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Testing for local adaptation in *Avena barbata*: a classic example of ecotypic divergence

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Abstract

Forty years ago, Robert Allard and colleagues documented that the slender wild oat, *Avena barbata*, occurred in California as two multi-locus allozyme genotypes, associated with mesic and xeric habitats. This is arguably the first example of ecotypes identified by molecular techniques. Despite widespread citation, however, the inference of local adaptation of these ecotypes rested primarily on the allozyme pattern. This study tests for local adaptation of these ecotypes using reciprocal transplant and quantitative trait locus (QTL) mapping techniques. Both ecotypes and 188 recombinant inbred lines (RILs) derived from a cross between them were grown in common garden plots established at two sites representative of the environments in which the ecotypes were first described. Across four growing seasons at each site, three observations consistently emerged. First, despite significant genotype by environment interaction, the mesic ecotype consistently showed higher lifetime reproductive success across all years and sites. Second, the RILs showed no evidence of a trade-off in performance across sites or years, and fitness was positively correlated across environments. Third, at QTL affecting lifetime reproductive success, selection favoured the same allele in all environments. None of these observations are consistent with local adaptation but suggest that a single genotype is selectively favoured at both moist and dry sites. I propose an alternative hypothesis that *A. barbata* may be an example of contemporary evolution – whereby the favoured genotype is spreading and increasing in frequency – rather than local adaptation.

Keywords: allozyme, ecotypes, fitness, genotype by environment interaction, selection

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Introduction

Certain examples in ecology and evolution achieve a classic status as textbook cases of the processes they exemplify. The Peppered moth, *Biston betularia* (Kettlewell 1956) and Darwin's Finches, *Geospiza* spp. (Grant & Grant 1993) are touchstone examples of natural selection. The reciprocal transplants of Clausen *et al.* (1941) along an elevational transect in California form the archetype of local adaptation. While such examples are often seen as definitive, some of the most insightful research can emerge from subsequent re-examination of the original example. For example, the selective elimination of melanic peppered moths following pollution control legislation is better documented than their original rise in frequency (Grant *et al.* 1996), and Turelli *et al.*'s (2001) re-evaluation of colour morph dynamics in *Linanthus parryae* indicated a strong role for fluctuating selection, in contrast to the original hypothesis of drift (Wright 1943).

Molecular ecology arguably dates from the development of allozyme electrophoresis and its initial application to wild populations (Lewontin & Hubby 1966). Along with *Drosophila*, *Avena barbata* (Pott, ex Link), Poaceae, was one of the first species to be examined with this technique (Marshall & Allard 1970). *Avena barbata* is an annual self-pollinating grass that is widespread in California, where it has been introduced from the Mediterranean. Inspired by early observations on morphological characters (Marshall & Jain 1969), Robert Allard and his colleagues surveyed allozyme variation in *A. barbata* ranging from a statewide (Clegg & Allard 1972) to micro-geographic scale (Hamrick & Holden 1979).

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Two observations were striking. First, wide-spread linkage disequilibrium was seen among allozyme loci, such that among five variable loci, only two multi-locus genotypes (out of a possible 32) were observed at any appreciable frequency (Allard *et al.* 1972). Second, strong spatial patterning of these genotypes was observed, such that one was consistently found in drier areas, while the other was observed in moister areas. This pattern was observed on multiple spatial scales (Clegg & Allard 1972; Hamrick & Allard 1972; Hamrick & Holden 1979). This led Allard *et al.* (1972) to hypothesize the existence of two ecotypes adapted to contrasting moisture regimes by the presence of co-adapted gene complexes.

The *A. barbata* example was both a classic and contentious example of ecotypic divergence. Seen through the prism of the selectionist–neutralist debate, which then dominated population genetics, many were sceptical of selection acting on allozymes (Lewontin 1974; Hedrick *et al.* 1976). Nevertheless, most agreed that the allozymes served as markers which had identified ecotypes adaptively diverged to contrasting environments. Numerous influential texts have included the *Avena* example (e.g. Grant 1981; Hoffman & Parsons 1991; Avise 1994; Linhart & Grant 1996), and the original three papers remain frequently cited. Thirty years after Lewontin (1974), Cox (2004), for example, cites *A. barbata* as an example of 'genetically based patterns of adaptation to local habitat conditions' in his discussion of adaptation of alien species to novel habitats.

Yet, despite this 'classic' status, the inference of adaptive divergence rests almost entirely on the spatial pattern of electrophoretic variation. Relatively few studies undertook either common garden or reciprocal transplant studies of this system. Hamrick & Allard (1975) conducted a common garden trial in the greenhouse, while Hutchinson (1982) undertook a more substantial reciprocal transplant/common garden study in the field. Both studies document divergent growth patterns of the ecotypes, but both studies revealed a consistently greater seed production of the mesic ecotype compared with the xeric. Jain & Rai (1980) also conducted a reciprocal transplant albeit with limited sample sizes. Their findings 'suggested weak selective forces consistent with those predicted from the observed patterns in natural populations' but fell well short of definitive proof.

