulations which later declined, and a slower increase in local adaptation in unidimensional metapopulations (Figure S4; Table S4). 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).

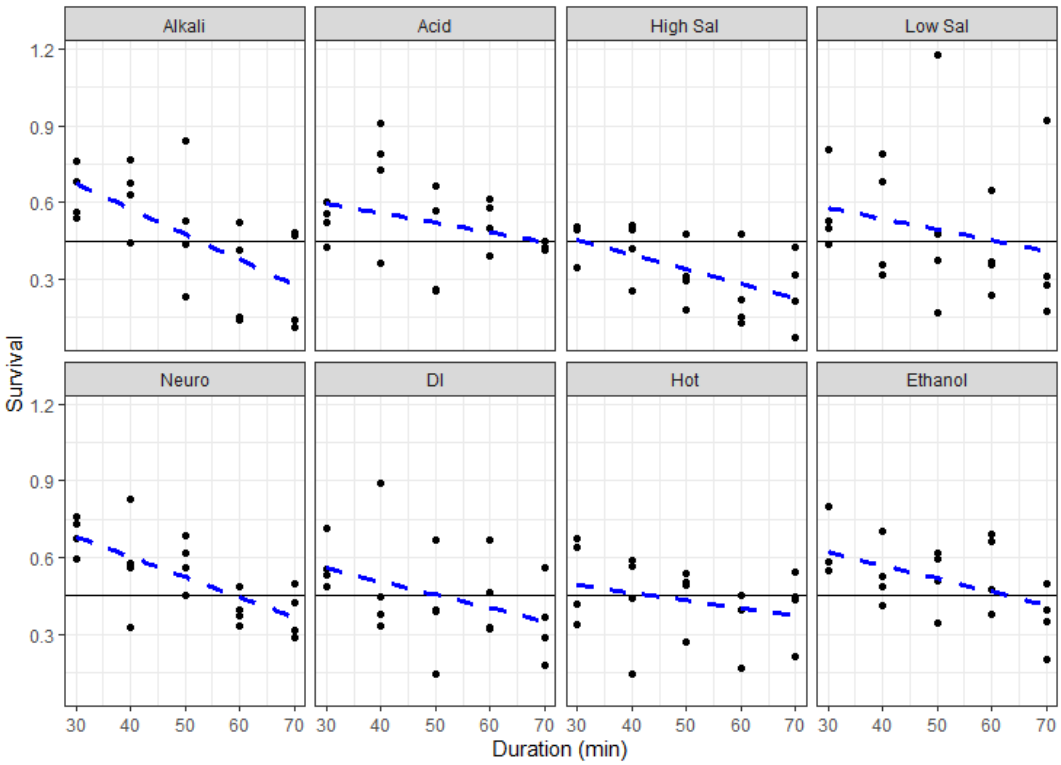

Figure S1: Pilot experiments. Survival rates of rotifer populations following shocks of variable duration from 8 independent stressors across four cycles. Black solid line represents the target survival value of 0.45 for use in experimental evolution. Blue dashed line represents the fitted relationship between duration and survival for each stressor.

