

1 **Environmental variation and biotic interactions limit adaptation at ecological margins:**
2 **lessons from rainforest *Drosophila* and European butterflies**

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17 **Summary**

18 Models of local adaptation to spatially varying selection predict that maximum rates of
19 evolution are determined by the interaction between increased adaptive potential due to
20 increased genetic variation, and the cost genetic variation brings by reducing population
21 fitness. We discuss existing and new results from our laboratory assays and field transplants
22 of rainforest *Drosophila* and UK butterflies along environmental gradients, which try to test
23 these predictions in natural populations.

24
25 Our data suggest that: (1) Local adaptation along ecological gradients is not consistently
26 observed in time and space, especially where biotic and abiotic interactions both affect
27 gradient steepness and genetic variation in fitness; (2) Genetic variation in fitness observed
28 in the laboratory is only sometimes visible to selection in the field, suggesting that
29 demographic costs can remain high without increasing adaptive potential; (3) Antagonistic
30 interactions between species reduce local productivity, especially at ecological margins. This
31 steepens gradients and may increase the cost of adaptation by increasing its dimensionality.
32 However, where biotic interactions do evolve, rapid range expansion can follow.

33
34 Future research should test how the environmental sensitivity of genotypes determines
35 their ecological exposure, and its effects on genetic variation in fitness, to predict the
36 probability of evolutionary rescue at ecological margins.

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38
39 *Key words: Range margins, genetic load, local adaptation, genetic variation, species*
40 *interactions, phenotypic plasticity, field transplants*

1 Introduction

2

3 Understanding how variation in genotypes, the phenotypes they create, and their
4 interactions with the environment and other phenotypes determine limits to evolution is
5 fundamental for identifying how biodiversity is generated and maintained. Without
6 constraints to local adaptation, populations would continue to evolve at their ecological
7 margins and occupy potentially unlimited areas of geographical and environmental space.

8

9 Rapid evolution into novel niches is certainly seen in some circumstances, such as the
10 colonisation of the land by plants, and the recolonisation of the ocean by terrestrial
11 mammals. However, limits to evolution are always observed at some spatial and temporal
12 scale, suggesting that adaptive radiation is associated with specialisation: alleles and
13 genotypes evolve behaviours that for some reason prevent their continual expansion into
14 novel environments. Ecological communities therefore consist of populations with
15 specialised phenotypes that transmit alleles effectively only within some habitats, and
16 species that only exist for short periods of time. Understanding the factors that allow and
17 (then) limit evolution is one of the most fundamental unanswered questions in biology.
18 When and why do narrow niches evolve? Why do species replace each other along
19 ecological gradients? And what determines how species' interactions evolve, especially
20 when such evolution allows species to co-exist in communities rather than replace each
21 other? Answering these questions is critical for understanding how organisms diversify
22 across space and time, as well as for understanding the persistence of ecological
23 communities in the increasingly unfamiliar environments generated by rapid climate change
24 and biodiversity loss [1].

25

26 In this review we summarise recent evolutionary models of ecological margins that
27 integrate population genetics with population ecology. We then explore what our studies of
28 rainforest *Drosophila*, and UK butterflies along ecological gradients tell us about how
29 environmental variation and heritable variation interact to determine patterns of local
30 adaptation and population density. In particular, we consider the ecology and evolution of
31 responses when phenotypes interact with the abiotic environment, as well as with members
32 of their own and other species.

33

34 Models of adaptation at ecological and genetic limits

35 The maximum rate of adaptation of any trait is determined by the amount of additive
36 genetic variance in fitness [2]. However, such genetic variance in fitness comes at a cost in
37 terms of population mean fitness because it also means that not all phenotypes match the
38 local optimum [3]. In later papers, Haldane [4, 5] introduced the concept of genetic load to
39 predict when and where alleles replace each other in time and space. Haldane [4] first
40 suggested that gene flow from the centre of a range causes maladaptation at ecological
41 margins, leading to a kind of "maladaptation" or "spatial lag" load, although the concept of
42 "lag load" was more clearly outlined by Maynard Smith [6], and then applied in terms of
43 adaptation to a temporally moving optimum by Pease *et al.*, [7] and Lynch and Lande [8].

44

45 Haldane [5] also asked how changing the frequency of one allele relative to another reduces
46 population mean fitness for a given number of generations (determined by the starting
47 allele frequency and selection strength). Although total "substitution load" is determined

1 only by the starting allele frequency, the increase in frequency (and the number of
2 generations required, determined by selection strength) also increases mean fitness (and
3 therefore population size once allele frequencies change) because it moves the population
4 trait mean closer to the local optimum.

5
6 Bulmer [9] then distinguished substitutional load from “standing or variance load”, which is
7 the cost in terms of population mean fitness of variance around the phenotypic optimum.
8 As we will see, the fact that the genetic component of standing load determines adaptive
9 potential, and is increased by gene flow along clines, but also reduces local density (and
10 therefore the efficiency of selection compared to drift), is the focus of the latest population
11 genetic models of ecological margins, and state-of-the-art predictions of where and when
12 adaptation will fail to track a changing optimum [10; Polechová, this issue].

13
14 In summary therefore, and in terms of understanding limits to adaptation, there are three
15 types of “genetic load”, all of which maintain actual population density below that of the
16 local carrying capacity [see 11 for a longer review].

- 17
18 1. *Standing or variance load*: The cost of genetic variation around the optimum,
19 determined by the amount of genetic variation within a population, and the strength
20 of selection on the trait (i.e. the fitness cost of being a certain distance from the
21 optimum). Such genetic variation is caused by mutation, gene flow and
22 recombination, and removed by stabilizing selection provided the local trait mean is
23 close to the optimum.
- 24 2. *Substitution load*: The cost of changing allele frequencies and trait values under
25 directional selection (i.e. after a change in the optimum), defined as the number of
26 deaths due to selection that are required to create changes in allele frequencies and
27 trait means.
- 28 3. *Maladaptation or lag load*: Defined by the size of the mismatch between the trait
29 mean and the local optima, maladaptation load represents the demographic cost of
30 evolutionary lags, for example where gene flow introduces sufficient maladapted
31 genotypes to prevent the population tracking the local optimum, or where the
32 optimum changes rapidly in time.

