1   **Title:** Identifying targets and agents of selection: Innovative methods to evaluate the processes

2   that contribute to local adaptation

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18   **Word counts**

19   Abstract: 335

20   Main text: 5272

21   References: 2846

22   Figure legends: 941

23   Tables: 381

Abstract:

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 toward 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 review how field experiments can be designed to 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. (A) 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. (B) Surprisingly few studies have identified the agents of selection that produce local adaptation, and nearly all have been 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.

Keywords: conditional neutrality, environmental gradient, genetic trade-off, field manipulation, 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 pattern toward testing the processes underlying local adaptation. Exploring these processes 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 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

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92   knowledge can be used to optimize crops, conserve endangered species, and predict how

93   anthropogenic forces will affect natural populations.

94

95   **<u>Question 1</u>: Does adaptation at individual loci result in fitness trade-offs across habitats?**

96   Local adaptation to one habitat typically comes at the cost of reduced fitness in

97   contrasting habitats (Clausen, Keck & Hiesey 1940; Kawecki & Ebert 2004). One key question

98   in evolutionary ecology is whether the genes and mutations underlying local adaptation mirror

99   the overall pattern of fitness trade-offs found at the organismal level (Lowry 2012; Savolainen,

100   Lascoux & Merila 2013; Tiffin & Ross-Ibarra 2014). Local adaptation can be caused by *genetic*

101   *trade-offs* at individual loci, such that local alleles confer a fitness advantage in their home

102   environments and experience a fitness disadvantage in the contrasting habitat (e.g., Lowry *et al.*

103   2009; Anderson *et al.* 2013). In contrast, local adaptation can also emerge when an individual

104   locus shows strong adaptive fitness effects in one habitat, but little or no evidence of a cost in

105   other habitats (*conditional neutrality*). Importantly, these two hypotheses are not mutually

106   exclusive, as both genetic trade-offs and conditional neutrality can occur within one species (Fig.

107   1). Determining the degree to which locally adapted loci exhibit genetic trade-offs or conditional

108   neutrality can help us to undersand the maintenance of genetic variation within and among

109   populations, the influence of gene flow on local adapation, and the potential for organisms to

110   evolve in pace with climate change. To test whether local adaptation proceeds via conditional

111   neutrality or genetic trade-offs, researchers need to identify the alleles underlying variation in

112   locally adaptive traits.

113   *Background: Genetic trade-offs versus Conditional neutrality*

114   Two major types of experiments have been conducted to test the genetic basis of local

115   adaptation. The first type involves reciprocally transplanting hybrid mapping populations into the

116   native habitats of the parental lineages (Tables 1 and 2). In this case, researchers cross

117   individuals derived from each of the habitats being evaluated to generate advanced generations

118   hybrids, including F2s, recombinant inbred lines (RILs), or near-isogenic lines (NILs). To

119   identify the loci involved in local adaptation, researchers then map quantitative trait loci (QTL)

120   for fitness components from field reciprocal transplant experiments and compare the relative

121   fitness effects of loci across habitats. In this way, researchers can detect loci that show genotype

122   by environment interactions for fitness, and classify these loci as causing a trade-off or being

123   conditionally neutral.

124   We are aware of only five biological systems in which reciprocal transplant experiments

125   have been performed in the native environments with hybrids to evaluate how individual loci

126   contribute to local adaptation. Across those experiments, 11 loci showed clear fitness trade-offs,

127   while 41 had sizable fitness advantages in one habitat with no detectable cost in other habitats

128   (Table 2). An additional nine loci showed a pattern of universal superiority, where one allele

129   outperformed the alternative allele across both habitats.

130   As an alternative to the use of hybrid mapping populations, researchers can map loci

131   underlying local adaptation by conducting Genome-Wide Association Studies (GWAS) of fitness

132   components across multiple habitats. GWAS use mixed linear models to find significant

133   associations between phenotype and genotype across a large panel of individuals of either known

134   or unknown relationship to one another (Korte & Farlow 2013). Chance associations due to

135   population structure are controlled with pedigrees, a kinship matrix, and/or population

136   membership model covariates (Price *et al.* 2010). As with QTL mapping, researchers can

137   compare the fitness effects of loci across habitats to test genetic trade-offs vs. conditional

138 neutrality. To date, few GWAS panels have been phenotyped for fitness across environments in

139 non-domesticated organisms. However, some early insights have come from the model plant

140 *Arabidopsis thaliana.* Fournier-Level et al. (2013) used a panel of 157 worldwide accessions to

141 identify markers associated with fitness components in common gardens in 4 different parts of

142 the native range of the species. They found that fitness was primarily linked to different genes

143 and different molecular processes in each location and that the frequency of SNPs associated

144 with fitness often co-varied with climate across the range. GWAS provides exciting

145 opportunities for species that are not amenable to experimental crossing, enabling tests of the

146 genetic basis of local adaptation in a more diverse array of species with a broader range of life

147 histories.

