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HOW TO MEASURE NATURAL SELECTION

Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation

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Summary

1. Extensive empirical work has demonstrated local adaptation to discrete environments, yet few studies have elucidated the genetic and environment mechanisms that generate it. Here, we advocate for research that broadens our understanding of local adaptation beyond pattern and towards process. We discuss how studies of local adaptation can be designed to address two unresolved questions in evolutionary ecology: Does local adaptation result from fitness trade-offs at individual loci across habitats? How do agents of selection interact to generate local adaptation to discrete contrasting habitats types and continuous environmental gradients?

2. To inform future investigations of the genetic basis of local adaptation, we conducted a literature review of studies that mapped quantitative trait loci (QTL) for fitness in native field environments using reciprocal transplant experiments with hybrid mapping populations or Genome-wide Association Study (GWAS) panels. We then reviewed the literature for field experiments that disentangle the contributions of various agents of selection to local adaptation. For each question, we suggest future lines of inquiry and discuss implications for climate change and agriculture research.

3. (i) Studies in the native habitats of five biological systems revealed that local adaptation is more often caused by conditional neutrality than genetic trade-offs at the level of the QTL. We consider the ramifications of this result and discuss knowledge gaps in our current understanding of the genetic basis of local adaptation. (ii) We uncovered only five studies that identified the agents of selection that contribute to local adaptation, and nearly all were conducted in discrete habitats rather than across the continuous environmental gradients that many species inhabit. We introduce a novel experimental framework for illuminating the processes underlying local adaptation.

4. A holistic view of local adaptation is critical for predicting the responses of organisms to climate change, enhancing conservation efforts, and developing strategies to improve crop resilience to environmental stress. Experiments that manipulate agents of selection in native field environments using pedigreed populations or GWAS panels offer unique opportunities for detecting the genetic and environmental mechanisms that generate local adaptation.

Key-words: conditional neutrality, environmental gradient, field manipulation, genetic trade-off, reciprocal transplant experiment

Introduction

Natural populations of the same species often display striking phenotypic diversity. These observed differences have inspired 75 years of reciprocal transplant studies in a diverse array of taxa (Leimu & Fischer 2008; Hereford 2009). These studies demonstrate the prevalence of local adaptation to contrasting environments, whereby local genotypes outperform foreign transplants (i.e. 'reciprocal home site advantage', Kawecki & Ebert 2004). Local adaptation arises when spatial variation in environmental conditions imposes divergent selection among populations across the range of a species. Reciprocal transplant experiments have demonstrated the fundamental influence of adaptation to local conditions on organismal diversity, yet we lack a basic understanding of the genetic and environmental mechanisms that contribute to local adaptation.

We advocate for research that goes beyond demonstrating patterns of local adaptation and towards testing the processes underlying local adaptation. Exploring these processes

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requires manipulative experiments conducted in the habitats in which species evolve (Table 1). Here, we focus on two outstanding questions in evolutionary ecology that can be addressed in a diversity of systems. First, we dissect the genetic basis of local adaptation to explore whether local adaptation at individual quantitative trait loci (QTL), and ultimately causal genes, comes at a fitness cost in alternative habitats. Second, we discuss how field studies can identify the individual agents of selection responsible for local adaptation across discrete habitats and along environmental gradients. By investigating processes that contribute to local adaptation, we will gain a better understanding of the environmental contexts under which local adaptation arises and the likelihood of adaptive responses to environmental change. From an applied perspective, this knowledge can be used to optimize crops, conserve endangered species, and predict how anthropogenic forces will affect natural populations.

Question 1: Does adaptation at individual loci result in fitness trade-offs across habitats?

Local adaptation to one habitat typically comes at the cost of reduced fitness in contrasting habitats (Clausen, Keck & Hiesey 1940; Kawecki & Ebert 2004). One key question in evolutionary ecology is whether the genes and mutations underlying local adaptation mirror the overall pattern of fitness trade-offs found at the organismal level (Lowry 2012; Savolainen, Lascoux & Merila 2013; Tiffin & Ross-Ibarra 2014). Local adaptation can be caused by *genetic trade-offs* at individual loci, such that local alleles confer a fitness advantage in their home environments and experience a fitness disadvantage in alternative habitats (e.g. Lowry *et al*. 2009; Anderson *et al*. 2013). In contrast, local adaptation can also emerge when an individual locus shows strong adaptive fitness effects in one habitat, but little or no cost in other habitats (*conditional neutrality*). Importantly, these two hypotheses are not mutually exclusive, as both genetic trade-offs and conditional neutrality can occur within one species (Fig. 1). Determining the degree to which locally-adapted loci exhibit genetic trade-offs or conditional neutrality can help us to undersand the maintenance of genetic variation within and among populations, the influence of gene flow on local adaptation, and the potential for organisms to evolve in pace with climate change. To test whether local adaptation proceeds via conditional neutrality or genetic trade-offs, researchers need to identify the alleles underlying variation in locally adaptive traits.

BACKGROUND: GENETIC TRADE-OFFS VS. CONDITIONAL NEUTRALITY

Two major types of experiments have been conducted to test the genetic basis of local adaptation. The first type involves reciprocally transplanting hybrid mapping populations into the native habitats of the parental lineages (Tables 1 and 2). In this case, researchers cross individuals derived from each of the habitats being evaluated to generate advanced generation hybrids, including F2s, recombinant inbred lines (RILs), or near-isogenic lines (NILs). To identify the loci involved in local adaptation, researchers then map quantitative trait loci (QTL) for fitness components from field reciprocal transplant experiments and compare the relative fitness effects of loci across habitats. In this way, researchers can detect loci that show genotype-by-environment interactions for fitness, and classify these loci as causing a trade-off or being conditionally neutral.