33
34 Above a critical rate of spatial environmental change, these components of load prevent the
35 population tracking the local optimum, because the amount of genetic variation needed to
36 track the optimum induces too great a demographic cost for selection to overcome genetic
37 drift as well as other stochastic effects on allele frequency (Fig 1a). At such points, as a
38 population fails to track the optimum in space or time, its lag load increases, causing local
39 reductions in population density. In terms of adaptation to spatial gradients, this effect is
40 extremely abrupt because, at such locations, *asymmetrical* gene flow from neighbouring
41 (and well-adapted) high density populations can then swamp adaptation, increasing the lag
42 load still further. At such key points along an ecological gradient, these processes generate a
43 range margin where density is too low for locally adapted alleles to increase in frequency,
44 even though (in fact, because) they are continually provided by gene flow from larger
45 populations within dispersal range.

1 Models of ecological margins that connect population genetics with population ecology in
2 this way provide an important framework to predict when and where evolution in response
3 to environmental change is possible [see reviews by 1, 12, 13-15]. Such models of ecological
4 margins can usefully be split into two types:

5
6 **(i) Ecological models of adaptation** assume a global or fundamental limit to niche width,
7 where the phenotypes accessible by evolution cannot prevent low density towards a range
8 margin [16, 17], or in some habitat patches compared to others [18]. Such models therefore
9 impose a higher demographic (and fitness) benefit to adaptation at the centre of a range,
10 because these regions (or some patches) are more productive than at the margins, even if
11 the optimum is matched everywhere. The role of such fundamental ecological limits in
12 predicting evolution is discussed in Chevin and Hoffmann [19] as variation in “habitat
13 quality”. When exploring these kinds of models, a key feature of their behaviour is that
14 adaptive evolution is inherently limited in its scope, because the phenotypic variation
15 available via gene flow and mutation does not allow populations to be equally productive
16 across all environments included in the model.

17
18 **(ii) Population genetic models of adaptation** explore evolution within a region of ecological
19 parameter space where the range of phenotypes available to selection could make
20 population growth equal everywhere. In such models, in contrast to ecological models, the
21 density gradient is entirely dependent on the efficiency of local adaptation. However, local
22 adaptation can only be achieved where evolution can vary allele frequencies sufficiently so
23 that all populations match the local optima, generating an infinitely wide niche with equal
24 population density. As we shall see, when and where this can be achieved depends on the
25 rate of population growth relative to the demographic cost of standing and substitutional
26 load. This determines whether selection can overcome genetic drift, and therefore the
27 maximum gradient steepness for which local adaptation can provide the necessary change
28 in trait mean.

29
30 *Connecting ecological and population genetic models:* Ecological models (i) explore the
31 consequences of local adaptation given underlying ecological constraints on evolutionary
32 responses. By contrast, population genetic models (ii) ask when and where adaptation, even
33 if theoretically possible, can sustainably match the local gradient. Population genetic models
34 therefore essentially operate somewhere within the region of parameter space considered
35 in ecological models.

36
37 However, additional constraints to adaptation emerge in population genetic models if real-
38 world ecological and genomic complexity are included. For example, trait correlations or
39 recombination may make certain regions of phenotypic space harder to reach than others,
40 especially for traits that are not highly polygenic [20]. Alternatively, ecological trade-offs can
41 limit evolution in particular areas of the ecological gradient if adaptation in one trait is
42 limited by fitness effects on other traits – for example, if increased thermal stress increases
43 the risk of disease infection, or increased competition reduces fecundity. Such trade-offs
44 between biotic and abiotic interactions will generate locally steep ecological gradients [see
45 e.g. 21], in regions of phenotypic space where adaptive potential does not increase in direct
46 proportion to segregating genetic variation, even though such variation continues to
47 increase standing load. In addition, if adaptation is demanded along more dimensions [see

22], the demographic cost of allelic substitution needed to access these phenotypes will be greater for a given amount of selection, leading to slower and more costly trait evolution (and increased size and duration of lag load). Here, understanding the population genetics and demography of range limits intersects with Fisher's geometric models of adaptation [2], and later explorations of the effects of phenotypic complexity on adaptive rates, demographic costs and genomic architecture by Orr [23], recently reviewed by Connallon and Hodgins [24].

Understanding evolutionary limits within a population genetic and ecological framework

Barton [25] extended Haldane's models to allow gene flow along a spatial gradient in trait mean to increase genetic variation in traits and in fitness, so increasing the adaptive potential of the population, as well as introducing standing (and potentially lag) load. Models that include this increased adaptive potential predict adaptation along virtually any ecological gradient, up to a critical limit where the standing load required to allow populations to match the gradient in trait mean required equals their maximum growth rate at the optimum (i.e. R_{\max}).

Bridle *et al.* [26] and Polechová and Barton [10] then showed that the effects of finite population size (K) prevent local adaptation before this critical limit, by reducing the effectiveness of selection relative to genetic drift. As the genetic load increases, the population either becomes extinct everywhere (in the case of a linear gradient), or is able to track the local optima and persist only in regions that are below a critical steepness (in the case of a non-linear gradient) (Fig 1a, left panel).

In addition to the effect of a steepening gradient, Bridle *et al.* [27] revealed an additional contribution in situations where the density of adjacent populations is not reduced by standing load (Fig 1a, right panel). In this formulation of spatially-varying selection, a linear ecological gradient is interrupted by a flat region that does not demand a change in trait mean (e.g. by an elevational gradient within a latitudinal one, or an area of local extinction of natural enemies or competitors). Within such flat regions, gene flow no longer increases standing load, meaning that local population density increases, almost to carrying capacity.