148   A clear understanding of how genomic change leads to local adaptation can only be

149 gained by examining the fitness consequences of the functional mutations that are subject to

150 selection. QTL studies rarely identify genomic regions that contain fewer than dozens of putative

151 adaptation genes. Even if a single QTL region shows evidence of genetic trade-offs, conditional

152 neutrality could still be the driving force behind local adaptation if two or more tightly-linked

153 underlying genes are conditionally neutral but opposite in the direction of their fitness effects

154 (Anderson *et al.* 2013). For many species, identifying local adaptation genes using GWAS panels

155 or fine mapping approaches is logistically challenging. In those cases, two types of studies

156 involving genome-wide or candidate gene re-sequencing can illuminate mutations underlying

157 local adaptation: 1) genome scans and 2) genotype-environment association studies (Rellstab *et*

158 *al.* 2015; Hoban *et al.* 2016).

159   To investigate the genomic basis of local adaptation, individuals from a diversity of

160 habitats can be analyzed by scanning the genome for regions with unusually high divergence

161 between environments (i.e. high Fst) and reduced nucleotide diversity indicative of recent

162 selection. High divergence in SNP frequencies between environments combined with a signature

163 of selection in one environment and its absence in another suggests that underlying genes may

164 control locally adaptive traits (Akey *et al.* 2002; Namroud *et al.* 2008; Shimada, Shikano &

165 Merilä 2011). In contrast, genotype-environment association studies infer the genetic basis of

166 local adaptation by identifying genetic polymorphisms that are statistically associated with

167 environmental variation across the landscape (Savolainen, Lascoux & Merila 2013; Lotterhos &

168 Whitlock 2015; Rellstab *et al.* 2015). Like GWAS, genome scans and genotype-environment

169 association studies have the advantage of identifying variants relevant across many populations

170 rather than those contained within a single cross. These methods can be applied to organisms

171 with or without sequenced genomes, even in the absence of phenotypic data ("reverse ecology,"

172 Li *et al.* 2008). Although genome scans do not directly test habitat-specific fitness associations,

173 they can provide important targets for follow-up field or greenhouse studies that validate allelic

174 effects and test for trade-offs.

175   Genome scans and genotype-environment association studies can rapidly detect candidate

176 genes that likely contribute to local adaptation. These methods have also been effectively

177 combined with mapping approaches to identify causal genes under broad QTL, which would

178 otherwise take years to resolve via positional cloning (Stinchcombe & Hoekstra 2008;

179 Hohenlohe *et al.* 2010; Wright *et al.* 2015; Gould, Chen & Lowry 2016). Genome scans have

180 identified genes that underlie locally adaptive traits such as flowering time (Horton *et al.* 2012;

181 Gould & Stinchcombe 2015), toxin resistance (Turner *et al.* 2010; Gould, McCouch & Geber

182 2014; Pfenninger *et al.* 2015), adaptation to elevation (Natarajan *et al.* 2015), organ gain or

183 loss(Hohenlohe *et al.* 2010; Bradic, Teotónio & Borowsky 2013) and salt tolerance (Baxter *et al.*

184 2010), to name a few. Similarly, genotype-environment association studies have uncovered

185 genes linked with adaptation to temperature, drought, precipitation, solar radiation, and pathogen

186 pressure in plants (Hancock *et al.* 2011a; Yoder *et al.* 2014). Hancock et al. (2011b) even found

187 strong climate-SNP associations in humans, worldwide.

188 *Recommendations for future studies*

189   The handful of QTL mapping studies conducted in field conditions have found most loci

190 to be conditionally neutral, but have also uncovered a few loci that incur fitness trade-offs across

191 habitats (Table 2). Interestingly, experimental evolution studies of adaptation to heterogeneous

192 environments in the lab have shown a higher prevalence of trade-offs than found in field studies

193 (reviewed in, Bono *et al.* 2017). Nevertheless, the frequency of conditional neutrality in field

194 studies suggests that local adaptation to one habitat may not reduce the ability to be successful in

195 alternative habitats. Additionally, this result implies that the level of gene flow among locally

196 adapted populations will play a prominent role in determining the types of mutations that are

197 involved in the evolution of local adaptation.

198   As evidence of conditional neutrality builds in field studies, it becomes important to

199 identify what genetic and physiological mechanisms underlie fitness asymmetries, which, to the

200 best of our knowledge, has not yet been done. Nevertheless, the high proportion of conditionally

201 neutral loci could result from ascertainment bias, as detecting genetic trade-offs requires

202 sufficient statistical power to uncover fitness consequences of allelic variation in at least two

203 habitats (Anderson *et al.* 2013). In contrast, detecting conditional neutrality only requires

204 significant fitness effects to emerge in one common garden; such a pattern could arise as an

205 artifact of poor growth conditions or similar constraints that occur sporadically (Anderson *et al.*

206 2013). Additionally, conditional neutrality could be overrepresented in current studies because of

207 the biology of the systems examined, which is heavily biased toward patchily distributed or self-

208 fertilizing plants that typically experience limited gene flow.