We are aware of only five biological systems in which reciprocal transplant experiments have been performed in the native environments with hybrids to evaluate how individual loci contribute to local adaptation. Across those experiments, 11 loci showed clear fitness trade-offs, while 41 had sizable fitness advantages in one habitat with no detectable cost in other habitats (Table 2). An additional nine loci showed a pattern of universal superiority, where one allele outperformed the alternative allele across both habitats.

As an alternative to the use of hybrid mapping populations, researchers can map loci underlying local adaptation by conducting Genome-wide Association Studies (GWAS) of fitness components across multiple habitats. GWAS use linear mixed models to find significant associations between phenotype and

Table 1. Experimental approaches of studies of local adaptation conducted in field settings. For each approach, we list the inferences that can be made about patterns of local adaptation and the processes that generate them and we include one example study

Experimental approach	Inference about local adaptation	Example citation
Two-site reciprocal transplant	Characterize patterns of local adaptation between contrasting habitats	Kim & Donohue (2013)*
Two-site reciprocal transplant with QTL mapping populations	Distinguish between conditional neutrality vs. genetic trade-offs underlying local adaptation	Lowry *et al*. (2009)
Provenance experiment	Characterize spatial patterns of local adaptation along environmental gradients	Wang, O'Neill & Aitken (2010)
Two-site reciprocal transplant + greenhouse/ laboratory study	Characterize patterns of local adaptation and identify putative agents of local adaptation	Anderson & Geber (2010)
Manipulative two-site or provenance reciprocal transplant	Characterize patterns of local adaptation and confirm agents of local adaptation	Macel *et al*. (2007)
Manipulations to simulate climate change in two-site or provenance reciprocal transplant	Evaluate whether climate change will disrupt patterns of local adaptation	Liancourt *et al*. (2013)

*Note that this study is an exemplary example of a two-site reciprocal transplant experiment between high and low altitudes because it includes three replicate sites for each elevation.

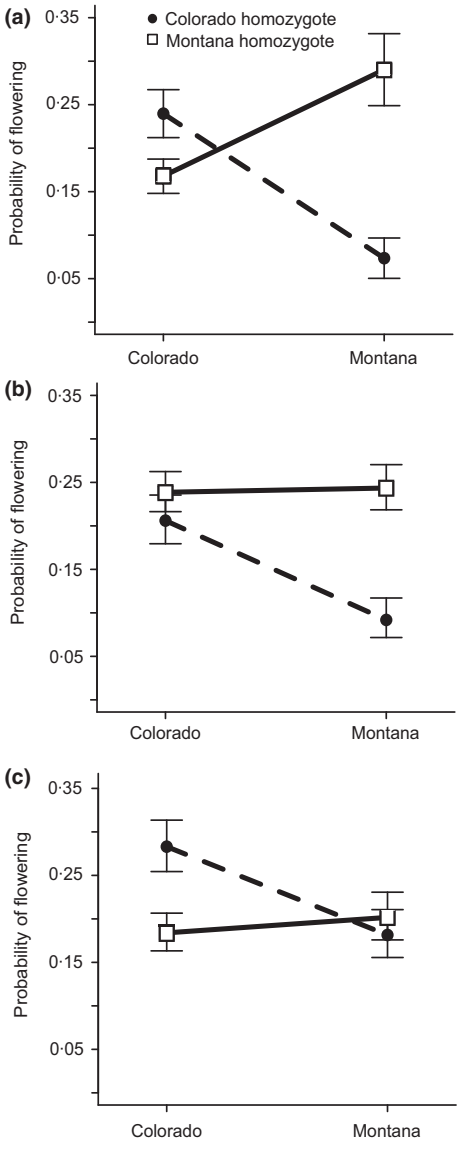


Fig. 1. Genetic trade-offs (a) and conditional neutrality (b + c) can both emerge in one study. These panels present published data from a field study that mapped QTL for fitness with recombinant inbred lines (RILs) of the forb, *Boechera stricta* (Brassicaceae), planted into the parental environments in Montana and Colorado (Anderson *et al.* 2013; Anderson, Lee & Mitchell-Olds 2014a). Panel (a) shows genetic trade-offs at a flowering phenology QTL (*nFT*) (redrawn from Anderson *et al.* 2013). Panel (b) depicts conditional neutrality at the *BCMA* locus, which controls the production of glucosinolates, a key anti-herbivore defense (Prasad *et al.* 2012): Montana genotypes at *BCMA* have a fitness advantage in the native habitat, but experience no cost in Colorado. Panel (c) depicts a QTL (*A12*) where the Colorado allele is conditionally advantageous in its home site, but neutral in the contrasting environment.

genotype across a large panel of individuals of either known or unknown relationship with one another (Korte & Farlow 2013). Chance associations due to population structure are controlled with pedigrees, a kinship matrix, and/or population membership model covariates (Price *et al.* 2010). As with QTL mapping, researchers can compare the fitness effects of loci across habitats to test genetic trade-offs vs. conditional neutrality. To date, few GWAS panels have been phenotyped for fitness across environments in non-domesticated organisms. However, some early insights have come from the model plant *Arabidopsis thaliana*. Fournier-Level *et al.* (2013) used a panel of 157 world-wide accessions to identify markers associated with fitness components in common gardens in four different parts of the native range of the species. They found that fitness was primarily linked to different genes and different molecular processes in each location and that the frequency of single nucleotide polymorphisms (SNPs) associated with fitness often co-varied with climate across the range. GWAS provide exciting opportunities for species that are not amenable to experimental crossing, enabling tests of the genetic basis of local adaptation in a more diverse array of species with a broader range of life histories.