These highly productive regions along the ecological gradient generate strongly asymmetrical gene flow that rapidly increases variance in adjacent populations, causing densities to collapse, and making local adaptation much less likely. Local regions of high population density created in this way within linear gradients seem to introduce additional stochasticity that prevents the establishment of clines in trait means, because (in contrast to the steepening and linear gradients), populations evolving from a central point often fail to expand beyond them into the linear gradient, even at parameter conditions where a linear gradient reliably permits range expansion and persistence. These results suggest (somewhat counter-intuitively) that local improvement of habitat at range centres may constrain adaptation along an ecological gradient, assuming spatial gradients are stable [27], by increasing the amount and consequences of gene flow for maladaptation load and standing load.

Polechová and Barton [10] (Fig 1b) analyse the effect of the steepness of a one-dimensional ecological gradient on evolutionary responses, and decompose the effects of gene flow

1 along the gradient, the strength of stabilising selection, and the R_{\max} into a single dimension
2 [A] **the efficacy of selection relative to drift**. This dimension is then compared to a second
3 dimension [B], **the effective environmental gradient**, which relates to the slope of the trait
4 mean along the environmental gradient that populations need to achieve to track the
5 optimum. Adaptation to very steep environmental gradients [B] is possible where selection
6 is highly effective at changing traits [A]. In addition, when movement in two dimensions is
7 possible along a one-dimensional ecological gradients (for example an elevational gradient),
8 gene flow has a more positive effect on local adaptation, provided conditions vary
9 sufficiently smoothly in space, and dispersal occurs via diffusion rather than across long
10 distances [28, 29].

11
12 Dimension [B] can be extended to include non-linear or patchy gradients, due to changes in
13 the environmental gradient itself [e.g. changing biotic interactions; 30]. However, changes in
14 [B] will also be affected by changes in dispersal along it, or due to particular forms of genetic
15 variation that allow easier access to the optimum phenotype (e.g. epistasis or dominance).
16 In such situations, a given amount of genetic variation could cause local adaptation with
17 relatively little standing load. Alternatively, if more resources are available at some locations
18 for population growth, selection would become more efficient relative to drift [A]. By
19 contrast, where there is little suitable genetic variation that allows local adaptation, or
20 where the genetic variation required carries a large standing load, or where populations
21 have a low rate of increase at the optimum (R_{\max}), adaptation will be prevented at a lower
22 value of [B] than expected.

23
24 The key empirical question posed by these models is how close natural populations are to
25 the critical line in Fig 1b, which defines their ability to either invade novel ecological regions,
26 or to survive where they are in novel environmental regimes. **Box 1** summarises the
27 ecological and genetic parameters that affect whether a given population can expand its
28 ecological niche (i.e. track a changing optimum).

29
30 To understand the ability of this conceptual framework to predict the population genetics of
31 local adaptation, we need to study environmental gradients in the field that affect mean
32 fitness, and populations that straddle the line in Fig 1b, by being associated either with
33 gradual or abrupt densities along these gradients. In particular, we need to understand the
34 stability of spatial environmental gradients in time, the effect of local adaptation along them
35 on density, and how such gradients affect alleles and genotypes, particularly in terms of
36 variation in fitness (and its demographic cost). Such information can test whether [A] and
37 [B] scale in the way predicted, and how this determines in which populations and
38 communities such adaptive limits are close to being exceeded.

39
40 Below we summarise lessons from our recent attempts to test theoretical models of
41 ecological margins empirically, firstly using Australian *Drosophila*, and then in UK butterflies.

42

43 **(1) Testing the population genetics of ecological margins in rainforest *Drosophila***

44 The *Drosophila* of eastern Australia provide a powerful system to test the limits to
45 adaptation across ecological gradients. They consist of more than 300 identified species,
46 many of which are restricted to the tropics [31]. They have diverse distributions, sexual
47 behaviour and ecology, which have been well-studied [31-33]. Many species are distributed

1 along both elevational and latitudinal gradients, suggesting limits to adaptation at ecological
2 margins, as well as local adaptation, given that several species show latitudinal clines in
3 some traits [e.g. 34, 35, 36]. In addition, extensive data are available on trait variation [37,
4 38], sexual behaviour [39, 40], phylogenetic relatedness [41, 42] and on covariances
5 between traits [e.g. 43], including the fitness costs of stress resistance [44, 45].

6
7 Elevational gradients in tropical Queensland are characterised by predictable changes in
8 temperature along tens of km that are as large as those seen across 1000s of km of latitude.
9 Variation in these abiotic factors is also associated with changes in the distributions and
10 abundances of species in these diverse *Drosophila* communities. This motivated our
11 sampling and detailed study of two closely-related species of *Drosophila* within the
12 montium subgroup (*D. birchii* and *D. bunnanda*). These two species show distinct
13 distributions and specific ecological requirements, but (unlike many tropical insect species)
14 can be reared on minimal media in the lab, and have generation times of c. 12-14 days at
15 25°C in the laboratory. *Drosophila bunnanda* is found at low elevations (below 500m) within
16 the distribution of *D. birchii*, which is common at higher elevations, but typically becomes
17 rarer above 900 m and below 500m elevation. In addition, where *D. bunnanda* is present, *D.*
18 *birchii* density is reduced, suggesting antagonistic (probably competitive) biotic interactions.

19
20 Our first set of experiments (2004-5 and 2010-12) set out to test some of the key
21 predictions of species' range models using *D. birchii*. In particular, we wanted to explore the
22 consequences of ecological gradient steepness for clinal divergence, the effects of such local
23 adaptation on population density, and the consequences of genetic variation for
24 demographic load along shallow compared to steep ecological gradients.