209   High levels of gene flow between populations experiencing contrasting environmental

210 conditions can counteract divergent selection, resulting in the dilution or loss of locally adapted

211 alleles (Bulmer 1972). This 'gene swamping' can even occur when gene flow is symmetrical

212 among populations, in which case the population experiencing the weakest selection will endure

213 the loss of locally adapted alleles (Lenormand 2002). Thus, gene flow among populations could

214 result in the rapid spread of conditionally advantageous alleles, homogenizing populations and

215 eroding local adaptation (Bulmer 1972; Lenormand 2002; Hall, Lowry & Willis 2010; Anderson

216 *et al.* 2013). With higher levels of gene flow, we expect genetic trade-offs to underlie

217 organismal local adaptation, otherwise gene flow would eliminate local adaptation. That is, only

218 alleles that confer fitness trade-offs will be maintained because conditionally neutral alleles

219 should spread by extensive gene flow into alternative habitats.

220   We expect the genetics of local adaptation to vary across species with different mating

221 systems, with obligate outcrossers exhibiting more evidence for genetic tradeoffs and

222 predominantly selfing species displaying a greater propensity for conditional neutrality. Future

223 work should determine whether species with restricted gene flow are likely to use a greater

224 proportion of available mutations for local adaptation because they can exploit alleles at

225 conditionally neutral loci. Furthermore, carefully constructed simulations can guide researchers

226 to potential rates of gene flow and patterns of selection under which conditional neutrality can be

227 maintained, which might allow hypotheses to be formed about the threshold levels of gene flow

228 above which conditional neutrality is unlikely. Ultimately, integrating data on population genetic

229 structure with data on the genetic basis of local adaptation will enable future tests of the

230   hypothesis that high levels of gene flow are associated with genetic tradeoffs whereas

231   conditional neutrality can be maintained under restricted gene flow.

232   Questions regarding the genetic basis of local adaptation are still largely unanswered

233   because few local adaptation QTLs have been identified and even fewer have been narrowed

234   down to individual mutations under selection. Genome scans and genotype-environment

235   association studies have identified putatively locally adaptive alleles, but few follow-up

236   experiments have been conducted to shed light on questions regarding trade-offs at the level of

237   individual mutations. Targeted follow-up studies are sorely needed, quantifying fitness

238   components under natural field conditions in organisms carrying candidate gene variants.

239   Smaller scale studies are also warranted, focusing on measuring the reciprocal fitness effects of

240   one or a few strong candidate genes in combination in a variety of field or controlled

241   environments. At a larger scale, the use of reciprocally transplanted GWAS populations would

242   reveal genome-wide patterns of trade-offs or conditional neutrality for individual mutations. For

243   example, in a study of *Arabidopsis,* Fournier-Level et al. (2011) found genome-wide negative

244   correlations (suggesting trade-offs) for the effect of SNPs on survival at some pairs of transplant

245   sites but not others. To dissect the complexity of adaptive allelic effects across space and time,

246   genotyped GWAS panels should be installed in experimental sites found along environmental

247   gradients and monitored across multiple years.

248   *Relevance for climate change and food security*

249   Industrialization and human activities have increased global temperatures, disrupted

250   precipitation regimes, and elevated atmospheric $[CO_2]$ (IPCC 2013). Detecting the genetic basis

251   of climatic adaptation is crucial for predicting whether local populations will persist. If selection

252   favors local alleles in contrasting climates (fitness trade-offs), ongoing climate change could

253   rapidly decrease the fitness advantage of local alleles relative to alleles from populations that

254   historically experienced hotter and drier conditions. In this case, declining fitness in response to

255   climate change could reduce local population growth rates and increase vulnerability to further

256   change (e.g., Kingsolver, Diamond & Buckley 2013; Anderson 2016). In contrast, if conditional

257   neutrality underlies local adaptation, local genotypes could maintain fitness if alleles that were

258   previously neutral begin to confer a fitness advantage under altered climates. In that way,

259   conditional neutrality may enable local populations to persist despite continued changes in

260   climatic patterns.

261   Novel environments can expose genetic variation that could potentially enable adaptive

262   responses to those conditions (Paaby & Rockman 2014). For example, in a laboratory

263   experiment, a marine population of the threespine stickleback exhibited higher additive genetic

264   variance for body size in a freshwater treatment than in high salinity conditions that more closely

265   reflected their native habitat (McGuigan *et al.* 2011). Cryptic genetic variation of this sort makes

266   it challenging to predict which species or populations will adapt to climate change, as genetic

267   variation may not be revealed until a population experiences novel environmental conditions.