A clear understanding of how genomic change leads to local adaptation can only be gained by examining the fitness consequences of the functional mutations that are subject to selection. QTL studies rarely identify genomic regions that contain fewer than dozens of putative adaptation genes. Even if a single QTL region shows evidence of genetic trade-offs, conditional neutrality could still be the driving force behind local adaptation if two or more tightly linked underlying genes are conditionally neutral but opposite in the direction of their fitness effects (Anderson *et al.* 2013). For many species, identifying local adaptation genes using GWAS panels or fine mapping approaches is logistically challenging. In those cases, two types of studies involving genome-wide or candidate gene resequencing can illuminate mutations underlying local adaptation: (i) genome scans and (ii) genotype–environment association studies (Rellstab *et al.* 2015; Hoban *et al.* 2016).

To investigate the genomic basis of local adaptation, individuals from a diversity of habitats can be analysed by scanning the genome for regions with unusually high divergence between environments (i.e. high Fst) and reduced nucleotide diversity indicative of recent selection. High divergence in SNP frequencies between environments combined with a signature of selection in one environment and its absence in another suggests that underlying genes may control locally-adaptive traits (Akey *et al.* 2002; Namroud *et al.* 2008; Shimada, Shikano & Merilä 2011). In contrast, genotype–environment association studies infer the genetic basis of local adaptation by identifying genetic polymorphisms that are statistically associated with environmental variation across the landscape (Savolainen, Lascoux & Merila 2013; Lotterhos & Whitlock 2015; Rellstab *et al.* 2015). Like GWAS, genome scans and genotype–environment association studies have the advantage of identifying variants relevant across many populations rather than those contained within a single cross. These methods can be applied to organisms with or without sequenced genomes, even in the absence of phenotypic data ('reverse ecology', Li *et al.* 2008). Although genome scans do not directly test habitat-specific fitness associations, they can provide important targets for follow-up field or greenhouse studies that validate allelic effects and test for trade-offs.

Genome scans and genotype–environment association studies can rapidly detect candidate genes that likely contribute to

Table 2. Results from reciprocal transplant experiments in a quantitative trait locus (QTL) mapping context to assess the effect QTL on fitness in native field habitats

Species	Trade-off	Conditional neutrality	Universal superiority	Citation
Avena barbata (Poaceae)	0	2	2	Gardner & Latta (2006)
Hordeum spontaneum (Poaceae)	0	11	2	Verhoeven *et al.* (2004, 2008)
Mimulus guttatus (Phyrmaceae)	1	7	0	Lowry *et al.* (2009), Hall, Lowry & Willis (2010) and Lowry & Willis (2010)
Boechera stricta (Brassicaceae)	1	8	0	Anderson *et al.* (2013) and Anderson, Lee & Mitchell-Olds (2014a)
Arabidopsis lyrata (Brassicaceae)	2	5	0	Leinonen *et al.* (2013)
Arabidopsis thaliana (Brassicaceae)	7	8	5	Ågren *et al.* (2013), Dittmar *et al.* (2014), Oakley *et al.* (2014) and Postma & Ågren (2016)

This table lists the number of QTLs identified per study that fit one of three classes: Trade-off loci, where local alleles had a fitness advantage in both habitats. Conditional neutrality loci, where there are fitness effects in one habitat and no detectable effects in the alternative habitat. Universal superiority loci, where one allele is superior to the alternative allele across both habitats.

local adaptation. These methods have also been effectively combined with mapping approaches to identify causal genes under broad QTL, which would otherwise take years to resolve via positional cloning (Stinchcombe & Hoekstra 2008; Hohenlohe *et al.* 2010; Wright *et al.* 2015; Gould, Chen & Lowry 2017). Genome scans have identified genes that underlie locally adaptive traits such as flowering time (Horton *et al.* 2012; Gould & Stinchcombe 2015), toxin resistance (Turner *et al.* 2010; Gould, McCouch & Geber 2014; Pfenninger *et al.* 2015), adaptation to elevation (Natarajan *et al.* 2015), organ gain or loss (Hohenlohe *et al.* 2010; Bradic, Teotónio & Borowsky 2013) and salt tolerance (Baxter *et al.* 2010), to name a few. Similarly, genotype–environment association studies have uncovered genes linked with adaptation to temperature, drought, precipitation, solar radiation, and pathogen pressure in plants (Hancock *et al.* 2011a; Yoder *et al.* 2014). Hancock *et al.* (2011b) even found strong climate–SNP associations in humans, worldwide.

RECOMMENDATIONS FOR FUTURE STUDIES

The handful of QTL mapping studies conducted in field conditions have found most loci to be conditionally neutral, but have also uncovered a few loci that incur fitness trade-offs across habitats (Table 2). Interestingly, experimental evolution studies of adaptation to heterogeneous environments in the laboratory have shown a higher prevalence of trade-offs than found in field studies (reviewed in Bono *et al.* 2017). Nevertheless, the frequency of conditional neutrality in field studies suggests that local adaptation to one habitat may not reduce fitness in alternative habitats. Additionally, this result implies that the level of gene flow among locally-adapted populations will play a prominent role in determining the types of mutations that are involved in the evolution of local adaptation.

As evidence of conditional neutrality builds in field studies, it becomes important to identify what genetic and physiological mechanisms underlie fitness asymmetries, which, to the best of our knowledge, has not yet been done. Nevertheless, the high proportion of conditionally-neutral loci could result from ascertainment bias, as detecting genetic trade-offs requires sufficient statistical power to uncover fitness consequences of allelic variation in at least two habitats (Anderson *et al.* 2013). In contrast, detecting conditional neutrality only requires significant fitness effects to emerge in one common garden; such a pattern could arise as an artefact of poor growth conditions or similar constraints that occur sporadically (Anderson *et al.* 2013). Additionally, conditional neutrality could be overrepresented in current studies because of the systems examined, which are heavily biased towards patchily distributed or self-fertilizing plants that typically experience limited gene flow.