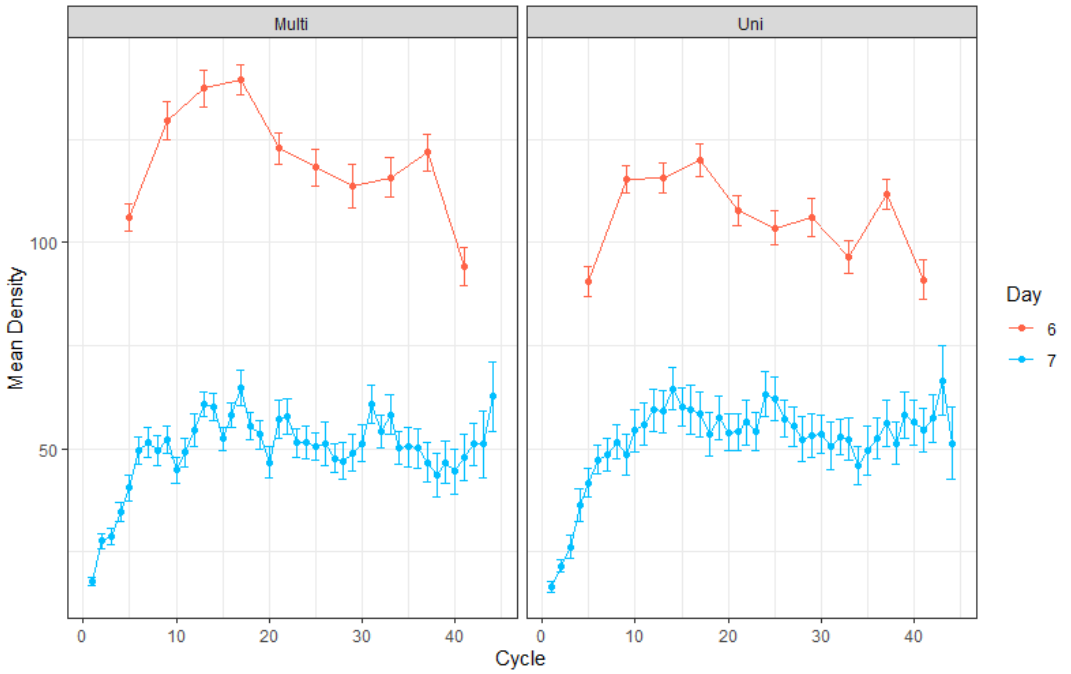

Figure S2: Laboratory adaptation. Mean population densities (rotifers per ml) ± one standard error at two timepoints within a cycle, grouped by dimensionality treatment. Day 6 counts taken immediately pre-shock treatment every four cycles, day 7 counts taken every cycle 24 hours post-shock treatment.

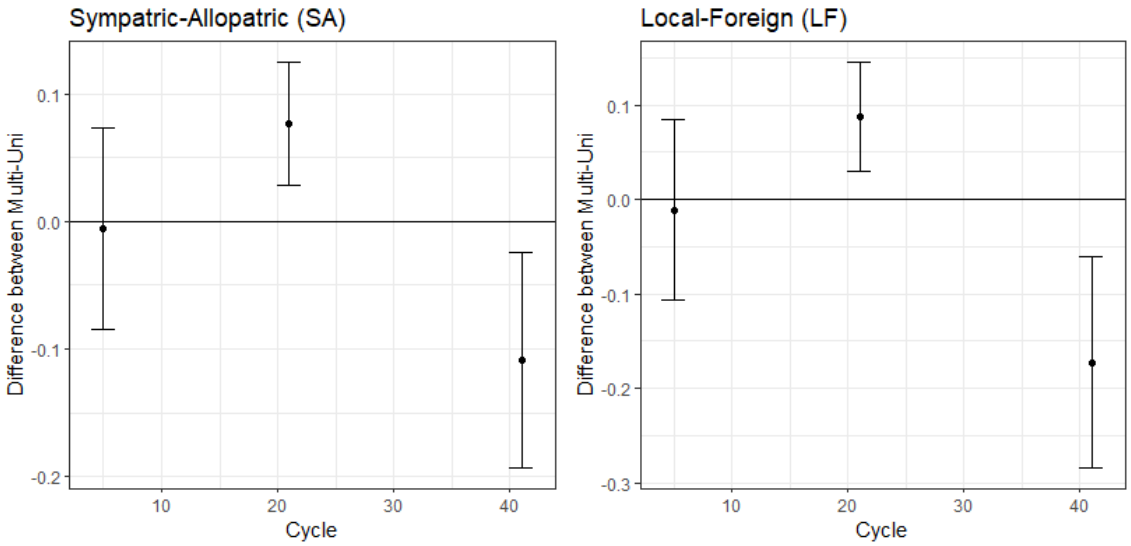

Figure S3: Contrasts between estimated marginal mean SA/LF values of metapopulations under unidimensional and multidimensional divergent selection at 3 timepoints. Estimates were derived from model with the square of cycle, stressor pair and their interaction as explanatory variables. Left plot uses an overall SA contrast, right plot uses individual LF contrasts.