25
26 Our second set of experiments (2017-19) was motivated by evidence for trade-offs between
27 abiotic and biotic stress resistance (developmental, physiological and genomic), and allowed
28 us to test whether gradients become locally steep where *D. birchii* and *D. bunnanda* overlap
29 in particular abiotic contexts, causing locally increased genetic load and reduced genetic
30 variation available for adaptation, and (therefore) abrupt ecological margins.

31
32 Our main findings are summarised below:

33
34 *(a) Local adaptation is observed along shallow but not steep ecological gradients*

35 Bridle et al. [11] established isofemale lines of *D. birchii* from field collections in 2004 along
36 two environmental gradients with differing rates of change in elevation (Paluma 871 m in
37 3.7 km; and Kirrama; 700 m in 10 km; Fig 2a) and compared population densities to genetic
38 divergence among elevations in heat and cold tolerance, body size, and starvation and
39 desiccation resistance, under constant conditions in the laboratory (Fig 2b). *D. birchii*
40 densities along both elevational transects were low at low (warm) elevations, peaked at
41 around 600-850 m, and declined again above 850 m (cooler environments), confirming that
42 these gradients represent the species' entire ecological range at these latitudes. Despite
43 apparently abundant genetic variation in most fitness traits across these gradients, stress-
44 resistance assays in the lab revealed evidence of adaptive divergence in only one trait: time
45 to recovery following a cold shock, which was significantly shorter at high (cool) elevations,
46 consistent with adaptation to the local thermal environment. In addition, such adaptive
47 divergence was observed along the shallower elevational gradient (Kirrama) but not along

1 the steeper one (Paluma), providing some evidence that gradient steepness limits adaptive
2 responses.

3
4 *(b) Local adaptation increases local population density provided genetic load is not too high*
5 Bridle et al. [11] also observed a greater reduction in population density at the warm end of
6 the steeper gradient compared to the shallower gradient (along which local adaptation was
7 observed) (Fig 2b). In addition, residuals at a given site from the regression line of cold
8 tolerance against elevation at Kirrama were negatively correlated with among-isofemale
9 line variation (broad sense heritability) in cold tolerance, suggesting that locally high genetic
10 variation improves tracking of the trait optimum [11]. These data are consistent with
11 Polechová and Barton [10] predictions that: (i) tracking the optimum of a trait across space
12 reduces lag load, which allows higher densities for a given elevation; (ii) local adaptation
13 along ecological gradients fails below a critical level of adaptive potential (assumed to be
14 correlated with genetic variation in this case). However, an alternate (and equally
15 interesting) explanation for this pattern could be that local adaptation (in terms of trait
16 means that matches the local optima) persists in both cases, but the standing load is higher
17 at sites along the steeper gradient, which reduces population density without any lag load.

18
19 *(c) Clines for traits are not consistent across different elevational gradients or years*

20 These findings from our 2004 collections motivated collections in 2010-11 at Kirrama and
21 Paluma and across two additional elevational gradients (Fig 2; Fig 3) to try to test the
22 relationship between gradient steepness, local adaptation to local trait optima, and genetic
23 (co)variation in stress resistance and fitness traits. Laboratory assays across elevation in
24 2010-11 indicated no clinal divergence across elevation in any individual fitness traits (Fig
25 2b; 4a), apart from a slight but significant reduction in wing size with elevation. However,
26 additive genetic variances were moderate to high in all traits (Fig 4b, see SI for methods). In
27 addition, field population density was reduced at low elevations compared to 2004, with
28 higher density at central elevations (Fig 2b), supporting the idea that local adaptation in cold
29 tolerance in 2004 reduced the density gradient towards the ecological margin, while
30 increasing standing load at the centre.

31
32 Despite observing no divergence in stress resistance traits, at two of the three gradients
33 studied in 2011, consistent clines in laboratory productivity at 25°C were observed, with
34 lines from higher elevations being 1.7 – 3.3 times as productive as those collected from low
35 elevation sites (Fig 3). This suggests either that high elevation increases egg to adult
36 mortality in the field (generating increased fecundity under lab conditions), or that
37 increased investment in (for example) larval competitive ability or immunity at low
38 elevation sites reduces their laboratory productivity.

39
40 The lack of consistency in clines across years (as well as across traits and gradients) suggests
41 that changes in environmental variation in space and time create sporadic local adaptation,
42 which may maintain standing variation in traits and fitness (see j below), especially if gene
43 flow is high along these gradients, as suggested by low levels of divergence in neutral alleles
44 among these sites [46]. According to [A], such gene flow along a one-dimensional gradient
45 will increase standing variance (maintaining high levels of evolutionary potential, but also
46 genetic load) until the environmental gradient [B] becomes sufficiently shallow, or
47 productivity at the optimum (R_{max}) increases sufficiently to allow local population density to

1 increase for a given amount of standing variance, which generates local adaptation because
2 selection overcomes drift. At the same time, locally steeper gradients may increase the
3 strength of selection relative to drift, given the increased rate of arrival of maladapted
4 genotypes per generation.

5
6 Such temporal variation in selection strength and gradient steepness, at least at the scales
7 where gene flow and selection interact, make theoretical models challenging to test in the
8 field, especially where selection is averaged across many generations, as well as varying in
9 intensity substantially within a genotype's lifetime. Such concerns, as revealed by our
10 studies on *D. birchii* (even across its entire ecological distribution at a given latitude), may
11 explain why celebrated empirical studies of selection in natural populations often study
12 traits that increase fitness in response to sudden and pervasive changes in the environment,
13 such as heavy metal or air pollution [47, 48], or seasonal and spatial pesticide application
14 [49, 50]. Although highly illuminating, such examples of strong directional selection, where
15 responses involve alleles of large (and typically dominant) effect, probably do not reflect
16 how limits to adaptation in polygenic fitness traits shape population persistence and
17 species' distributions more generally.