More recently, I have begun to employ *A. barbata* as a study system in which the genetic basis of fitness variation can be examined in detail (for an overview, see Latta *et al.* 2007). A mapping population of recombinant inbred lines (RILs) has been developed from a cross between the ecotypes, leading to an initial linkage map of amplified fragment length polymorphisms (AFLP) markers (Gardner & Latta 2006). In addition, common garden plots have been established at two sites typical of the habitats with which the allozyme ecotypes were associated. Both the parental ecotypes and RILs have been examined for their performance in each of these environments (Gardner & Latta 2006, 2008; Johansen-Morris & Latta 2006, 2008), and these experiments have now been replicated over several years. As a result, there is now a substantial body of empirical data with which to evaluate whether the ecotypes are in fact divergently adapted to the contrasting environments in which they were originally described. I here synthesize that data for evidence of such local adaptation by testing three predictions: (i) the mesic ecotype will have higher fitness than the xeric ecotype at the moist site, while the reverse will be true at the dry site; (ii) the RILs will show a negative correlation between fitness at the dry site and fitness at the moist site; and (iii) quantitative trait loci (QTL) mapping will reveal genotype by environment interactions for loci related to fitness such that the allele favoured at the dry site will be disfavoured at the moist site and vice versa.

Materials and methods

Genotypes

Seeds of the mesic and xeric ecotypes were kindly provided by Dr Pedro Garcia from collections he made in northern California during the mid- to late 1980s. Six accessions of the xeric genotype and 15 of the mesic were available. These accessions were screened to verify their allozyme genotype before proceeding. Seeds were planted in the McGill University greenhouse to propagate the seed stocks, and to perform the cross needed to produce the RILs. These accessions, referred to here as 'parental lines' (as opposed to the recombinant hybrids), were screened for numerous AFLP markers for use in genetic mapping. While approximately 200 such markers showed fixed differences between the ecotypes, only one showed any indication of polymorphism within ecotypes (Gardner & Latta 2006). In addition, the ecotypes are strongly diverged for a suite of quantitative traits, but there is generally little heritable variation among accessions within either ecotype, despite a substantial release of genetic variation among the RILs due to recombination (Latta *et al.* 2004; Gardner & Latta 2008).

Crosses between the ecotypes were performed by the hand pollination techniques of Brown (1980). Several F_1 individuals were produced, each of which produced abundant F_2 seeds. One such family of F_2 (i.e. the selfed progeny of a single F_1) was propagated by single seed descent in the Dalhousie University greenhouse for six generations, giving 188 F_6 inbred lines (details in Gardner & Latta 2006). These lines are almost completely

homozygous, each for a unique novel combination of the alleles that differentiate the parents. As the selfed progeny of a homozygote are themselves all homozygous, each recombinant genotype can thus be precisely replicated within and between environments. The genotype of each RIL was determined for a panel of 129 AFLP markers to create a genetic map (Gardner & Latta 2006).

Environments

Experimental plots were established at the Hopland Research and Extension Center and at the Sierra Foothills Research and Extension Center, both operated by the Division of Agriculture and Natural Resources at the University of California, Davis (http://danrrec.ucdavis.edu/). Each is a reserve of some 2500 ha of rangeland, at roughly the same latitude and altitude. Each of these sites was included in the allozyme surveys of the 1970s (Hutchinson 1982). Sierra Foothills, on the east side of California's Central Valley, north-east of Sacramento was monomorphic for the xeric allozyme genotype (Hutchinson 1982), as were many neighbouring sites in the foothills (e.g. Clegg & Allard 1972). Hopland, in the California Coast Ranges, north of San Francisco, is just to the north of the Calistoga populations studied intensively for micro-geographic variation (Hamrick & Allard 1972; Hamrick & Holden 1979). Thus, Hopland lies within the mesic region of *A. barbata*'s range (Clegg & Allard 1972).

Hopland receives 40% more rainfall on average than does Sierra Foothills along with a less severe summer drought. In addition, the two common garden plots were sited to accentuate differences in moisture availability, by locating the Hopland common garden in a shallow swale near a seasonal stream, while the Sierra Foothills garden was located on a stony hilltop. Such micro-topographical distinctions have been shown to associate with the relative frequencies of the two genotypes (Hamrick & Holden 1979). I verified that the specific locations of our gardens were occupied by the mesic and xeric ecotypes, respectively, using a visible morphological marker. (Stem pubescence is consistently observed in the mesic ecotype and is absent in the xeric, and this trait shows strict genetic inheritance among the RILs, showing the 3:1 ratio expected for a two-locus trait in inbred lines.)

Year-to-year variation in climate was considerable across the 4 years of the study. Detailed weather data (http://www.cimis.org) from each site show that the growing seasons ranged from among the wettest (2005–2006) to the driest (2006–07) of the last 15 years (Fig. 1), and are typical of the range seen since the stations were established in the 1950s.