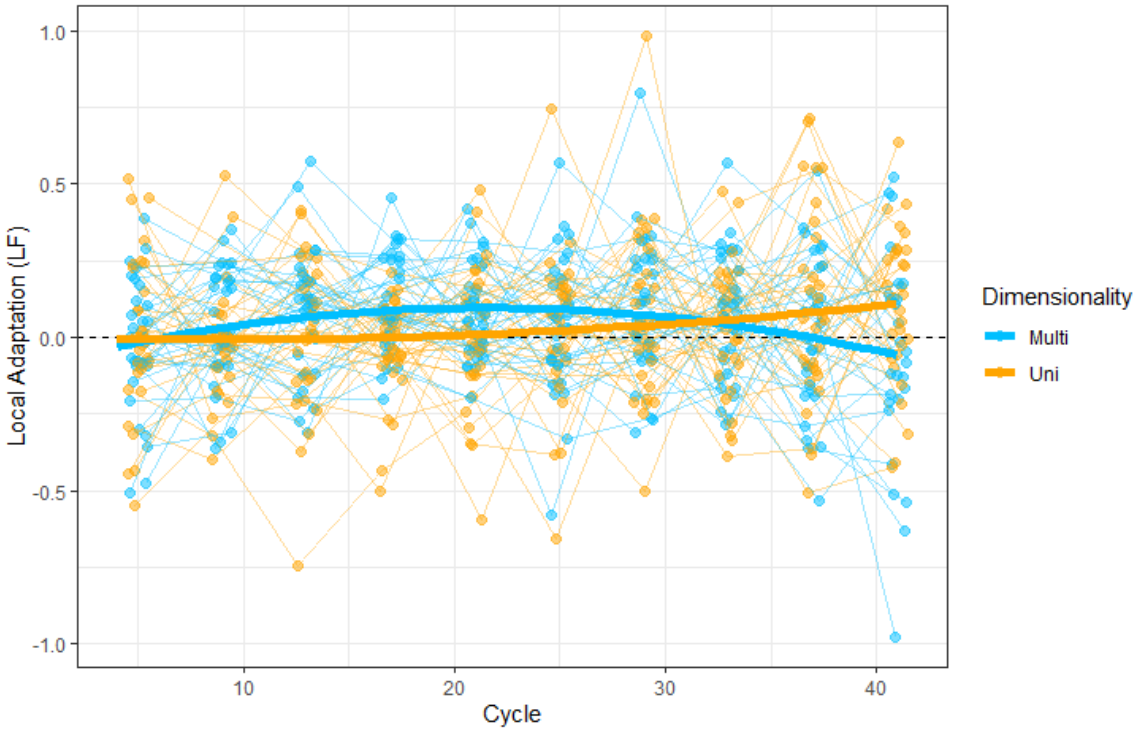

Figure S4: Local-foreign measures of local adaptation. Curved thick lines display model fit using the first and second order polynomial of cycle, dimensionality, and their interaction. Random effects not presented in this Figure.