268   Indeed, marine populations of the threespine stickleback appear to harbor little genetic variation

269   in body size and other functional traits, yet this species has repeatedly colonized freshwater

270   systems perhaps because novel habitats augmented genetic variation upon which selection could

271   act (McGuigan *et al.* 2011). Two potential mechanisms could explain why genetic variation

272   changes under new environments: (1) novel selection favors previously rare alleles, which

273   increase in frequency, and (2) cryptic genetic variation is expressed in new environmental

274   conditions (McGuigan *et al.* 2011). Conditional neutrality is consistent with this second

275   mechanism, wherein previously neutral, but possibly common, alleles increase fitness under

276 novel conditions.

277   If conditional neutrality underlies climatic adaptation, environment-specific effects of

278 alleles could reduce the risk of local population extinction and hasten adaptation. Nevertheless,

279 few empirical studies have explicitly examined the extent to which cryptic genetic variation

280 emerges in novel environments, especially in the context of climate change. Furthermore, under

281 rapid environmental change, gene flow could spread beneficial mutations (Bell & Gonzalez

282 2011), enhance genetic variation, and introduce pre-adapted alleles (Kremer *et al.* 2012; Aitken

283 & Whitlock 2013). If populations have adapted locally to climatic variation, then genetic

284 variation may already exist within meta-populations that would enable continued adaptation to

285 climate change. Species at risk of decline may be those with limited gene flow and strong fitness

286 costs when exposed to the climatic factors that are changing the most rapidly. Knowledge of the

287 genetic basis of local adaptation should provide us with a predictive framework for identifying

288 populations and species that are particularly vulnerable to global change.

289   The Food and Agriculture Organization of the United Nations outlines plant genetic

290 resources as a cornerstone for maintaining global food security and states that greater intra-

291 varietal diversity is needed to cope with climate change predictions (Jarvis *et al.* 2015).

292 Currently, increasing production of crops such as sorghum and millet includes simple genetic

293 manipulations such as gene insertion for pest resistance or drought tolerance (Godfray *et al.*

294 2010). Crop scientists are also looking toward genetic engineering to functionally characterize

295 the alleles associated with stress tolerance in the laboratory, and subsequently to manipulate

296 individual genes to produce new varieties to increase crop yield (Mittler & Blumwald 2010).

297 Genetic engineering efforts should focus on conditionally neutral alleles that confer a fitness

298 advantage in novel temperature, precipitation, and $[CO_2]$ regimes projected for a specific region,

299 but are not disadvantageous under contemporary conditions. A better understanding of

300 mechanisms underlying local adaptation to climate in crops could provide researchers and

301 stakeholders with tools to identify alleles influenced by environmental changes and genetically

302 engineer crop varieties to produce greater yields as the climate continues to change.

303

304 **Question 2: How do agents of selection interact to generate local adaptation across discrete**

305 **habitats and environmental gradients?**

306   Multiple biotic and abiotic factors exert selection on natural populations (Lowry *et al.*

307 2009; Calsbeek & Cox 2010; Garrido, Andraca-Gomez & Fornoni 2012; Kim & Donohue 2013;

308 Franks *et al.* 2016). Isolating the specific agents of selection that contribute to local adaptation

309 (hereafter refered to as agents of local adaptation) is a major challenge that requires innovative

310 field manipulations, often complemented by laboratory experiments, to determine or verify the

311 importance of a putatively causal environmental factor (Table 1). By identifying agents of local

312 adaptation, studies can reveal whether the mechanisms that generate adaptation are consistent

313 across habitats and through time, while facilitating the conservation of populations vulnerable to

314 extinction, and providing guidance to maximize crop yields across different growing regions.

315 Although many studies have demonstrated patterns of adaptation to local conditions, few have

316 identified individual agents of selection responsible for the fitness trade-offs underlying local

317 adaptation (Cheplick 2015).

318   Initial experiments in controlled laboratory conditions can pinpoint probable agents of

319 local adaptation that can then be targeted for manipulation under field conditions. Knowledge of

320 life-history characteristics and the environmental conditions faced by populations in the wild

321 should help researchers decide what treatments to implement in controlled conditions. For

322   example, imposing flooding vs. drought treatments in a greenhouse would be an obvious first

323   step for studying local adaptation in a plant species that inhabits dry upland vs. flood-prone

324   bottomland environments (Porter 1966; Anderson & Geber 2010). However, subsequent field

325   manipulations are necessary to confirm reputed agents of local adaptation (Agrawal 2011), as

326   laboratory and greenhouse environments can be poor proxies for natural conditions (Kellermann

327   *et al.* 2015; Poorter *et al.* 2016).

328   Here, we review the handful of studies that have identified agents of local adaptation by

329   manipulating environmental variables in common garden and reciprocal transplant experiments

330   in the field. We then highlight the ways in which this approach can be improved to illuminate the

331   evolutionary processes that generate local adaptation to discrete environments and along

332   environmental gradients.