High levels of gene flow between populations experiencing contrasting environmental conditions can counteract divergent selection, resulting in the dilution or loss of locally-adapted alleles (Bulmer 1972). This 'gene swamping' can even occur when gene flow is symmetrical among populations, in which case the population experiencing the weakest selection will endure the loss of locally-adapted alleles (Lenormand 2002). Thus, gene flow among populations could result in the rapid spread of conditionally-advantageous alleles, homogenizing populations and eroding local adaptation (Bulmer 1972; Lenormand 2002; Hall, Lowry & Willis 2010; Anderson *et al.* 2013). With higher levels of gene flow, we expect genetic trade-offs to underlie organismal local adaptation, otherwise gene flow would eliminate local adaptation. That is, only alleles that confer fitness trade-offs will be maintained because conditionally neutral alleles should spread by extensive gene flow into alternative habitats.

We expect the genetics of local adaptation to vary across species with different mating systems, with obligate outcrossers exhibiting more evidence for genetic trade-offs and predominantly selfing species displaying a greater propensity for conditional neutrality. Future work should determine whether species with restricted gene flow are likely to use a greater proportion of available mutations for local adaptation because they can exploit alleles at conditionally neutral loci. Furthermore, carefully constructed simulations can guide researchers to potential rates of gene flow and patterns of selection under which conditional neutrality can be maintained, which might

allow hypotheses to be formed about the threshold levels of gene flow above which conditional neutrality is unlikely. Ultimately, integrating data on population genetic structure with data on the genetic basis of local adaptation will enable future tests of the hypothesis that high levels of gene flow are associated with genetic trade-offs whereas conditional neutrality can be maintained under restricted gene flow.

Questions regarding the genetic basis of local adaptation are still largely unanswered because few local adaptation QTLs have been identified and even fewer have been narrowed down to individual mutations under selection. Genome scans and genotype–environment association studies have identified putatively locally adaptive alleles, but few follow-up experiments have been conducted to shed light on questions regarding trade-offs at the level of individual mutations. Targeted follow-up studies are sorely needed, quantifying fitness components under natural field conditions in organisms carrying candidate gene variants. Smaller scale studies are also warranted, focusing on measuring the reciprocal fitness effects of one or a few strong candidate genes in combination in a variety of field or controlled environments. At a larger scale, the use of reciprocally transplanted GWAS populations would reveal genome-wide patterns of trade-offs or conditional neutrality for individual mutations. For example, in a study of *Arabidopsis*, Fournier-Level *et al*. (2011) found genome-wide negative correlations (suggesting trade-offs) for the effect of SNPs on survival at some pairs of transplant sites but not others. To dissect the complexity of adaptive allelic effects across space and time, genotyped GWAS panels should be installed in experimental sites found along environmental gradients and monitored across multiple years.

RELEVANCE FOR CLIMATE CHANGE AND FOOD SECURITY

Industrialization and human activities have increased global temperatures, disrupted precipitation regimes, and elevated atmospheric [CO_2] (IPCC 2013). Detecting the genetic basis of climatic adaptation is crucial for predicting whether local populations will persist. If selection favours local alleles in contrasting climates (genetic trade-offs), ongoing climate change could rapidly decrease the fitness advantage of local alleles relative to alleles from populations that historically experienced hotter and drier conditions. In this case, declining fitness in response to climate change could reduce local population growth rates and increase vulnerability to further change (e.g. Kingsolver, Diamond & Buckley 2013; Anderson 2016). In contrast, if conditional neutrality underlies local adaptation, local genotypes could maintain fitness if alleles that were previously neutral begin to confer a fitness advantage under altered climates. In that way, conditional neutrality may enable local populations to persist despite continued changes in climatic patterns.

Novel environments can expose genetic variation that could potentially enable adaptive responses to those conditions (Paaby & Rockman 2014). For example, in a laboratory experiment, a marine population of the threespine stickleback exhibited higher additive genetic variance for body size in a freshwater treatment than in high salinity conditions that more closely reflected their native habitat (McGuigan *et al*. 2011). Cryptic genetic variation in this sort makes it challenging to predict which species or populations will adapt to climate change, as genetic variation may not be revealed until a population experiences novel environmental conditions. Indeed, marine populations of the threespine stickleback appear to harbour little genetic variation in body size and other functional traits, yet this species has repeatedly colonized freshwater systems perhaps because novel habitats augmented genetic variation upon which selection could act (McGuigan *et al*. 2011). Two potential mechanisms could explain why genetic variation changes under new environments: (i) novel selection favours previously rare alleles, which increase in frequency, and (ii) cryptic genetic variation is expressed in new environmental conditions (McGuigan *et al*. 2011). Conditional neutrality is consistent with this second mechanism, wherein previously neutral, but possibly common, alleles increase fitness under novel conditions.

If conditional neutrality underlies climatic adaptation, environment-specific effects of alleles could reduce the risk of local population extinction and hasten adaptation. Nevertheless, few empirical studies have explicitly examined the extent to which cryptic genetic variation emerges in novel environments, especially in the context of climate change. Furthermore, under rapid environmental change, gene flow could spread beneficial mutations (Bell & Gonzalez 2011), enhance genetic variation, and introduce pre-adapted alleles (Kremer *et al*. 2012; Aitken & Whitlock 2013). If populations have adapted locally to climatic variation, then genetic variation may already exist within meta-populations that would enable continued adaptation to climate change. Species at risk of decline may be those with limited gene flow and strong fitness costs when exposed to the climatic factors that are changing the most rapidly. Knowledge of the genetic basis of local adaptation should provide us with a predictive framework for identifying populations and species that are particularly vulnerable to global change.