18
19 *(d) Genetic variation does not decline at ecological margins, even when local density is low*

20 In Figure 4 we present new results from a lab experiment (see SI for methods) that crossed
21 lines collected in 2011 from sites along elevation gradients to estimate spatial variation in
22 trait means and density of *D. birchii* (Fig 4a), which were then related to additive genetic
23 variation in body size (performance) and cold tolerance (stress resistance) (Fig 4b). These
24 data indicate no reduction in narrow-sense heritability for either body size or cold tolerance
25 towards warm or cold ecological margins, and that levels of genetic variation remain high
26 (h^2 between 0.08 and 0.28 for each gradient for both cold tolerance and wing size) even
27 within very low-density populations at ecological margins. As predicted by the models,
28 these data suggest that spatial genetic variation in traits towards the margin is dominated
29 by gene flow among populations, rather than by local population density.

30
31 *(e) No evidence for genetic correlations between stress resistance and fitness:*

32 Surprisingly, these new data (Fig 4b) also provide no evidence for the expected negative
33 correlations between stress resistance (cold tolerance) and performance (body size),
34 suggesting no detectable cost (under lab conditions) of increased stress resistance in terms
35 of reducing resources allocated to larval growth. These data therefore do not detect trade-
36 offs that would make parts of these elevational gradients locally steeper, as we might
37 predict where density declines suddenly (but see k below). Lab assays by O'Brien *et al.* [51]
38 also found no significant genetic correlations between heat and cold tolerance, or between
39 either of these traits and wing size. Furthermore, these (nonsignificant) estimates of genetic
40 correlation were always positive between these traits in the laboratory, suggesting that
41 trade-offs between heat and cold tolerance do not limit evolutionary responses along these
42 gradients. This could be partly due to *Drosophila* distributions being more determined by
43 cold than heat tolerance [52].

44
45 *(f) Field transplants across elevational gradients provide no evidence for local adaptation*

46 In 2012 we reared 35 isofemale lines collected from 8 locations across two elevational
47 gradients. We then transplanted virgin males and females from known families into 591

1 cages at 10 sites across the gradient at Paluma and 9 sites along the gradient at Mt Edith
2 [53]. Ample food was provided, and surviving adults removed after 5 days. After 30 days,
3 the number of adult offspring produced per cage was assayed, which equates to the mean
4 fitness of each cage including mating and oviposition success, as well as egg-adult survival.
5 Elevational variation in temperature within and across sites during the experiment was
6 typical of that observed across a 2 year period [53], representing a 5°C and 2 °C reduction in
7 mean temperature from the bottom to the top of Mount Paluma and Mount Edith
8 respectively.

9
10 Surprisingly, data from these cage transplants revealed that the clinal divergence in
11 productivity (and probably parental investment) detected in the laboratory along these
12 gradients (c) was not reflected by variation in cage productivity in the field, even when
13 families were transplanted away from their elevations of origin, and to opposite ends of
14 their entire ecological niche. This suggests that local adaptation underlying genetic
15 divergence is generated by occasional periods of extreme environmental conditions, or the
16 average of selection across several generations, meaning that its consequences for fitness
17 are not necessarily captured by snapshots of fitness variation estimated using field cages
18 that exclude most biotic interactions.

19
20 *(g) Strength of selection towards an optimal phenotype is weak for most traits*

21 We used relatives of the offspring reared from each family that were used in the transplant
22 cages to estimate variation in cold tolerance, heat tolerance and body size [51]. This meant
23 that we could connect trait means for each family measured in the laboratory with the
24 fitness of their relatives transplanted to each cage in the field. The 2012 transplant
25 experiment could therefore test whether mean trait values of families significantly
26 explained fitness variation at sites along each elevation gradient, even if no local adaptation
27 was observed.

28
29 The standardised selection differentials ranged from 0.01-0.48 for cold tolerance, heat
30 tolerance and wing size [51]. This is similar to the range of values observed in a large review
31 of phenotypic selection in the wild (most estimates between 0-0.3, median selection
32 gradient = 0.16) [54]. In our study, estimates of selection at individual sites were never
33 significantly different from zero for any of the traits, which is likely due to the relatively low
34 sample size (15 – 20 families) per site. However, for cold tolerance (assayed as offspring
35 production following a cold shock), selection differentials varied with elevation at one of the
36 gradients in a way that would drive divergence of the trait between elevational extremes.
37 Combining selection strength with heritability estimates for this trait generated a predicted
38 response to selection of an increase of 0.12 SD per generation at the lowest elevation and a
39 decrease of 0.44 SD per generation at the highest elevation [51]. This predicted adaptive
40 response is interesting given clines in cold shock recovery time were observed in 2004 (see a
41 above). However, the response to selection from these 2012 estimates is a reduction in
42 productivity following cold shock at high elevations, suggesting either trade-offs in the field
43 with other traits that are not obvious in the lab (see i below), or that delaying reproduction
44 during cold periods is an adaptive strategy at high elevations [51].

45
46 *(h) Genetic variation in fitness in the lab does not correspond to variation in the field*

1 Mean fitness in the field cages varied significantly among families in the 2012 experiment,
2 suggesting abundant genetic variation in fitness in the field at any given location on the
3 gradient [53]. However, the ranking of these lines did not correlate with those estimated in
4 the laboratory, demonstrating that the relative fitness of lines in the lab is a poor predictor
5 of their relative fitness in the field, and that genetic variation in the field (Fig 1b and Box 1)
6 is highly contingent on local conditions. The same may also apply to trade-offs (c above).
7 Ecological gradients are therefore likely to vary substantially in their steepness from one
8 year or one generation to the next, making clines in fitness traits likely to appear and
9 disappear across generations.