333   *Background: Abiotic and biotic agents of local adaptation*

334   Climatic factors are considered to be key drivers of local adaptation (Clausen, Keck &

335   Hiesey 1940). To address the role of climate in shaping adaptive population divergence,

336   Liancourt et al. (2013) manipulated temperature, soil water availability, and plant density on

337   upper and lower slopes of the Mongolian steppe after transplanting *Festuca lenensis* (Poaceae)

338   individuals from both habitats into each environment. Addition of water on the upper slope

339   increased the fitness of plants that originated from the moist, lower slope, but decreased fitness

340   in those from the dry, upper slope. This manipulative study disentangled several potential agents

341   of selection and identified variation in water regimes as the agent of local adaptation. The results

342   enabled predictions of plant fitness under climate change (see also,Anderson 2016), which would

343   not have been possible if the specific driving force for adaptation had not been identified.

344   Light, another important abiotic factor, can also be manipulated in the field to isolate the

345   dominant selective pressures causing local adaptation. Inspired by results from previous studies

346   and observations of rapid ozone depletion, Williamson et al. (1997) exposed reciprocally

347   transplanted yellow perch (*Perca flavescens*) from contrasting lakes to manipulated levels of

348   solar radiation and demonstrated that individuals are locally adapted to specific exposure to high

349   levels of ultraviolet radiation. Other abiotic agents of local adaptation depend on the study

350   system. For example, soil nutrients, such as nitrogen, are important for local adaptation in plants

351   (Maes *et al.* 2014). Ultimately, identifying appropriate factors for manipulation requires

352   information on spatiotemporal variation in environmental conditions that natural populations

353   experience across their life cycle.

354   Species interactions can impose strong selection on natural populations (e.g., Nosil &

355   Crespi 2006; Calsbeek & Cox 2010), and manipulative field studies have implicated biotic

356   factors as potent agents of local adaptation (Bischoff *et al.* 2006; Liancourt *et al.* 2013). For

357   example, by placing dark vs. light colored plasticine mouse models in inland and beach habitats,

358   Vignieri et al. (2010) demonstrated that predation is a causal agent of local adaptation for cryptic

359   coloration in *Peromyscus polionotus*. Emerging evidence also suggests that local adaptation in

360   plant-herbivore interactions can vary across generations (Kalske *et al.* 2016), implying that

361   studies of these interactions should be replicated across growing seasons or generations to

362   evaluate dynamic patterns of local adaptation. Furthermore, local adaptation need not be

363   reciprocal when there are antagonistic interactions between species. In a study of plant-herbivore

364   interactions, Garrido and colleagues (2012) found that if one plant or herbivore species showed

365   elevated fitness in the presence of its interacting partner, the partner was either locally

366   maladapted or simply not locally adapted.

367   Individual agents of selection can interact in intricate ways to generate complex patterns

368   of local adaptation. Accordingly, Morris et al. (2007) suggest that the impact of mutualistic and

369   antagonistic agents of selection on plant performance should be gauged under field conditions

370   where all biotic interactions occur simultaneously. The detection of local adaptation can also be

371   contingent upon manipulating reputed agents of local adaptation at specific ontogenetic stages.

372   Indeed, Hereford's (2009) meta-analysis revealed that the magnitude of local adaptation can

373   change across life-history and may be stronger in its effects on fecundity than on viability. In

374   reciprocal transplants of serpentine and riparian populations of the Californian sunflower

375   *Helianthus exilis*, local adaptation via seed production was only observed in treatments where

376   competitors were removed, suggesting population differentiation in response to disturbance

377   (Sambatti & Rice 2006). When selection was examined via survival instead of fecundity, local

378   adaptation was only detected in serpentine sites without competitors. Similarly, manipulative

379   reciprocal transplants of the native bunchgrasses *Elymus glaucus* and *Nassella pulchra*

380   demonstrated that competition enahnced local adaptation, and that home-site advantages were

381   most apparent when examining fecundity rather than viability components of fitness (Rice &

382   Knapp 2008). These studies suggest that the extent of local adaptation can vary across life-

383   history and be influenced by multiple agents of selection.

384   Interactions between abiotic and biotic drivers of local adaptation can be challenging to

385   distinguish, particularly when biotic and abiotic factors vary at different spatial scales (McGill

386   2010; Fraterrigo, Wagner & Warren 2014). The geographic selection of sites for transplant

387   experiments is crucial for disentangling putative agents of divergent selection among populations

388   and for evaluating the spatial scale of local adaptation. It is important to consider both abiotic

389   and biotic agents of local adaptation when predicting the impact of environmental change on

390   locally adapted populations. To dissect the influence of climate and biotic and abiotic soil

391   properties on local adaptation, Macel and colleagues (2007) reciprocally transplanted a perennial

392   grass (*Holcus lanatus*) and a legume (*Lotus corniculatus*) across three sites, while also

393   reciprocally transplanting soil origin among sites for two years. The grass species exhibited local

394   adaptation to climate in both years for most fitness components (survival, size, and infection rate

395   by a rust fungus), but was not adapted to local soil. In contrast, the legume showed local

396   adaptation via fruit production to climate in one year and to soil environment in both years.