The Food and Agriculture Organization of the United Nations outlines plant genetic resources as a cornerstone for maintaining global food security and states that greater intravarietal diversity is needed to cope with climate change predictions (Jarvis *et al*. 2015). Currently, increasing production of crops such as sorghum and millet includes simple genetic manipulations such as gene insertion for pest resistance or drought tolerance (Godfray *et al*. 2010). Crop scientists are also looking towards genetic engineering to functionally characterize the alleles associated with stress tolerance in the laboratory, and subsequently to manipulate individual genes to produce new varieties to increase crop yield (Mittler & Blumwald 2010). Genetic engineering efforts should focus on conditionally neutral alleles that confer a fitness advantage in novel temperature, precipitation, and [CO_2] regimes projected for a specific region, but are not disadvantageous under contemporary conditions. A better understanding of mechanisms underlying local adaptation to climate in crops could provide

researchers and stakeholders with tools to identify alleles influenced by environmental changes and genetically engineer crop varieties to produce greater yields as the climate continues to change.

Question 2: How do agents of selection interact to generate local adaptation across discrete habitats and environmental gradients?

Multiple biotic and abiotic factors exert selection on natural populations (Lowry *et al*. 2009; Calsbeek & Cox 2010; Garrido, Andraca-Gomez & Fornoni 2012; Kim & Donohue 2013; Franks *et al*. 2016). Isolating the specific agents of selection that contribute to local adaptation (hereafter refered to as agents of local adaptation) is a major challenge that requires innovative field manipulations, often complemented by laboratory experiments, to determine or verify the importance of a putatively causal environmental factor (Table 1). By identifying agents of local adaptation, studies can reveal whether the mechanisms that generate adaptation are consistent across habitats and through time, while facilitating the conservation of populations vulnerable to extinction and providing guidance to maximize crop yields across different growing regions. Although many studies have demonstrated patterns of adaptation to local conditions, few have identified individual agents of selection responsible for the fitness trade-offs underlying local adaptation (Cheplick 2015).

Initial experiments in controlled laboratory conditions can pinpoint probable agents of local adaptation that can then be targeted for manipulation under field conditions. Knowledge of life-history characteristics and the environmental conditions faced by populations in the wild should help researchers decide what treatments to implement in controlled conditions. For example, imposing flooding vs. drought treatments in a greenhouse would be an obvious first step for studying local adaptation in a plant species that inhabits dry upland vs. flood-prone bottomland environments (Porter 1966; Anderson & Geber 2010). However, subsequent field manipulations are necessary to confirm reputed agents of local adaptation (Agrawal 2011), as laboratory and greenhouse environments can be poor proxies for natural conditions (Kellermann *et al*. 2015; Poorter *et al*. 2016).

Here, we review the handful of studies that have identified agents of local adaptation by manipulating environmental variables in reciprocal transplant experiments in the field. We then highlight the ways in which studies can illuminate the evolutionary processes that generate local adaptation to discrete environments and along environmental gradients.

BACKGROUND: ABIOTIC AND BIOTIC AGENTS OF LOCAL ADAPTATION

Climatic factors are considered to be key drivers of local adaptation (Clausen, Keck & Hiesey 1940). To address the role of climate in shaping adaptive population divergence, Liancourt *et al*. (2013) manipulated temperature, soil water availability, and plant density on upper and lower slopes of the Mongolian steppe after transplanting *Festuca lenensis* (Poaceae) individuals from both habitats into each environment. Addition of water on the upper slope increased the fitness of plants that originated from the moist, lower slope, but decreased fitness in those from the dry, upper slope. This manipulative study disentangled several potential agents of selection and identified variation in water regimes as the agent of local adaptation. The results enabled predictions of plant fitness under climate change (see also Anderson 2016), which would not have been possible if the specific driving force for adaptation had not been identified.

Light, another important abiotic factor, can also be manipulated in the field to isolate the dominant selective pressures causing local adaptation. Inspired by results from previous studies and observations of rapid ozone depletion, Williamson *et al*. (1997) exposed reciprocally transplanted yellow perch (*Perca flavescens*) from contrasting lakes to manipulated levels of solar radiation and demonstrated that individuals are locally adapted to high levels of ultraviolet radiation. Other abiotic agents of local adaptation depend on the study system. For example, soil nutrients, such as nitrogen, are important for local adaptation in plants (Maes *et al*. 2014). Ultimately, identifying appropriate factors for manipulation requires information on spatiotemporal variation in environmental conditions that natural populations experience across their life cycle.

Species interactions can impose strong selection on natural populations (e.g. Nosil & Crespi 2006; Calsbeek & Cox 2010), and manipulative field studies have implicated biotic factors as potent agents of local adaptation (Bischoff *et al*. 2006; Liancourt *et al*. 2013). For example, by placing dark vs. light coloured plasticine mouse models in inland and beach habitats, Vignieri, Larson & Hoekstra (2010) demonstrated that predation is a causal agent of local adaptation for cryptic coloration in *Peromyscus polionotus*. Emerging evidence also suggests that local adaptation in plant–herbivore interactions can vary across generations (Kalske *et al*. 2016), implying that studies of these interactions should be replicated across growing seasons or generations to evaluate dynamic patterns of local adaptation. Furthermore, local adaptation need not be reciprocal when there are antagonistic interactions between species. In a study of plant–herbivore interactions, Garrido, Andraca-Gomez & Fornoni (2012) found that if one plant or herbivore species showed elevated fitness in the presence of its interacting partner, the partner was either locally maladapted or simply not locally adapted.

Individual agents of selection can interact in intricate ways to generate complex patterns of local adaptation. Accordingly, Morris *et al*. (2007) suggest that the impact of mutualistic and antagonistic agents of selection on plant performance should be gauged under field conditions where all biotic interactions occur simultaneously. The detection of local adaptation can also be contingent upon manipulating reputed agents of local adaptation at specific ontogenetic stages. Indeed, Hereford's (2009) meta-analysis revealed that the magnitude of local adaptation can change across life history and may be stronger in its effects on fecundity than on viability. In reciprocal

transplants of serpentine and riparian populations of the Californian sunflower *Helianthus exilis*, local adaptation via seed production was only observed in treatments where competitors were removed, suggesting population differentiation in response to disturbance (Sambatti & Rice 2006). When selection was examined via survival instead of fecundity, local adaptation was only detected in serpentine sites without competitors. Similarly, manipulative reciprocal transplants of the native bunchgrasses *Elymus glaucus* and *Nassella pulchra* demonstrated that competition enahnced local adaptation, and that home-site advantages were most apparent when examining fecundity rather than viability components of fitness (Rice & Knapp 2008). These studies suggest that the extent of local adaptation can vary across life history and be influenced by multiple agents of selection.