Common gardens

Common gardens were established in the fall of 2002. Plots approximately 35×25 m were fenced to exclude grazing livestock but were otherwise unmanipulated, with the goal of exposing the genotypes to as much of the natural environment as possible. At each site, three randomized complete blocks were established. Six mesic and six xeric accessions were each replicated eight times across the three blocks (two to three individuals per accession per block, for 48 of each parental type total). Three individuals of each RIL were planted, one in each block. In addition, a subset of 25 of the RILs

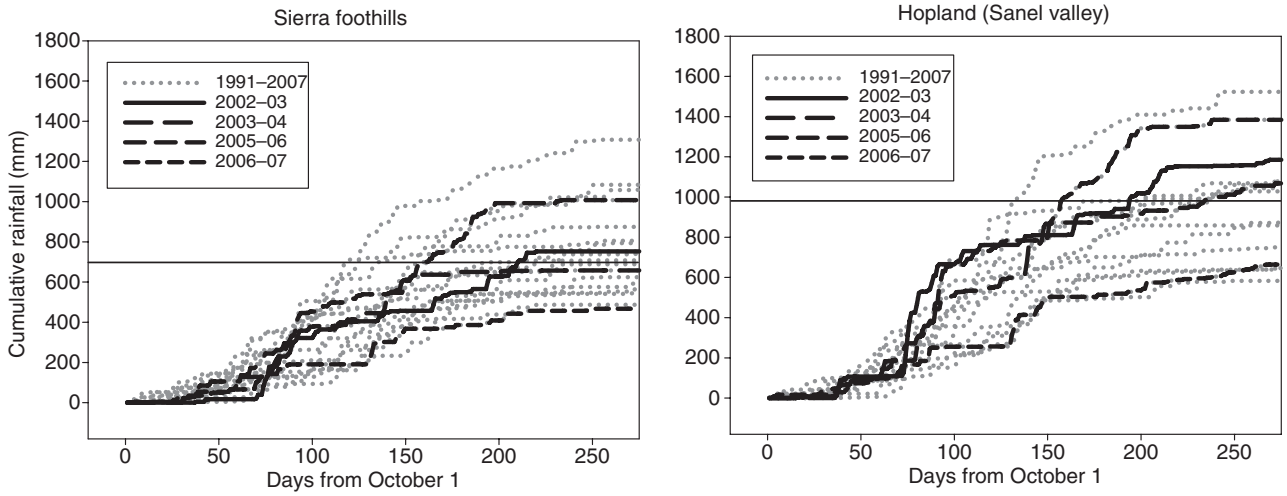

Fig. 1 Rainfall data for the field sites since 1991 (http://www.cimis.org). The 4 years during which field trials were conducted are highlighted against the background of inter-annual climate variation. Long-term annual averages are indicated by horizontal lines.

were investigated more intensively at 16 individuals per line (five to six per line in each block) for a separate experiment (Johansen-Morris & Latta 2006, 2008). Each individual was randomly located within each block.

In early November 2002, coincident with the first germinating rain of the winter growing season, seeds were germinated on filter paper following Latta *et al.* (2004). Germinated seedlings were planted into 5-cm-diameter Ray-Leach Cone-tainers (Stuewe & Sons, Corvallis OR, USA) filled with Sunshine germination mix (Sun-Gro Horticulture, Vancouver, BC, Canada), and allowed to establish in the Hopland greenhouse. Once seedlings were established, they were transplanted to the field as follows. A hole of the same diameter as the Cone-tainer was punched in the soil surface with an iron spike, leaving the surrounding vegetation undisturbed. The bottom of each Cone-tainer was cut off below the root, and the cut Cone-tainer was placed within the punched hole such that the roots could grow down through the end of the Cone-tainer into the natural soil.

At the end of the growing season, and the onset of summer drought (early June 2003), the experimental plants were identified by the rim of the Cone-tainers. The height of the plants and the number of reproductive tillers and spikelets were recorded. Plants were cut off at ground level, and placed in paper bags in a drying oven at 55 °C for 4 days to remove any remaining moisture before above ground dry weights were taken.

The same procedure was repeated (at the same sites, and using the same genotypes) in three subsequent growing seasons with the following variations. In 2003–2004, as the fence excluded grazing livestock there was considerable standing dead vegetation on the plots. The consequence of this was that a thatch formed over the seedlings which apparently exacerbated mortality that year. In 2004–2005, the plots were left fallow, although the thatch was burned off in fall 2004 as a fire prevention measure, resulting in minimal standing dead vegetation in 2005–2006. In 2005–2006, sample sizes were reduced to three for all RILs because many of our seed stocks were running low, especially for the 25 intensively studied RILs. Also in this year, Cone-tainer-liners with open sides became available, and these were used to increase the contact with the natural soil below ground.

Finally, for the 2006–2007 growing season, F_7 RILs were propagated by self-fertilization from each of the F_6 to replenish seed numbers. Note that the only genetic difference between these generations is that any residual heterozygosity is reduced by half. Sample size in the common garden was increased to five per RIL. In addition, seed germination was examined under more natural environmental conditions than in previous years, as follows. Natural soil was dug from the garden plots and sifted to remove any *A. barbata* seed bank. The soil was placed in smaller 2.5-cm Cone-tainers and seeds were placed directly into the soil (as opposed to germinating on filter paper as in previous seasons). Racks containing the cones were placed in the field immediately prior to the first germinating rain of 2006 and left until seedlings emerged (racks were covered with plastic mesh to simulate the protection of the dead vegetation from the direct impact of the rain while still allowing germination to be monitored). Once germination was complete, cones were planted as before. In total, eight field trials (two sites × 4 years) were conducted.

Statistical analysis

Individuals that failed to survive were assigned a fitness of zero. However, a few individuals in the first year showed signs of having been either broken or eaten by herbivores and it was not clear whether this occurred before or after seeds were matured (observations in 2007 suggests that it was before – not shown). These individuals were treated as missing data.