10
11 In addition, although mean fitness in cages varied substantially among families [53], when
12 we plotted the change in fitness against elevation for each family, these lines did not cross,
13 as would be required for selection to generate local adaptation. Instead, were selective
14 conditions sufficiently consistent, the highest ranked families should spread into all
15 elevations at the expense of all other families. Standing genetic variation (in the lab and
16 field) is therefore shaped by historical responses to selection [55], and is not explained by
17 current environmental variation, again suggesting that ecological gradients are highly
18 dynamic at these spatial scales.

19
20 *(i) Environmental variation in the field increases load but may reduce genetic variation*

21 In addition to estimating selection on trait variation in the field, we estimated heritability for
22 wing size at each field site using the correlation between lab-reared parents and their cage-
23 reared offspring [51]. A key message is that although heritability for the same set of
24 genotypes in field cages at different sites varies substantially, its size is not predicted by
25 elevation, or in a way that makes evolution in an adaptive direction more likely at one end
26 of the gradient than the other. For example, at Paluma, heritabilities at the highest and
27 lowest elevation sites were both high and significant ($h^2 = 0.94 - 1.00$; $P < 0.01$) [51]. A
28 similar dependence on the environment for genetic variances and covariances in key fitness
29 traits was recorded in recent lab assays of *D. birchii* and *D. serrata* [56].

30
31 This effect of environmental variation on the visibility of genetic variation to selection
32 makes predicting evolutionary responses difficult, at least over a short period. This finding
33 also suggests that phenotypic variance in the field can generate high levels of standing
34 *phenotypic* load (which still reduces mean fitness), without concomitant increases in the
35 amount of this load that is *genetic*, leading to faster responses to selection (i.e. a change in
36 allele frequency). Such an effect would increase where more loci underlie a trait, or where
37 more traits are involved in adaptation (e.g. in response to biotic interactions or trade-offs).
38 The potential for environmental variation to determine the amount of genetic variation in
39 traits is a classic issue in quantitative genetics [see reviews by 57, 58]. Understanding how
40 genotypes vary in the extent to which they can minimise their exposure to environmental
41 variation through plastic responses, including by movement in space (dispersal) or time
42 (phenology), or by changes in the phenotype itself, is a key issue for future research [59,
43 Noer et al., this issue].

44
45 Temporal environmental variation may also reduce the productivity (R_{\max}) of genotypes at a
46 given point in a gradient, even where they match the phenotypic optimum, further reducing
47 the gradient steepness at which adaptation fails [see 60; see also Holt et al., this issue], as

1 may stochastic environmental variation [61]. Environmental effects on R_{\max} that are
2 insurmountable given the genetic variation available (i.e. “ecological” or “fundamental”
3 limits; see 1 above) may therefore limit species to particular places along environmental
4 gradients, regardless of the effects of standing variation on local adaptation. Such effects
5 may include antagonistic interactions with closely-related species [62; see below], or sexual
6 interactions within species [63].

7
8 For example, Saxon *et al.*, [46] show that in *D. birchii*, the (shade) levels of diurnal thermal
9 variation routinely experienced at their lower elevation limit (i.e. where *D. bunnanda* is
10 more common), reduce male larval fertility by up to 30%. Similar results have been seen in
11 other insect species [64, 65], suggesting that upper thermal limits to male fertility may
12 represent a fundamental limit for species at the warm ends of their distribution [66]. This is
13 in addition to the increased intensity of interactions with other species at warm margins,
14 which is suggested by the transplant experiment of [53; j below], and subsequently
15 confirmed in the transplant experiment of [62; k below].

16 17 *(j) Effect of elevational gradient on mean fitness*

18 The first transplant experiment [53] showed that all lines had increased productivity at
19 lower elevations (i.e. higher temperatures), indicating (h) that the abiotic gradient had a
20 strong and consistent effect on fitness [53]. However, this increased productivity (in cages)
21 towards lower elevations did not match the gradient in field population density, which
22 consistently declined at lower elevations. This finding suggests that biotic interactions
23 (which were mostly absent from the cages) are crucial in determining fitness and in reducing
24 local density of *D. birchii* at low elevations in the field, and may also affect the expression of
25 genetic variation in key fitness traits.

26 27 *(k) Antagonistic biotic interactions are stronger towards ecological margins*

28 Further collections of *D. birchii* and *D. bunnanda* in 2017 were motivated by the findings in
29 (j), as well as evidence for trade-offs between stress resistance and biotic resistance in other
30 *Drosophila* species. We also wanted to test whether including biotic interactions revealed
31 previously cryptic adaptive divergence (a, g) along transects in the ability of genotypes to
32 mitigate antagonistic interactions with their own and other species, in different abiotic
33 contexts. 80 isofemale lines of *D. birchii* were collected from 8 locations along two
34 elevational gradients in 2017 and established in large numbers in the laboratory. As in 2012,
35 lab-reared virgin males and females from these localities were then transplanted into 972
36 vials in field cages at low, middle and high elevations at Paluma. However, this time these
37 field vials were established for a range of densities of *D. bunnanda* in relation to *D. birchii*,
38 as well as variation in overall density, at different positions along an elevation gradient [62].

39
40 Results from these 2017 transplants revealed that: (i) both intra and interspecific
41 competition have stronger effects on productivity overall at lower elevations, consistent
42 with the findings of a recent review [67]; and (ii) inter-specific interactions have bigger
43 effects on fitness at ecological margins than at the range centre for a given species. This
44 provides some of the first empirical evidence (first suggested in Darwin’s “tangled bank”
45 concept) that inter-specific interactions limit niche width, so causing species replacement
46 along ecological gradients. These findings also suggest that fitness consequences of biotic

1 and abiotic environments interact to limit adaptation at ecological margins, as reviewed by
2 Alexander et al., (this issue).