397   Although they found no interaction between climate and soil environment on local adaptation,

398   this study illustrates the complexity of interactions between multiple drivers of local adaptation.

399   *Recommendations for future studies*

400   The frequent discussion of the processes contributing to local adaptation in the literature

401   may lead many to believe that they are well-investigated. To the best of our knowledge, only 4

402   studies have manipulated aspects of the abiotic or biotic environment in the field to identify

403   agents of local adaptation (Williamson *et al.* 1997; Bischoff *et al.* 2006; Liancourt *et al.* 2013;

404   Maes *et al.* 2014, see Supplemental Methods for literature survey protocol). It is not surprising

405   that very few field experiments have employed this approach, given the unpredictable nature of

406   field studies (e.g., deer can breach fences placed around experimental gardens, Stinchcombe &

407   Rausher 2001), the large sample sizes needed to detect local adaptation (e.g., Postma & Ågren

408   2016), the intractability of non-model study organisms, and the time and labor involved with

409   manipulative field experiments. Moreover, it may not be immediately obvious which agents of

410   selection interact to drive adaptation to local environments, and it may not be possible to

411   manipulate all agents of local adaptation in a field setting. Nevertheless, multifactorial

412   manipulative studies in native field environments provide the most robust mechanism for

413   disentangling the various abiotic and biotic factors that contribute to local adaptation (Box 1).

414   The few studies that have examined putative agents of local adaptation have done so

415   through two-site reciprocal transplant experiments, whereby the fitness of individuals native to a

416   particular habitat is compared with that of transplants from an environmentally contrasting

417   locale. This experimental approach provides powerful and direct tests of adaptive divergence to

418   disparate habitats. However, many species are distributed broadly across complex gradients

419   (Lowry *et al.* 2014). Forestry professionals have long employed common garden experiments

420   across environmental gradients to identify genotypes that are suitable for production and

421   reforestation efforts (Langlet 1971). These provenance trials assess the performance of the

422   progeny of economically-valuable tree species from multiple geographic origins in common

423   gardens located throughout the species' range (Reznick & Ghalambor 2005). This approach has

424   several crucial advantages over traditional two-site reciprocal transplant experiments. For one,

425   the inclusion of a diverse collection of accessions incorporates a broader array of evolutionary

426   histories shaped by unique combinations of abiotic and biotic factors than would be found in

427   genotypes collected from two contrasting environments (Wilczek *et al.* 2014). In addition,

428   provenance trials are well primed to investigate the spatial scale of local adaptation, the adaptive

429   context of clinal trait variation, the extent of phenotypic plasticity within and among populations,

430   and the degree to which gene flow can constrain local adaptation (Richardson *et al.* 2014;

431   Boshier *et al.* 2015; Tomiolo, van der Putten & Tielbörger 2015). Lastly, the use of multiple

432   common gardens enables researchers to disentangle the genetic and environmental factors that

433   promote or impede local adaptation along climatic gradients (Mátyás 1996; Wang, O'Neill &

434   Aitken 2010). However, these experiments must incorporate treatments that manipulate relevant

435   abiotic and biotic agents of selection to reveal the causal environmental factors that generate

436   local adaptation.

437   To examine the processes contributing to local adaptation, we propose that researchers

438   employ manipulative provenance trials with pedigreed populations or GWAS panels transplanted

439   into multiple gardens arrayed across environmental gradients. This approach can identify the

440   genomic regions linked to variable selection across those gradients, test whether the fitness

441   effects of individual loci change non-linearly in response to varying environmental conditions

442   along gradients, and examine whether there are thresholds where the environment shifts

443   dramatically over short spatial scales. Moreover, this approach would generate novel insights

444   into the interacting evolutionary processes that shape population divergence. To the best of our

445   knowledge, provenance trials have not yet been utilized for mapping QTL for fitness along

446   environmental gradients, which is necessary to understand how the individual loci confer their

447   adaptive effects across space.