Genotype by environment interactions were examined using general linear models in SPSS 15 (SPSS Inc.). Parental lines were examined separately from the RILs. Parental ecotype was treated as a fixed factor, as was site, while year and accession within ecotype were random factors. Fitness measures were log-transformed (log[no. of spikelets + 0.5]) prior to analysis, and residuals were examined for normality. The analysis of the RILs was similar, except that RIL was a random factor, and there was no accession effect nested within RILs. Genetic correlations of mean RIL performance across environments were calculated using RIL line means.

I repeated the analysis of QTL under selection from Gardner & Latta (2006), with the additional data from 2006 and 2007. In addition to the composite interval mapping procedure reported in our previous paper, I employed joint interval mapping (Jiang & Zeng 1995) to pool statistical power across multiple year–site combinations. However, a full analysis of epistatic and pleiotropic effects is beyond the scope of the present study which is restricted to estimation of additive allelic effects on fitness.

Results

Environmental variation between sites and years translated into markedly different mean absolute fitness expressed by the plants. Overall survivorship and fecundity varied substantially across the two sites and 4 years of the study (Fig. 2). In the drier years, mortality was substantial, especially at Hopland, where 94%

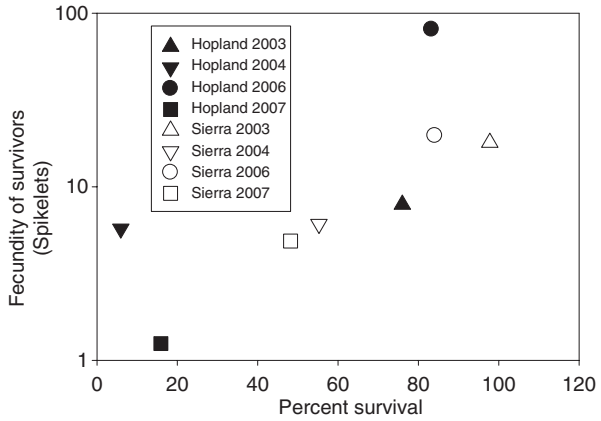

Fig. 2. Mean survival and fecundity of survivors across all individuals at each site each year.

mortality occurred in 2004 and 85% in 2007. In these years, survival represented a major component of fitness variation. In other years survival was high, and fecundity was the major determinant of fitness. Mean spikelet production per survivor ranged from a low of 1.25 in Hopland 2007 to a high of 80 in Hopland 2006.

Parental ecotypes

The mean fitness of the mesic ecotype was consistently higher than that of the xeric, across all sites and years (Fig. 3), and the main effect of ecotype was statistically significant (Table 1). Variation among accessions within ecotypes was minimal, although marginally significant. There were no significant main effects of either year or site, and all environmental variance was accounted for by the year by site interaction. Significant genotype by environment interaction was observed, in the three-way interaction of genotype by site by year. Residuals approximated a normal distribution showing little skew but some leptokurtosis.

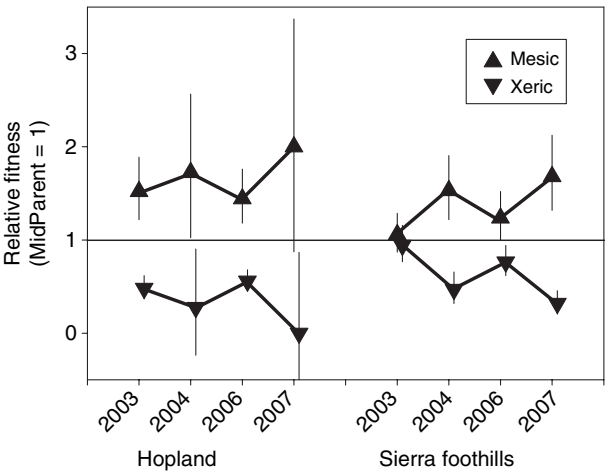

Fig. 3. Mean relative fitness with 95% confidence limits of the mesic and xeric ecotypes of *Avena barbata* across two sites in each of 4 years. Least squares means of log(no. of spikelets + 0.5) were back-transformed to an arithmetic scale and standardized to a mid-parent fitness of 1.

Table 1 Analysis of variance in fitness effects attributable to variation in environment (site, year) and genotype (ecotype, accession, RIL)

Source	d.f.	MS	*F*	*p*
Mesic and xeric ecotypes				
Site	1	54.691	0.584	0.501
Year	3	383.874	4.648	0.142
Site × year	3	93.680	11.261	0.022
Block (site × year)	16	2.953	1.629	0.056
Ecotype	1	63.072	36.213	0.001
Accession (ecotype)	11	3.243	1.789	0.052
Site × ecotype	1	0.148	0.021	0.895
Ecotype × year	3	0.313	0.045	0.985
Site × ecotype × year	3	7.171	3.956	0.008
Residual	778	1.813		
Recombinant inbred lines (RILs)				
Site	1	170.777	0.343	0.599
Year	3	1954.093	3.839	0.149
Site × year	3	510.844	61.596	0.000
Block (site × year)	16	8.736	6.948	0.000
RIL	192	5.315	2.564	0.000
Site × RIL	191	1.525	0.943	0.684
Year × RIL	560	2.227	1.357	0.000
Site × year × RIL	558	1.641	1.305	0.000
Residual	4595	1.257		

Dependent variable was log(0.5 + no. of spikelets), where individuals that failed to survive to maturity had zero spikelets.