3
4 Surprisingly however, O'Brien *et al.* [62] found no evidence that the biotic environment
5 affected the amount of among population variation in fitness, suggesting that intra and
6 inter-specific interactions in *D. birchii* have little effect on genetic load, or on the response
7 to selection, even though they both have large and consistent effects on local productivity,
8 and their effects varied depending on the abiotic environment.

9
10 ***(1) Local adaptation has only limited capacity to mitigate competition***

11 O'Brien *et al.* [62] also tested the consequences of transplant and exposure to intra and
12 inter specific competition relative to site of origin for *D. birchii* populations. This revealed
13 some evidence that local adaptation at high elevations (where intraspecific competition
14 among *D. birchii* is high) has selected for a reduced effect of competition on fitness.
15 However, where there was evidence of local adaptation, it was only associated with a
16 reduced cost of competition on adult body size (a key predictor of individual fecundity in
17 *Drosophila*), but did not increase cage productivity *per se* for that elevation and level of
18 competition. This supports the idea that there are fundamental limits to the adaptation
19 possible from existing genetic variation along these gradients (as explored in the ecological
20 models i) – i.e. adaptive divergence can reduce the cost of competition for individual fitness
21 at some elevations, but it cannot alter the effect of competitive interactions on productivity,
22 even at native elevations.

23
24 ***(2) Biotic interactions steepen ecological gradients in UK Brown Argus butterflies***

25 European Lepidoptera also provide exceptional opportunities for testing the evolution of
26 populations in response to environmental change because their historical distributions,
27 ecology and life history are well known. In particular, the breadth of larval food plants
28 exploited by European butterflies affects their responses to abiotic change by limiting
29 habitat availability at their climatic margins, slowing or preventing range shifts [68-70].
30 Evidence suggests that although many species that use a wide variety of host plants have
31 shifted their distributions to track climate change, only c. 25% of UK host plant specialist
32 species have expanded their ranges to occupy thermally suitable environments at higher
33 latitudes and elevations. This indicates an inability to evolve novel biotic interactions (e.g.
34 changes in host plant use) to overcome locally steep (and patchy) ecological gradients (in
35 terms of [A] and [B]) at their margins. As with *D. birchii*, antagonistic biotic interactions (this
36 time among, rather than within trophic levels) seem to confine populations to narrow
37 ecological niches, and to increasingly warm and fragmented locations in their historical
38 range, despite abiotic conditions that are rapidly becoming suitable elsewhere.

39
40 The UK Brown Argus butterfly (*Aricia agestis*) is an exception that tests this rule. Historically,
41 populations have mostly been confined to chalk downland habitats in southern England,
42 where it uses common rockrose (*Helianthemum*, Cistaceae) as a larval host plant. By contrast,
43 in southern and locally warm coastal regions, populations of *A. agestis* mainly use *Geranium*
44 species as host plants. Since the 1980s, Brown Argus populations have rapidly shifted
45 northwards to colonise newly-suitable warming microclimates associated with climate
46 change, even though this has meant colonising areas further north that mostly lack rockrose
47 host plants [71, 72].

1
2 Population genomic data reveal that this response to climate change has been associated
3 with rapid evolutionary responses [73]. In addition, field experiments involving transplants
4 of host plants and individual mothers across sites demonstrate that: (a) range expansion has
5 involved the spread of *Geranium* preferring phenotypes; (b) these phenotypes are likely to
6 be more mobile than southern populations [74]; and (c) although females from southern
7 populations are able to use both rockrose and *Geranium* host plants, northern populations
8 have lost the ability to use their ancestral rockrose hosts, and lay only on *Geranium* plants
9 [75].

10
11 These data suggest that where biotic interactions are able to evolve (in this case, through
12 specialisation of oviposition preference onto a more widespread host plant), they generate
13 a shallower (or less locally steep) ecological gradient [B] that has allowed the Brown Argus
14 to expand its range to colonise thermally suitable regions that lacked its ancestral host
15 plant. Such a biotic shift, allowing use of previously inaccessible abiotic environmental
16 conditions, increases the productivity of the populations and should therefore increase their
17 adaptive potential, leading to further range and population expansion [A]. In the Brown
18 Argus case however, rapid evolution involves specialisation on a single (albeit more
19 widespread) host plant family, and the loss of the ability to use both host plants that is
20 observed in southern populations. This may reduce future adaptive potential [A], at least
21 until gene flow from southern populations on rockrose provides genetic variation that
22 allows the northern populations to return to using rockrose host plants.

23
24 *Smoothing ecological gradients in time vs space:* One key question in the Brown Argus story
25 is: Why do southern populations retain the ability to use *Geranium* as well as the locally
26 dominant host plant when maintaining both forms of preference is apparently costly (given
27 we observe a loss of rockrose use during expansion into habitats where rockrose is rare)?
28 One explanation is that although rockrose may provide a reliable host plant for larval
29 growth in years when springs are wet and cold, it may limit productivity in years when
30 climates are more clement (Stewart et al., this issue). In such warmer years, eggs laid on
31 *Geranium* plants found in low abundance at field margins may produce many adults,
32 allowing local expansion of Brown Argus populations into neighbouring fields, followed by
33 local contractions into rockrose habitats in cooler years. Certainly, *Geranium* leaves are
34 highly productive food plants under high laboratory temperatures [76], and oviposition
35 preferences in Brown Argus are highly sensitive to local microclimates, highlighting once
36 again how individual behaviour and plasticity affects their exposure to environmental
37 gradients [77].