Interactions between abiotic and biotic drivers of local adaptation can be challenging to distinguish, particularly when biotic and abiotic factors vary at different spatial scales (McGill 2010; Fraterrigo, Wagner & Warren 2014). The geographic selection of sites for transplant experiments is crucial for disentangling putative agents of divergent selection among populations and for evaluating the spatial scale of local adaptation. It is important to consider both abiotic and biotic agents of local adaptation when predicting the impact of environmental change on locally adapted populations. To dissect the influence of climate and biotic and abiotic soil properties on local adaptation, Macel *et al.* (2007) reciprocally transplanted a perennial grass (*Holcus lanatus*) and a legume (*Lotus corniculatus*) across three sites, while also reciprocally transplanting soil origin among sites for 2 years. The grass species exhibited local adaptation to climate in both years for most fitness components (survival, size, and infection rate by a rust fungus), but was not adapted to local soil. In contrast, the legume showed local adaptation via fruit production to climate in 1 year and to soil environment in both years. Although they found no interaction between climate and soil environment on local adaptation, this study illustrates the complexity of interactions between multiple drivers of local adaptation.

RECOMMENDATIONS FOR FUTURE STUDIES

The frequent discussion of the processes contributing to local adaptation in the literature may lead many to believe that they are well investigated. To the best of our knowledge, only four studies have manipulated aspects of the abiotic or biotic environment in the field to identify agents of local adaptation (Williamson *et al.* 1997; Bischoff *et al.* 2006; Liancourt *et al.* 2013; Maes *et al.* 2014; see Data S1, Supporting Information, for literature survey protocol). It is not surprising that very few field experiments have employed this approach, given the unpredictable nature of field studies (e.g. deer can breach fences placed around experimental gardens, Stinchcombe & Rausher 2001), the large sample sizes needed to detect local adaptation (e.g. Postma & Ågren 2016), the intractability of non-model study organisms, and the time and labour involved with manipulative field experiments. Moreover, it may not be immediately obvious which agents of selection interact to drive adaptation to local environments, and it may not be possible to manipulate some agents of local adaptation in a field setting. Nevertheless, multifactorial manipulative studies in native field environments provide the most robust mechanism for disentangling the various abiotic and biotic factors that contribute to local adaptation (Box 1).

The few studies that have examined putative agents of local adaptation have done so through two-site reciprocal transplant experiments, whereby the fitness of individuals native to a particular habitat is compared with that of transplants from an environmentally contrasting locale. This experimental approach provides powerful and direct tests of adaptive divergence to disparate habitats. However, many species are distributed broadly across complex gradients (Lowry *et al.* 2014). Forestry professionals have long employed common garden experiments across environmental gradients to identify genotypes that are suitable for production and reforestation efforts (Langlet 1971). These provenance trials assess the performance of the progeny of economically-valuable tree species from multiple geographic origins in common gardens located throughout the species' range (Reznick & Ghalambor 2005). This approach has several crucial advantages over traditional two-site reciprocal transplant experiments. For one, the inclusion of a diverse collection of accessions incorporates a broader array of evolutionary histories shaped by unique combinations of abiotic and biotic factors than would be found in genotypes collected from two contrasting environments (Wilczek *et al.* 2014). In addition, provenance trials are well primed to investigate the spatial scale of local adaptation, the adaptive context of clinal trait variation, the extent of phenotypic plasticity within and among populations, and the degree to which gene flow can constrain local adaptation (Richardson *et al.* 2014; Boshier *et al.* 2015; Tomiolo, van der Putten & Tielbörger 2015). Lastly, the use of multiple common gardens enables researchers to disentangle the genetic and environmental factors that promote or impede local adaptation along climatic gradients (Mátyás 1996; Wang, O'Neill & Aitken 2010). However, these experiments must incorporate treatments that manipulate relevant abiotic and biotic agents of selection to reveal the causal environmental factors that generate local adaptation.

To examine the processes contributing to local adaptation, we propose that researchers employ manipulative provenance trials with pedigreed populations or GWAS panels transplanted into multiple gardens arrayed across environmental gradients. This approach can identify the genomic regions linked to variable selection across those gradients, test whether the fitness effects of individual loci change non-linearly in response to varying environmental conditions along gradients, and examine whether there are thresholds where the environment shifts dramatically over short spatial scales. Moreover, this approach would generate novel insights into the interacting evolutionary processes that shape population divergence. To the best of our knowledge, provenance trials have not yet been utilized for mapping QTL for fitness along environmental gradients, which is necessary to understand how the individual loci confer their adaptive effects across space.

Box 1. Multifactorial field manipulations can identify the environmental agents of natural selection that drive local adaptation. Consider a hypothetical herbaceous plant species that inhabits the understorey of both dry and mesic forests. Initial reciprocal transplant experiments document clear patterns of local adaptation at the organismal level (panel a). In this hypothetical example, dry forests are high light environments prone to drought, whereas mesic forests have low light levels and minimal water stress. To disentangle the contributions of light and drought to local adaptation, researchers might use shadecloth to depress light levels and rainout shelters to depress precipitation.