In no site or year was the xeric ecotype more fit than the mesic ecotype. Thus, the genotype by environment interaction seen for the parental ecotypes derives from changes in the size of the fitness difference between them, rather than the rank order (Fig. 3). Selection favoured the mesic ecotype by a greater margin at Hopland than at Sierra Foothills, but surprisingly also by a wider margin in dry years than in wet.

Recombinant inbred lines

There was strongly significant fitness variation among recombinant genotypes (RILs) (Table 1). That is to say, certain genotypes consistently outperformed others across the eight field trials. Significant interaction was also observed between Genotype and year as well as a three-way interaction of Genotype by site by year. The Genotype by site by year interaction reflects the vastly different variance in fitness between years when mortality was high (and consequently most individuals and

Table 2 Genetic correlations (correlation among line means) of fitness across sites and years in 188 recombinant inbred lines of the xeric and mesic ecotypes of *Avena barbata*

	Hopland				Sierra Foothills			
	2003	2004	2006	2007	2003	2004	2006	2007
Hopland								
2003		−0.0177	0.1783	0.0398	0.4222	0.1789	0.1478	0.1146
2004	0.0534		−0.0479	−0.0304	0.0819	0.1922	−0.0019	0.1586
2006	0.2324	−0.0072		0.1465	0.2843	0.1619	0.2163	0.1719
2007	0.0847	0.0321	0.2271		0.1681	0.0445	0.1103	0.2322
Sierra Foothills								
2003	0.4418	0.1610	0.4623	0.2373		0.2661	0.1285	0.3043
2004	0.1495	0.1843	0.2639	0.0348	0.3428		0.1204	0.3357
2006	0.1212	0.0900	0.3405	0.1548	0.2358	0.1921		0.3100
2007	0.2182	0.1654	0.2867	0.2905	0.3602	0.3888	0.3358	

Arithmetic above diagonal, log-transformed below.

RILs had zero fitness giving low fitness variance) and years when mortality was low and fitness variance among lines was greater (discussed in detail in Johansen-Morris & Latta 2008). Again, residuals were somewhat leptokurtic but not skewed.

The genetic correlation of fitness across environments was generally positive both across years within sites, or across sites within years (Table 2, Fig. 4). The only cases of negative correlations were very small and not significantly different from zero. All such instances involve the fitness at Hopland in 2004, where there was little variation in fitness due to extreme mortality.

It is widely recognized that positive genetic correlations can mask the presence of underlying trade-offs if a large number of genotypes have low fitness in both environments (Houle 1991; Fry 1993; Mackenzie 1996). Scatterplots of fitness in one environment vs. another occasionally suggest the presence of genotypes with high fitness in one environment, but intermediate fitness in another. For example, the best fit RIL at Sierra Foothills in 2003 had quite low fitness the following year (Fig. 4, lower panel). However, this result must be treated cautiously because a low sample size (three per RIL) means that the estimate of fitness for any specific line in a specific environment will have large error, especially when mortality is high. Indeed, for those lines for which larger sample sizes (16 per RIL) are available, the correlation across environments becomes more positive – $r = 0.742$ for the 25 RILs intensively studied by Johansen-Morris & Latta (2008) in 2003, vs. $r = 0.421$ for all RILs (Fig. 4, top panel).

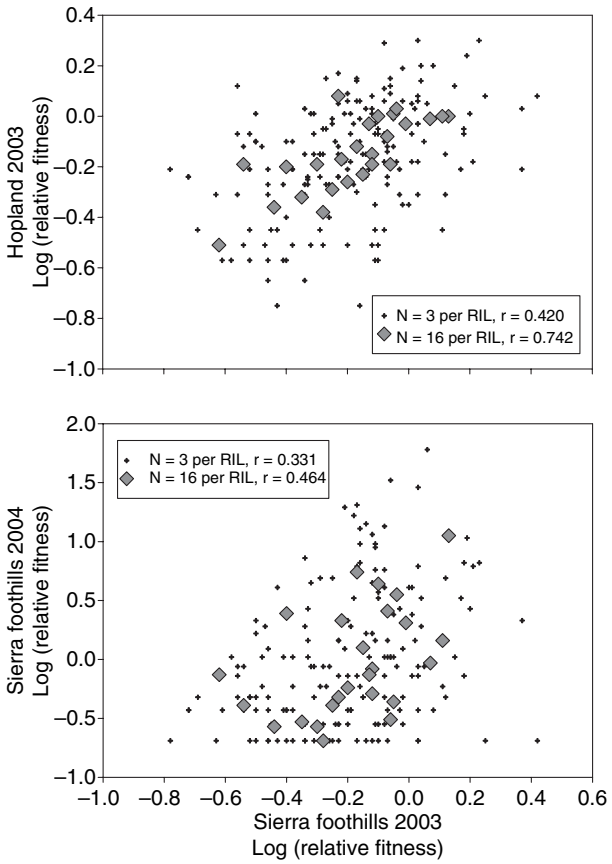

Fig. 4. Scatterplots of F_6 recombinant inbred line fitness across separate environments. Genotypes for which larger sample sizes (and therefore more precise estimates of genotype fitness) were available from Johansen-Morris & Latta (2008) are highlighted.