448   *Relevance for climate change and agricultural studies*

449   Unprecedented rates of climate change may already be out-pacing the process of local

450   adaptation by favoring foreign genotypes from historically warmer sites (equatorial latitudes and

451   lower elevations) over local genotypes (Fig. 2, (Wilczek *et al.* 2014). Local maladaptation may

452   become more pronounced as climate change continues and individuals no longer display optimal

453   fitness in their local environment (Wang, O'Neill & Aitken 2010). Furthermore, locally adapted

454   populations may not have sufficient genetic variation for adaptation to novel climates (Kelly,

455   Sanford & Grosberg 2012). Provenance trials that manipulate climatic conditions or include sites

456   beyond the current range boundary of a species are valuable for determining the key agents of

457   selection that govern adaptation to climate change and for predicting the evolutionary potential

458   of populations (Griffith & Watson 2006; Wang, O'Neill & Aitken 2010; Wilczek *et al.* 2014;

459   Wadgymar, Cumming & Weis 2015).

460   The manipulative provenance trial approach could also be applied to understand the

461   genetic and environmental basis of local adaptation in agricultural settings to enable researchers

462   to predict which crop varieties will thrive in various locations under climate change and breed

463   regionally adapted varieties. Crop species vary in their response to climate change, yet general

464   trends indicate that increasing temperature and altered precipitation patterns will reduce

465   agriculture productivity (Fedoroff *et al.* 2010). At the same time, increasing human populations

466   are placing pressure on crop breeders to enhance yield (Fedoroff *et al.* 2010). Microclimate

467   changes in precipitation have caused drought in semi-arid and arid developing nations resulting

468   in loss of soil fertility and increasing food insecurity (St.Clair & Lynch 2010). Partnerships

469   between evolutionary biologists and crop scientists has great potential to improve decisions with

470   regard to breeding and management in order to maximize crop resilience under future climate

471   change scenarios.

472

473   **CONCLUSIONS**

474   In spatially heterogeneous landscapes, species often consist of mosaics of populations

475   that have adapted to local biotic and abiotic conditions (e.g., Savolainen, Pyhajarvi & Knurr

476   2007; Leimu & Fischer 2008; Hereford 2009). Here, we recommend that researchers move from

477   establishing patterns of local adaptation to uncovering the processes that generate it. In this

478   review, we have illustrated how studies of local adaptation can be designed to reveal the

479   mechanisms that drive adaptation to local conditions. Our survey of the literature revealed the

480   need to examine interactions among the multitude of environmental factors often involved in

481   local adaptation. Ultimately, researchers may be able to merge approaches by conducting

482   research at multiple transplant sites across environmental gradients, and manipulating key

483   environmental factors to dissect agents of local adaptation, and using mapping and genomic

484   approaches to identify the alleles that control fitness variation. Studies that couple genetic,

485   genomic, and molecular techniques with manipulative field experiments will propel us forward

486   in our understanding of local adaptation in a changing world.

487

493

494   **Data Accessibility:** This manuscript does not include unpublished data. Data underlying Fig. 1

495   are available on the Dryad Digital Repository (DOI: http://dx.doi.org/10.5061/dryad.rp3pc).

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776   **Table 1.** Experimental approaches of studies of local adaptation conducted in field settings. For

777   each approach, we list the inferences that can be made about patterns of local adaptation and the

778   processes that generate them and we include one example study.

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Experimental approach	Inference about local adaptation	Example citation
Two-site reciprocal transplant	Characterize patterns of local adaptation between contrasting habitats	(Kim and 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 et al. 2010)
Two-site reciprocal transplant + greenhouse/laboratory study	Characterize patterns of local adaptation and identify putative agents of local adaptation	(Anderson and 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)

780   *Note that this study is an exemplary example of a two-site reciprocal transplant experiment

781   between high and low altitudes because it includes three replicate sites for each elevation.

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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. 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.

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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; Verhoeven *et al.* 2008)
Mimulus guttatus (Phyrmaceae)	1	7	0	(Lowry *et al.* 2009; Hall, Lowry & Willis 2010; Lowry & Willis 2010)
Boechera stricta (Brassicaceae)	1	8	0	(Anderson *et al.* 2013; Anderson, Lee & Mitchell-Olds 2014)
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; Postma & Ågren 2016)

Box legend

800 **Box 1:** Multifactorial field manipulations can identify the environmental agents of natural

801 selection that drive local adaptation. Consider a hypothetical herbaceous plant species that

802 inhabits the understory of both dry and mesic forests. Initial reciprocal transplant experiments

803 document clear patterns of local adaptation at the organismal level (panel A). In this hypothetical

804 example, dry forests are high light environments prone to drought, whereas mesic forests have

805 low light levels and minimal water stress. To disentangle the contributions of light and drought

806 to local adaptation, researchers might use shadecloth to depress light levels and rainout shelters

807 to depress precipitation.

808   Panel B: Light and water levels under experimental treatments. Light levels are highest

809 under ambient (control) conditions in the dry forest (bright yellow), are reduced but equivalent

810 under the shadecloth treatment in the dry forest and ambient conditions in the mesic forest

811 (medium yellow), and are lowest in the shadecloth treatment in the mesic forest (dark yellow).

812 Similarly, rainfall levels are highest under ambient conditions in the mesic forest (dark blue), are

813 reduced under the rainout shelter treatment in the mesic forest and under ambient conditions in

814 the dry forest (medium blue), and are lowest in the rainout shelter treatment in the dry forest

815 (light blue). The relative differences among habitats and treatments should serve as guides for

816 predictions of local adaptation. Below, we outline various predictions.