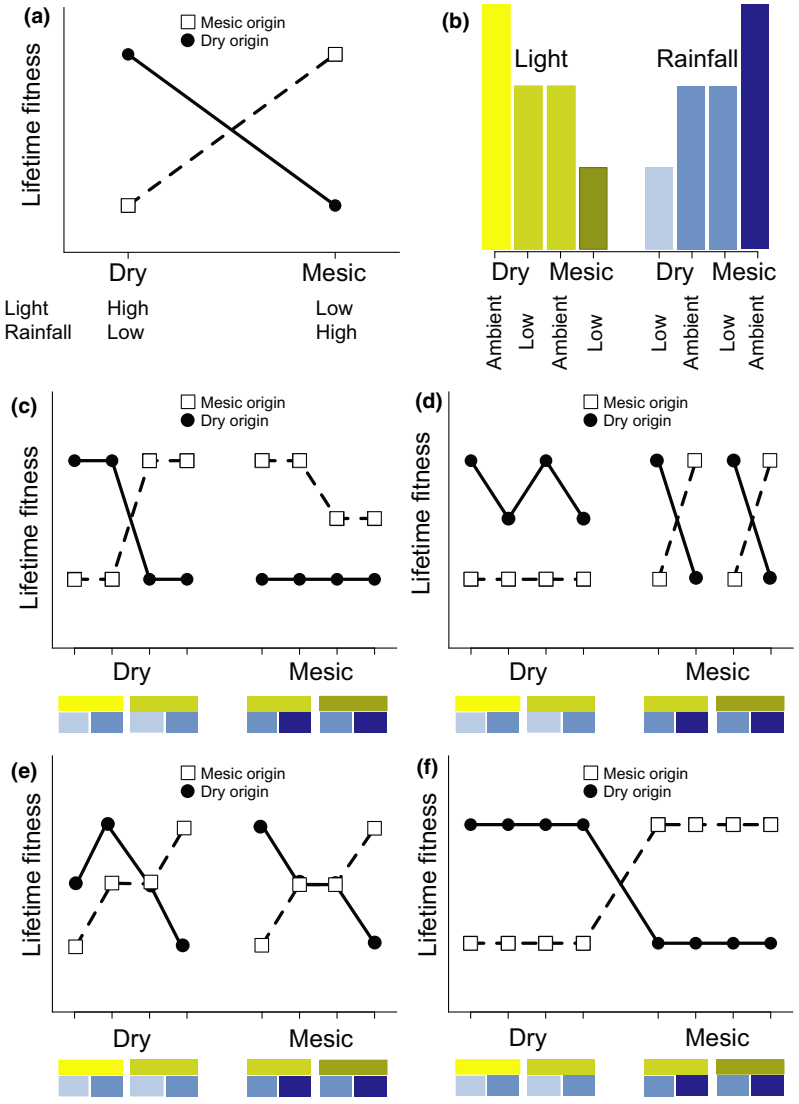
Panel b: Light and water levels under experimental treatments. Light levels are highest under ambient (control) conditions in the dry forest (bright yellow), are reduced but equivalent under the shadecloth treatment in the dry forest and ambient conditions in the mesic forest (medium yellow), and are lowest in the shadecloth treatment in the mesic forest (dark yellow). Similarly, rainfall levels are highest under ambient conditions in the mesic forest (dark blue), are reduced under the rainout shelter treatment in the mesic forest and under ambient conditions in the dry forest (medium blue), and are lowest in the rainout shelter treatment in the dry forest (light blue). The relative differences among habitats and treatments should serve as guides for predictions of local adaptation. Below, we outline various predictions.

Panel c: If local adaptation is driven purely by light level, low light levels under shadecloth in the dry habitat would favour mesic genotypes. While shadecloth would depress fitness for mesic origin genotypes in the low light mesic habitat, it would not shift genotypic ranking, nor would rainout shelters alter genotypic rankings in either habitat.

Panel d: If water stress underlies local adaptation, light treatments would no longer influence the relative fitness rankings of genotypes in either habitat, but rainout shelters would strongly favour upland over local genotypes in the mesic environment. In that case, rainout shelters would not alter genotypic rankings in the dry habitat despite reducing fitness for dry origin genotypes.

Panel e: If both light and water stress contribute to local adaptation, low light levels would favour mesic genotypes under control conditions, but not in rainout shelters, in the dry habitat. Conversely, rainout shelters would favour dry origin genotypes in control, but not low light conditions in mesic forests. The predictions in this panel represent one depiction of the combined effects of light and water stress on adaptation. There are alternative signatures of local adaptation could arise from the same experimental design.

Panel f: If treatments do not alter the relative fitness of genotypes in either habitat, researchers would conclude that neither water stress nor light level factor into patterns of local adaptation in this system.