Quantitative trait loci

As mortality was so high at Hopland in 2004 and 2007, most families had zero fitness, and the variation of mean fitness among lines was very low. Consequently, these year/site combinations were omitted from the QTL analysis (see Gardner & Latta 2006). Our earlier

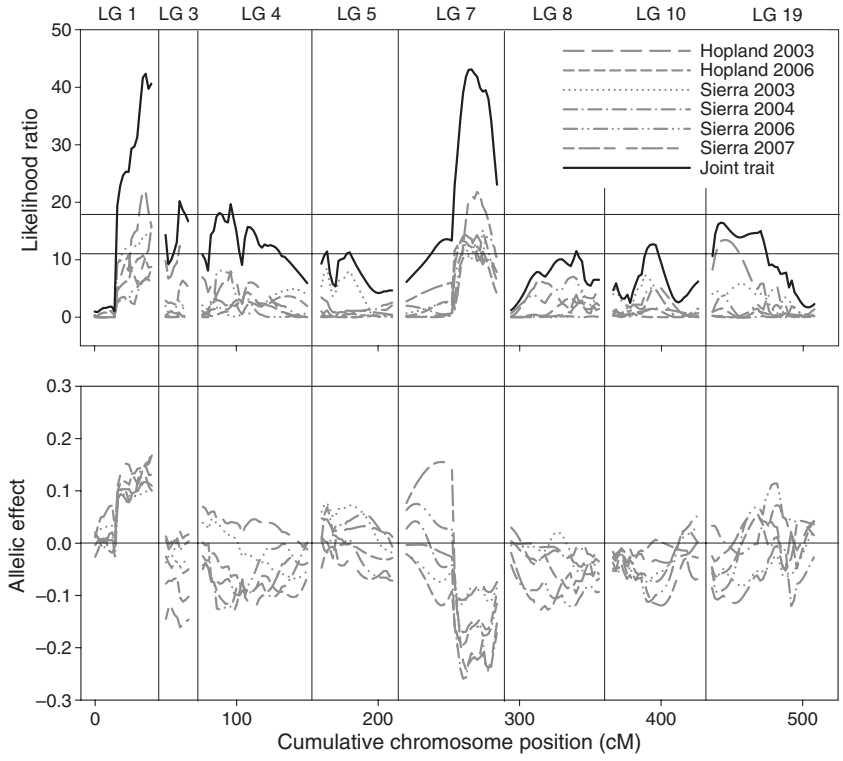

Fig. 5. Joint interval mapping of quantitative trait loci (QTL) for relative fitness in each environment. Top: likelihood ratio plots. Horizontal lines represent significance thresholds (based upon permutation) for individual traits (lower line) and joint trait (upper line) analysis. Bottom: allelic effects. A positive value indicates that the xeric allele increases fitness, a negative value indicates that the xeric allele decreases fitness. Hopland 2004 and 2007 are omitted due to lack of variation caused by high mortality. Linkage groups without significant QTL are omitted.

study found two primary QTL with effects on fitness across field environments. These loci, on LG 1 and LG 7 also affected fitness in the 2006 and 2007 (Fig. 5). Few other loci show such strong associations with fitness, but the joint analysis reveals several other chromosomal regions (e.g. LG 3 and LG 19) that associate significantly with aggregate fitness across all years. For the two strongly significant QTL, allelic effects are in the same direction in all years and environments (Fig. 5). The mesic allele at LG 7 is associated with higher fitness, while it is the xeric allele at LG 1 that associates with higher fitness – these effects are constant across environments. The secondary QTL (significant in the joint analysis) also show this trend (e.g., LG 19). None of these loci show a significant reversal of fitness effects across environments.

Discussion

The key observation documenting local adaptation is a change in the rank order of fitness among genotypes such that within each environment, local genotypes are consistently more fit than conspecific immigrants (Kawecki & Ebert 2004). A long tradition of reciprocal transplant experiments has identified numerous cases where this criterion is met (e.g. Clausen *et al.* 1941; Antonovics *et al.* 1971; Turkington & Harper 1979; Schemske 1984; Via 1991; Nagy 1997). Although *A. barbata* enjoys a certain 'classic' status as the original example of genetic markers identifying ecotypic divergence, there is – surprisingly – no evidence for local adaptation. Three observations consistently emerge. First, the mesic ecotype consistently outperforms the xeric ecotype in both survival and fecundity (Fig. 3), whereas one would expect the xeric ecotype to have higher fitness in drier environments. Second, the recombinant progeny of the cross between the ecotypes fail to show evidence of a trade-off in performance between environments – the genetic correlations are generally positive (Fig. 4, Table 2), and never significantly negative as would be expected if there were fitness trade-offs across environments. Third, the QTL results show that the same alleles are consistently favoured in all environments (Fig. 5), where local adaptation predicts that alternate alleles would be favoured in different environments.