38
39 Polyphagy in Brown Argus may therefore act as a bet-hedging strategy for populations in
40 the south, smoothing temporal variation in the ecological gradient by retaining rockrose as a
41 reliable (albeit relatively unproductive) host for larval growth, during years when wet and
42 cold springs would cause high larval mortality on *Geranium* hosts. By contrast, although
43 specialisation on the more widespread *Geranium* hosts smooths the environment in space
44 (by making suitable host plants less patchily distributed), this loss of polyphagy increases the
45 fitness costs of temporal environmental variation, making local range contractions more
46 likely in poor years (Stewart et al., this issue). Once again, detailed study of organisms and
47 their interactions in natural populations and climates highlights that we need to understand

1 ecological gradients and their steepness from the point of view of the alleles and
2 phenotypes that experience them.

3

4 **An organism's view of ecological gradients: understanding biotic and plastic interactions**

5 Theory predicts that the niche width and geographical range of species are determined by
6 the environmental conditions that: (1) reduce genetic variation in relative fitness; (2)
7 increase the demographic cost of genetic or phenotypic variation (and so reduce mean
8 fitness); and (3) cause rapid reductions in fitness across short spatial and temporal
9 distances, especially if caused by antagonistic interactions with other species, which may
10 also demand adaptation along a very limited number of phenotypic trajectories (so
11 increasing the effects of 1 and 2) (Fig 1b; Box 1).

12

13 We have presented two empirical systems that test maximum rates of evolution in response
14 to environmental variation. Two key conclusions emerge. Firstly, real environmental
15 gradients are rarely stable in time and space at the scale at which local adaptation occurs,
16 and their steepness and consistency are affected both by external (abiotic) variation, as well
17 as by the movement and behaviour of the genotypes, which determines fitness variation
18 and adaptive potential. Secondly, gradient steepness may increase locally via antagonistic
19 interactions, including between sexes, and with predators and competitors [e.g. 21, 30,
20 Alexander et al., this issue]. Alternatively, ecological gradients may be made shallower
21 where beneficial interactions (e.g. between legumes and bacteria, or plants and pollinators)
22 increase adaptive potential. Evolutionary responses in turn will fundamentally alter the
23 relationship between (A) and (B) (Fig 1b) – e.g. by allowing a virus or a herbivore to colonise
24 a new host, increasing its population size and genetic variation (A), as well as altering the
25 pattern and rate of environmental change (B). The rapid evolution of biotic interactions can
26 therefore bring parameter space into (and out) of the province where population genetic
27 models (i) rather than ecological models of range margin (ii) are most relevant for predicting
28 adaptive responses.

29

30 Biotic interactions are also likely to increase the difficulty of changing trait means in the
31 ways demanded by a given ecological gradient. If it is difficult for populations to access
32 regions of phenotypic space required for adaptation, then the speed of adaptation will be
33 reduced, for a given strength of selection. The point in parameter space where gradients
34 become locally steep due to constraints in genomic or ecological possibilities marks a
35 boundary between “population genetic models” of maintaining local adaptation (i.e. where
36 adaptation everywhere is possible in theory), to “ecological models” of parameter space
37 where some phenotypes can never evolve due to fundamental constraints. In such regions
38 of parameter space, standing variation can only mitigate some fitness consequences, but
39 cannot prevent a density gradient at margins, as may be the case in local adaptation to
40 competition in *D. birchii* (k above), but is not the case in Brown Argus butterflies (above),
41 where evolutionary responses to biotic interactions have allowed fundamental shifts in the
42 ecological niche, and biological invasion into novel habitat and latitudes.

43

44 *Understanding genotype sensitivity to the environment:* Evolution along ecological gradients
45 depends on [A] the efficiency of selection relative to drift (which depends on the adaptive
46 potential of standing variation as well as its effect in reducing mean fitness); and on [B],
47 which represents the change in trait mean demanded by the change in the environment

1 [10; Fig. 1b]. However, both dimensions [A] and [B] are shaped by the way that alleles and
2 genotypes are sensitive to environmental variation, and how this affects their experience of
3 ecological gradients, particularly in terms of the phenotypes that these genotypes create,
4 and their behaviour in distributing these phenotypes in space (dispersal) or time
5 (phenology) [59], as we have seen through variation in Brown Argus oviposition preference,
6 both at a regional [75] and microclimate [77] scale. Such non-random movement of
7 gametes (by mate or fertilisation choice) or genotypes (by seed dispersal, or by oviposition
8 preference) to areas where they have high fitness may make ecological gradients very
9 patchy (i.e. locally steep) at their edges, but highly productive at their centres [27]. Including
10 such behaviour in our measurements of [A] and [B] steepness may make local adaptation
11 less (or sometimes more) likely at ecological margins than we might otherwise predict, as
12 has been demonstrated in experimental studies of local adaptation in unicellular ciliates
13 [78-80].

14

15 These considerations remind us that traits that come under selection in the field are the
16 product of differences in the sensitivity among genotypes and populations, which tend to
17 evolve to make environmental variation as predictable (and often as smooth) as possible
18 [81, 82]. Increasing awareness of this has focussed attention on how genotypes respond to
19 environments within and outside their historical distribution and to test the prediction that
20 non-adaptive changes in phenotype may reduce absolute fitness but increase relative
21 fitness in novel environments. Such non-adaptive plasticity may increase adaptive potential
22 in novel environments, even if many (most) decisions by genotypes when outside their
23 familiar environment may reduce fitness, as has been demonstrated by our recent
24 transplants of genotypes of *Senecio* daisies along elevational gradients in Sicily [83-85]. In
25 addition, our recent work on *Drosophila* [63, 86, 87] reveals their complex responses to
26 different sexual and social environments, all of which are likely to affect population mean
27 and relative fitness, and therefore adaptive potential in natural populations. Effective
28 modelling of evolutionary responses depends on incorporating such plastic responses, and
29 their effect on adaptive potential, load and gradient steepness into theoretical models
30 (Eriksson and Rafajlovic, this issue) as well as empirical tests of plastic responses and their
31 effects on fitness in the field.

32

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43

44

45

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