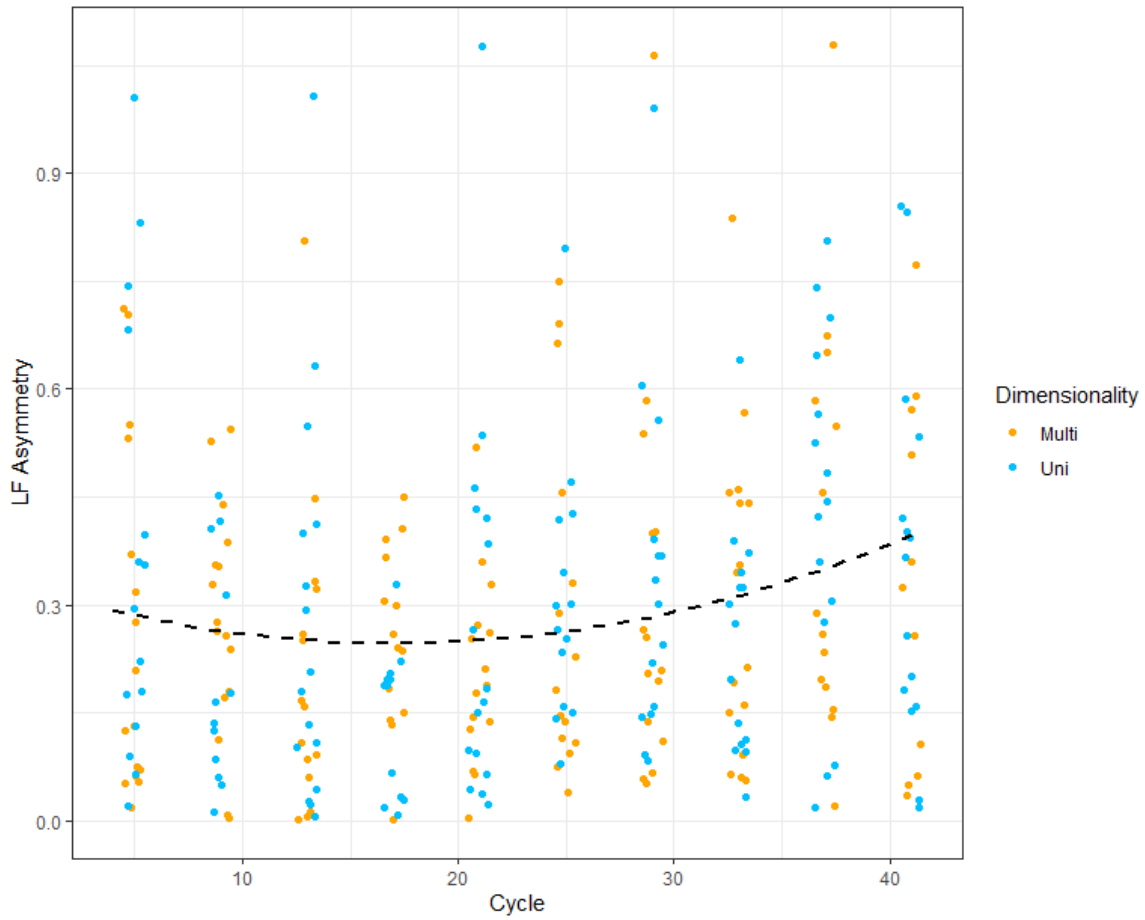

Figure S5: Asymmetry of local adaptation (absolute difference of local-foreign comparisons between demes in a metapopulation) in relation to time (cycle number), coloured by dimensionality. Black dashed line shows quadratic effect of cycle on asymmetry. No interaction with stressor-pair or dimensionality was identified.

Alkali	Acid	HS	LS	Neuro	DI	Hot	Ethanol
53	69	31	61	59	51	44	63

Table S1: Incubation duration (min) for all stressors used in this experiment.

Variable	Estimate	St. Error	DF	t value	p-value
Intercept	0.052	0.012	29.71	4.16	2.47×10^{-4}
Cycle	0.061	0.186	38.14	0.33	0.744
$Cycle^2$	-0.520	0.177	70.30	-2.94	4.4×10^{-3}
Unidimensional	-0.021	0.018	29.80	-1.20	0.239
Cycle : Unidimensional	0.498	0.260	36.59	1.92	0.063
$Cycle^2$: Unidimensional	0.775	0.244	65.02	3.18	2.3×10^{-3}

Table S2: Fixed effects from a linear mixed effects model explaining variation in SA using the second order polynomial of cycle, dimensionality, and their interaction. t values derived using Satterthwaite's method from the R package '*lmerTest*'(Kuznetsova, Brockhoff and Christensen, 2017).

Cycle	Estimate	St. Error	DF	Lower CI	Upper CI	t ratio	p value
0	-0.00593	0.0383	23.97	-0.085	0.073	-0.155	0.878
21	0.0763	0.0235	24.20	0.028	0.125	3.248	0.003
41	-0.108	0.0409	23.01	-0.193	-0.024	-2.653	0.014

Table S3: Contrasts between estimated marginal means of unidimensional and multidimensional metapopulations' local adaptation (SA) at three time points.

Variable	Estimate	St. Error	DF	t value	p-value
Intercept	0.041	0.014	64.47	3.02	3.6×10^{-3}
Cycle	-0.306	0.344	44.69	-0.89	0.378
$Cycle^2$	-1.177	0.330	181.06	-3.57	4.5×10^{-4}
Unidimensional	-0.012	0.019	65.14	-0.61	0.546
Cycle : Unidimensional	1.190	0.486	45.04	2.44	0.018
$Cycle^2$: Unidimensional	1.509	0.464	181.88	3.25	1.4×10^{-3}

Table S4: Fixed effects from a linear mixed effects model explaining variation in LF using the second order polynomial of cycle, dimensionality, and their interaction. t values derived using Satterthwaite's method from the R package '*lmerTest*'. Additional degrees of freedom compared with Table S2 are due to there being two measurements per metapopulation, whereas SA aggregates these into a single measure.

Cycle	Estimate	St. Error	DF	Lower CI	Upper CI	t ratio	p value
0	-0.011	0.046	23.79	-0.107	0.085	-0.238	0.814
21	0.088	0.028	24.06	0.030	0.146	3.149	4.33×10^{-3}
41	-0.173	0.054	23.93	-0.284	-0.061	-3.188	3.96×10^{-3}

Table S5: Contrasts between estimated marginal means of unidimensional and multidimensional metapopulations' local adaptation (LF) at three time points.