Experimental considerations

Four general explanations could potentially be advanced to explain this lack of evidence if local adaptation does indeed occur in *A. barbata*. However, I believe all four can be largely refuted. First, the experiment may have lacked the necessary power to detect significant local adaptation, and much of the foregoing has been presented to document the extent of efforts to find evidence for local adaptation. Over 6500 individuals of 190 known genotypes have been examined for their lifetime reproductive success. The experiment was

replicated over four growing seasons (corresponding to four generations for wild populations), which span the range of inter-annual climate variation. Evidence for local adaptation was sought at several levels from the parental genotypes to individual QTL. In this, it greatly exceeds the power of previous field studies of these ecotypes (cf. Jain & Rai 1980). If local adaptation has gone undetected due to a lack of statistical power, we must infer that the effect is small.

Second, one could argue that local adaptation occurs through some fitness component or selective agent not studied in this experiment. For this reason, special care was taken to expose the genotypes to all aspects of the environment – both above and below ground and both abiotic and biotic. In the final year, I included germination success under field conditions (seeds which failed to germinate were retrieved at the end of the growing season and tested to demonstrate nonviability, and thus the absence of an appreciable seed bank – not shown). Although it is conceivable that 2006–2007 was an unusual year for germination and seedling establishment – fitness components which were less thoroughly explored than growth and fecundity – it was nevertheless a year with substantial opportunity for selection through these traits, given the degree of seedling mortality. In addition, one of the advantages of *A. barbata* as a study organism is the ease with which fitness can be directly measured. As *A. barbata* produces two self-fertilized seeds per spikelet and the glumes are retained after the seeds drop, a count of spikelets provides a direct measure of lifetime reproductive success. The only remaining component of fitness not directly examined here is over-summer seed survival. It would be an unexpected mechanism that could drive ecotypic differentiation between moist and dry environments through differential survival of dormant seeds.

Range of genotypes and environments

Third, it is possible that a greater range of genotypes would include some which outperform the mesic genotype at Sierra Foothills. This possibility is somewhat discounted by the inclusion of the RILs in this study, as 188 different multilocus combinations were assayed in addition to the parental mesic and xeric types. Some of the recombinants do outperform the mesic parent under greenhouse conditions (Johansen-Morris & Latta 2006), but this effect is greatly reduced in the field (Johansen-Morris & Latta 2008). In addition, as the RILs represent novel combinations of only that variation that separates the parents of the cross, there is the possibility of further allelic variation in other accessions not used here. While there is little variation within ecotypes among the accessions from northern California (Gardner & Latta 2008), there is evidence of divergence between northern and southern accessions of the xeric allozyme genotype. Hutchinson (1982) reports substantially earlier flowering in southern California xeric accessions grown at Sierra Foothills, compared with northern Californian accessions, which flower consistently later. Comparing the northern California accessions used here to a collection from the Riverside area of southern California (under greenhouse conditions) shows a similar result (personal observation), indicating that there is indeed greater genetic variation at a statewide geographic scale than the allozymes indicate. However, in Hutchinson's (1982) study, the southern California xerics were no more capable of outperforming the mesics at Sierra foothills than were the northern California xeric accessions.

The final possibility is that the experiments were not conducted in appropriate environments to bring out local adaptation. The four growing seasons examined here span the range of inter-annual climatic variation, but it may be that the Sierra Foothills site is not typical of the environments to which the xeric genotype is adapted. However, the Sierra Foothills site is within a monomorphic region of the xeric ecotype (Clegg & Allard 1972) and was explicitly surveyed by Hutchinson (1982) confirming monomorphism for the xeric ecotype on this site. The use of only one dry and one moist site was in some degree a logistical choice given the difficulty of maintaining multiple simultaneous experiments in geographically disparate eco-regions (compounded by the need for access to appropriate ecological reserves). We have also conducted a number of greenhouse investigations in which we have altered the watering and fertilizer regime (Sherrard & Maherali 2006; Johansen-Morris & Latta 2008), as well as the competitive environment (Latta *et al.* 2004) to expand the range of environments in which the ecotypes were compared. In none of these environments did the xeric exhibit higher fitness than did the mesic.

The two sites used here do not, however, represent the extremes of the environments in which *A. barbata* is found. For example, *A. barbata* can be found in semi-desert environments in southern California and extending into Mexico. Such environments not only receive substantially less rainfall, but the onset of summer drought is considerably sooner, creating a much shorter growing season. It is therefore possible that the mesic genotype would lose its advantage in southern California.

Two conclusions

These latter two possibilities are, of course, not mutually exclusive. If we accept that the results reported here accurately reflect a lack of local adaptation, within *northern* California, there may be local adaptation

between northern and southern populations. Such a hypothesis would suggest that *A. barbata* accessions from southern California which exhibit earlier flowering than their northern counterparts (Hutchinson 1982) have an advantage in the shorter growing season of southern California. However, if indeed such a pattern of local adaptation occurs, it must be emphasized that it is not reflected in the allozyme genotypes with which the system was first identified as a potential case of ecotypic divergence. The results of the common garden studies make clear that the distribution of electrophoretic allozyme variation described by Allard *et al.* (1972) does not accurately predict a pattern of local adaptation and ecotypic divergence. The widespread occurrence of the xeric genotype in the foothills of the Sierra Nevada (Clegg & Allard 1972) does not, in fact, indicate an adaptation to that environment.