817   Panel C: If local adaptation is driven purely by light level, low light levels under

818 shadecloth in the dry habitat would favor mesic genotypes. While shadecloth would depress

819 fitness for mesic origin genotypes in the low light mesic habitat, it would not shift genotypic

820 ranking, nor would rainout shelters alter genotypic rankings in either habitat.

821   Panel D: If water stress underlies local adaptation, light treatments would no longer

822   influence the relative fitness rankings of genotypes in either habitat, but rainout shelters would

823   strongly favor upland over local genotypes in the mesic environment. In that case, rainout

824   shelters would not alter genotypic rankings in the dry habitat despite reducing fitness for dry

825   origin genotypes.

826   Panel E: If both light and water stress contribute to local adaptation, low light levels

827   would favor mesic genotypes under control conditions, but not in rainout shelters, in the dry

828   habitat. Conversely, rainout shelters would favor dry origin genotypes in control, but not low

829   light conditions in mesic forests. The predictions in this panel represent one depiction of the

830   combined effects of light and water stress on adaptation. There are alternative signatures of local

831   adaptation could arise from the same experimental design.

832   Panel F: If treatments do not alter the relative fitness of genotypes in either habitat,

833   researchers would conclude that neither water stress nor light level factor into patterns of local

834   adaptation in this system.

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Figure legends

846 **Figure 1**: Genetic trade-offs (A) and conditional neutrality (B + C) can both emerge in one

847 study. These panels present published data from a field study that mapped QTL for fitness with

848 recombinant inbred lines (RILs) of the forb, *Boechera stricta* (Brassicaceae), planted into the

849 parental environments in Montana and Colorado (Anderson *et al.* 2013; Anderson, Lee &

850 Mitchell-Olds 2014). Panel A shows genetic trade-offs at a flowering phenology QTL (*nFT*)

851 (redrawn from Anderson *et al.* 2013). Panel B depicts conditional neutrality at the *BCMA* locus,

852 which controls the production of glucosinolates, a key anti-herbivore defense (Prasad *et al.*

853 2012): Montana genotypes at *BCMA* have a fitness advantage in the native habitat, but

854 experience no cost in Colorado. Panel C depicts a QTL (*A12*) where the Colorado allele is

855 conditionally advantageous in its home site, but neutral in the contrasting environment.

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857 **Figure 2:** Provenance trial experiments provide powerful tests of local adaptation across

858 environmental gradients and can evaluate the extent to which climate change could disrupt local

859 adaptation. Here, we explore a scenario in which many ecotypes from across a broad gradient

860 have been transplanted into one hypothetical common garden. Panel (A) depicts the performance

861 of a particular ecotype against the degree of environmental dissimilarity between an ecotype's

862 home site and the conditions in the transplant garden *under pre-industrial climates*. This

863 environmental dissimilarity could represent differences in temperature, precipitation, or even

864 surrounding community composition. The fitness of a particular ecotype in the focal transplant

865 garden could reflect any measure of performance, including population growth, survival, and

866 reproductive output. If ecotypes are locally adapted, fitness will be highest for ecotypes

867 transplanted into a site that resembles their home environment. In contrast, local maladaptation

868   arises if fitness increases with the degree of environmental dissimilarity between origin and

869   transplant sites. Lastly, genotypes show no local adaptation or maladaptation if fitness is not

870   associated with environmental similarity. Panel (B) reflects the influence of climate change on

871   the magnitude of environmental dissimilarity over time. Here, there is an increasing difference

872   between the historical environmental conditions a genotype experienced in its source

873   environment and the current environmental conditions it is experiencing in the transplant

874   environment. For ease of presentation, we have plotted a linear relationship, although it could be

875   curvilinear depending on the rate of climate change. Panel (C) demonstrates that local

876   maladaptaion may become more pronounced as climate change progresses. As future climates

877   warm and diverge from contemporary conditions in this transplant garden (lines ranging from

878   dark blue ➔light blue ➔dark purple ➔light purple ➔red), foreign genotypes from hotter and

879   drier home sites achieve a fitness advantage over local genotypes. In that case, the overarching

880   pattern changes from highly locally adapted (negative relationship between fitness and *pre-*

881   *industrial* environmental dissimilarity) to highly maladapted (positive relationship between

882   fitness and *pre-industrial* environmental dissimilarity). Note that fitness maxima decrease

883   through time as accelerated rates of climate change promote the continued suppression of local

884   adaptation.

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891 **Supporting Information**

892 <u>Supplemental Methods</u>: Protocol for literature survey on investigations of agents of local

893 adaptation, including search terms, and inclusion and exclusion criteria.

894

(A)

Local adaptation
No local adaptation
Local maladaptation

Fitness

0 | Difference in environmental condition |

(B)

| Difference in environmental condition |

-- Future conditions -->

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Time

(C)

Fitness

0 | Difference in environmental condition |