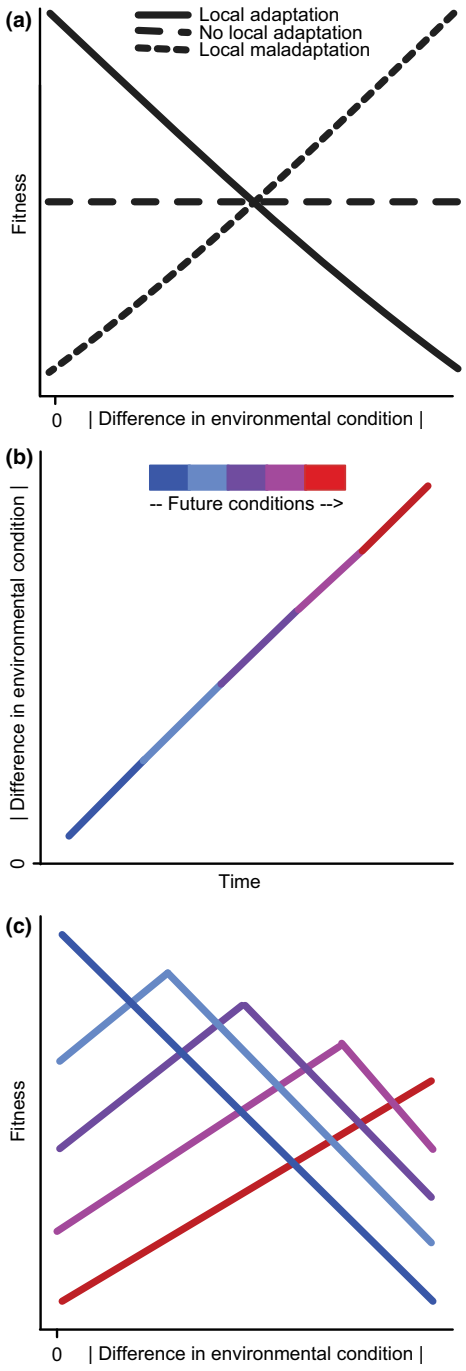


Fig. 2. Provenance trial experiments provide powerful tests of local adaptation across environmental gradients and can evaluate the extent to which climate change could disrupt local adaptation. Here, we explore a scenario in which many ecotypes from across a broad gradient have been transplanted into one hypothetical common garden. Panel (a) depicts the performance of a particular ecotype against the degree of environmental dissimilarity between an ecotype's home site and the conditions in the transplant garden *under pre-industrial climates*. This environmental dissimilarity could represent differences in temperature, precipitation, or even surrounding community composition. The fitness of a particular ecotype in the focal transplant garden could reflect any measure of performance, including population growth, survival and reproductive output. If ecotypes are locally adapted, fitness will be highest for ecotypes transplanted into a site that resembles their home environment. In contrast, local maladaptation arises if fitness increases with the degree of environmental dissimilarity between origin and transplant sites. Lastly, genotypes show no local adaptation or maladaptation if fitness is not associated with environmental similarity. Panel (b) reflects the influence of climate change on the magnitude of environmental dissimilarity over time. Here, there is an increasing difference between the historical environmental conditions a genotype experienced in its source environment and the current environmental conditions it is experiencing in the transplant environment. For ease of presentation, we have plotted a linear relationship, although it could be curvilinear depending on the rate of climate change. Panel (c) demonstrates that local maladaptaion may become more pronounced as climate change progresses. As future climates warm and diverge from contemporary conditions in this transplant garden (lines ranging from dark blue → light blue → dark purple → light purple → red), foreign genotypes from hotter and drier home sites achieve a fitness advantage over local genotypes. In that case, the overarching pattern changes from highly locally adapted (negative relationship between fitness and *pre-industrial* environmental dissimilarity) to highly maladapted (positive relationship between fitness and *pre-industrial* environmental dissimilarity). Note that fitness maxima decrease through time as accelerated rates of climate change promote the continued suppression of local adaptation.

RELEVANCE FOR CLIMATE CHANGE AND AGRICULTURAL STUDIES

Unprecedented rates of climate change may already be outpacing the process of local adaptation by favouring foreign genotypes from historically warmer sites (equatorial latitudes and lower elevations) over local genotypes (Fig. 2, Wilczek *et al.* 2014). Local maladaptation may become more pronounced as climate change continues and individuals no longer display optimal fitness in their local environment (Wang, O'Neill & Aitken 2010). Furthermore, locally-adapted populations may not have sufficient genetic variation for adaptation to novel climates (Kelly, Sanford & Grosberg 2012). Provenance trials that manipulate climatic conditions or include sites beyond the current range boundary of a species are valuable for determining the key agents of selection that govern adaptation to climate change and for predicting the evolutionary potential of populations (Griffith & Watson 2006; Wang, O'Neill & Aitken 2010; Wilczek *et al.* 2014; Wadgymar, Cumming & Weis 2015).

The manipulative provenance trial approach could also be applied to understand the genetic and environmental basis of local adaptation in agricultural settings to enable researchers to predict which crop varieties will thrive in various locations under climate change and breed regionally adapted varieties. Crop species vary in their response to climate change, yet general trends indicate that increasing temperature and altered precipitation patterns will reduce agriculture productivity (Fedoroff *et al.* 2010). At the same time, increasing human populations are placing pressure on crop breeders to enhance yield (Fedoroff *et al.* 2010). Microclimate changes in precipitation have caused drought in semi-arid and arid developing nations resulting in loss of soil fertility and increasing food insecurity (St.Clair & Lynch 2010). Partnerships between evolutionary biologists and crop scientists have great potential to improve decisions with regard to breeding and management in order to maximize crop resilience under future climate change scenarios.

Conclusions

In spatially heterogeneous landscapes, species often consist of mosaics of populations that have adapted to local biotic and abiotic conditions (e.g. Savolainen, Pyhajarvi & Knurr 2007; Leimu & Fischer 2008; Hereford 2009). Here, we recommend that researchers move from establishing patterns of local adaptation to uncovering the processes that generate them. In this review, we have illustrated how studies of local adaptation can be designed to reveal the mechanisms that drive adaptation to local conditions. Our survey of the literature revealed the need to examine interactions among the multitude of environmental factors often involved in local adaptation. Ultimately, researchers may be able to merge approaches by conducting research at multiple transplant sites across environmental gradients, manipulating key environmental factors to dissect agents of local adaptation, and using mapping and genomic approaches to identify the alleles that control fitness variation. Studies that couple genetic, genomic, and molecular techniques with manipulative field experiments will propel us forward in our understanding of local adaptation in a changing world.

Authors' contributions

All authors contributed equally to this manuscript. J.T.A. initiated and coordinated the project.

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Data accessibility

This manuscript does not include unpublished data. Data underlying Fig. 1 are available on the Dryad Digital Repository https://doi.org/10.5061/dryad.rp3pc (Anderson, Lee & Mitchell-Olds 2014b).

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Supporting Information

Details of electronic Supporting Information are provided below.

Data S1. Protocol for literature survey on investigations of agents of local adaptation, including search terms and inclusion and exclusion criteria.