Rather, the common garden experiment indicates that as the mesic ecotype is more fit at Sierra Foothills – and presumably sites like it – this genotype should be spreading throughout northern California. I would suggest that this is indeed occurring, and that the mesic genotype is increasing in frequency and range as it displaces the (northern) xeric genotype through natural selection. Alternatively, a recombinant genotype combining the selectively advantageous features of each may be displacing both the original ecotypes. Our prior experiments have documented the occurrence of high fitness recombinants from within our mapping population (Johansen-Morris & Latta 2006), although this advantage is more pronounced in novel environments than it is in the field (Johansen-Morris & Latta 2008). The QTL mapping results provide a clear mechanism for such a transgressive segregation in that the xeric parent has the favoured allele on LG1, while the mesic parent has the favoured allele on LG7 (Fig. 5; Gardner & Latta 2006). One in four recombinants will have the favoured allele at both loci and (holding all else constant) will have a selective advantage over both ecotypes (cf. Rieseberg *et al.* 2003).

This hypothesis can be tested by comparing past genotype distributions documented by Allard with the present genotypes extant in California. A full re-survey of the state remains to be undertaken, but one morphological character can be easily scored and is readily compared with past records. The mesic genotype exhibits a pubescent leaf sheath as opposed to a glabrous sheath on the xeric genotype (Rai 1972; personal observation). This trait is easily scored and is clearly true breeding in the RILs. If the mesic ecotype is spreading, then pubescent stems should be more abundant today than they were in the past.

Most herbarium records at UC Davis and at the Jepson Herbarium collected prior to about 1950 were glabrous, whereas pubescent stems become more common in the more recently collected specimens (M.A. Blumer, personal communication; personal observation). Specimens collected from Hopland and Sierra Foothills, ca. 1950 are all glabrous. Rai (1972) undertook a microgeographic survey at Hopland, recording the frequency of pubescent stems at 38 locations around the station. The mean frequency of the glabrous morph was 0.48. When the same (to within ca. 100 m) locations were resurveyed in 2007, all but one of the locations were monomorphic for the pubescent morph (personal observation). Hutchinson (1982) documents that in 1978, plants at Sierra Foothills were monomorphic for the glabrous stem, but, in 2007, pubescent morphs are present over many parts of the station (personal observation), as well as other sites in the Sierra Foothills. Similarly, the glabrous morph is now largely absent from the Calistoga area, although this region was described as highly polymorphic in the 1970s (Hamrick & Allard 1972).

These observations demonstrate that the frequency of a genetically controlled phenotype has changed over approximately 30–40 generations of this annual species – or in other words, that evolution has occurred. The important evolutionary forces at play seem to be migration – driving the spread of the favoured genotype(s) marked by the pubescent trait into drier areas – and selection favouring the increased frequency of those genotype(s) once they arrive. We cannot determine at this point whether the change represents simply a replacement of xeric ecotypes by their mesic counterpart, or whether novel recombinants (marked by pubescent stems) are increasing in frequency. Such recombinants could entail either the introgression of favoured mesic alleles into the xeric ecotype, or the spread of a broadly adapted genotype that is displacing both of the original ecotypes (cf. Hedge *et al.* 2006).

The broader implication for molecular ecology is thus not to deny that local adaptation can occur but to document that it is not always accurately reflected in the pattern of electrophoretically detectable variation. This contrast between the traditional interpretation of the allozyme patterns in *A. barbata* and the experimental evidence presented here is striking. At the time of the original allozyme surveys (Allard *et al.* 1972), population genetics was focussed on the selectionist–neutralist debate (Lewontin 1974, 1991). Indeed most early discussions of allozyme variation in *A. barbata* concerned whether the allozymes were themselves the targets of selection or simply markers linked by genetic hitchhiking (Hedrick & Holden 1979). Local adaptation may have been readily accepted because the targets of selection were under such heated debate. To a lesser extent, debate over the importance of interactions between loci (the possibility of 'coadapted gene complexes') may

have made the inference of local adaptation seem uncontroversial.

However, the inference of local adaptation (or indeed any evolutionary process) from static electrophoretic patterns assumes that those patterns have reached equilibrium with natural selection. In the 1970s, most population genetic theory explored analytical models of equilibrium conditions (Crow & Kimura 1970). Only more recently have theoretical advances (such as, for example, coalescent theory, Kingman 1982; Hudson 1990) allowed us to explore nonequilibrium population genetics. In addition, it is only relatively recently that biologists have begun to fully appreciate the rapid rate of contemporary evolution (Hendry & Kinnison 1999), often in response to the rapid changes that humans are currently causing in the natural environment. Indeed, Allard (1996) fully recognized the rapidity of *A. barbata*'s adaptation to its introduced range in California, where it has occurred for only about 150–200 generations. In this regard, *A. barbata* seems a particularly likely candidate for continued evolution to be occurring in the present day.

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Robert Latta's main research focus is to understand the genetic basis of fitness variation in natural populations. He has been intrigued by the *Avena barbata* system ever since reading the original studies as an undergraduate, and adopted it as a study system in which RILs could be easily created. Other aspects of this work address the role of genetic correlations, hybridization and recombination, dominance and epistasis, phenotypic plasticity, physiological ecology and transgressive segregation in determining the fitness and niche breadth of genotypes.
