An evolutionary approach to studying the relative importance of plant–plant interactions along environmental gradients

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Summary

1. Abiotic stress and interactions with neighbours are major selective forces but their relative importance for local adaptation has rarely been separated. Plant community theory predicts increasing importance of competition with decreasing stress. We experimentally separated the role of neighbours and drought stress for local adaptation of two annual plant species. We predicted that neighbours amplify patterns of local adaptation with adaptation to competition prevailing in benign habitats.

2. During one growing season, we combined reciprocal transplants with neighbour removals along a gradient of more than an eightfold increase in annual rainfall using populations from sites representative of Arid, Semi-arid, Mediterranean and Mesic Mediterranean ecosystems. We evaluated statistical interactions between origin and planting site with and without neighbours and quantified plant–plant interaction importance.

3. An extreme drought ($< 63\%$ of the average annual rainfall at the arid sites) reduced the probability of detecting neighbour effects. There was weak but non-significant evidence for local adaptation and for the amplification of adaptation patterns with neighbours for populations from wetter sites of the two species. Our results together with previous findings in similar gradients suggest that detecting local adaptation to neighbours in unpredictable environments depends on the climatic conditions of the study season.

4. We coupled a central concept from plant community ecology with an evolutionary approach to separate the role of abiotic vs. biotic factors for local adaptation. This permits quantifying the importance of interactions with neighbours and we advocate its use in future studies of local adaptation, which should be conducted over several years.

Key-words: annual plants, competition, environmental gradient, facilitation, local adaptation, plant–plant interaction importance

Introduction

Understanding the forces that control species distributions and community structure has been a central goal of plant ecology (Barton 1993). Two major classes of 'ecological filters' (e.g. Keddy 1992) determining the occurrence and performance of plants are abiotic constraints ('stress' *sensu* Grime 1977) and interactions with neighbours (i.e. competition and facilitation). Ample evidence from studies conducted along environmental gradients shows that these two filters are not independent, i.e. the intensity and importance of plant–plant interactions depend on the level of abiotic stress and the productivity of the environment (Goldberg

et al. 1999; Brooker *et al*. 2008). However, the spatial and temporal scales at which these major constraints prevail and their relative roles as selective factors are not yet clear (see Pearson & Dawson 2003; Hampe 2004).

Widely distributed species encounter large spatial variation in biotic and abiotic factors across their range. In response, single genotypes may show phenotypic adjustments to the experienced conditions, i.e. phenotypic plasticity (Bradshaw 1965; Sultan 2000; Alpert & Simms 2002), or populations may undergo genetic differentiation and evolve traits that result in higher relative fitness in their native habitats than genotypes from other habitats, i.e. they show local adaptation (Williams 1966). The expression of local adaptation in plants is well-documented (Leimu & Fischer 2008) and a considerable number of reciprocal transplant studies

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has evaluated adaptive genetic differentiation among populations in response to soil and climate-related factors (e.g. Joshi *et al*. 2001; Becker *et al*. 2006; Abdala-Roberts & Marquis 2007; Macel *et al*. 2007). Studies focusing on specific biotic selective forces are less common (Crémieux *et al*. 2008) but provide evidence of adaptation to local herbivory levels (Schemske 1984; Sork, Stowe & Hochwender 1993), neighbour's allelochemicals (Grøndahl & Ehlers 2008), neighbour identity (Turkington & Harper 1979) and to the presence of symbiotic soil nitrogen-fixing bacteria (Lie *et al*. 1987; Parker 1995). Remarkably scarce are field studies specifically conducted to evaluate the role of plant–plant interactions (competition and facilitation) as selective factors in forming locally adapted populations (e.g. Kindell, Winn & Miller 1996; Knight & Miller 2004; Bischoff *et al*. 2006; Sambatti & Rice 2006; Lankau & Strauss 2007; Rice & Knapp 2008). Also, these have yielded ambiguous results with competition amplifying patterns of local adaptation (Knight & Miller 2004; Rice & Knapp 2008), having no effect (Kindell, Winn & Miller 1996; Sambatti & Rice 2006) or having a species-specific amplifying effect (Bischoff *et al*. 2006). Density-dependent interactions are among the most studied biotic processes and their role on the composition, structure and function of communities has been repeatedly recognized. It is therefore surprising that reciprocal transplants have only rarely been combined with neighbour manipulations for separating the importance of abiotic factors and interactions among plants.

A useful framework for predicting the relative importance of the above two filters for local adaptation stems from plant community ecology. Namely, it is commonly assumed that facilitation and competition act simultaneously but dominate at opposite ends of gradients of abiotic stress because net-positive interactions are most often observed in physically stressful and low productive habitats (e.g. Bertness & Callaway 1994; Greenlee & Callaway 1996; Brooker & Callaghan 1998; Pugnaire & Luque 2001; Callaway *et al*. 2002; but see Tielbörger & Kadmon 2000; Maestre & Cortina 2004). Conversely, as interactions among plants become more frequent with increasing biomass, competition should prevail in fertile habitats (e.g. Grime 1977; Wilson & Keddy 1986; Campbell & Grime 1992; Twolan-Strutt & Keddy 1996). Yet, empirical data from neighbour manipulation studies have supported all possible trends of either increasing or decreasing competition (or facilitation) intensity along stress gradients, or have detected no trend at all (see Goldberg *et al*. 1999; Maestre, Valladares & Reynolds 2005). Such conflicting results have fuelled a classical 'evergreen' in plant community ecology, the 'Grime–Tilman debate', which has been recently expanded to facilitation in the context of the so-called stress-gradient hypothesis (Bertness & Callaway 1994; evidence reviewed in Brooker *et al*. 2008).

Despite the perpetual debate about *intensity* of interactions along environmental gradients, there seems to be consensus about their *importance* relative to abiotic factors. Specifically, importance of facilitation is expected to dominate in harsh environments, whereas competition should be more impor-

tant in habitats with low abiotic stress (Grime 2001; Brooker *et al*. 2005). Interaction intensity is usually measured in experiments and is expressed as the relative difference in performance of isolated plants vs. plants grown with neighbours. The importance of interactions describes the magnitude of neighbour effects on plant performance relative to all other factors (e.g. abiotic stress, herbivory). It is not amenable to direct measurement but can be calculated from experiments measuring interaction intensity (Welden & Slauson 1986; Corcket *et al*. 2003; Brooker *et al*. 2005; Brooker & Kikividze 2008; Seifan *et al*. 2010). 'Importance' is the appropriate concept underlying the initial assumptions of Grime (Grace 1991; Grime 2007; Brooker & Kikividze 2008; Kikvidze & Brooker 2010), and thus it should be quantified more often.

Here, we introduce a novel aspect to the above discussion by addressing 'importance' from an evolutionary point of view. Interaction importance reflects the result of past competitive interactions (Welden & Slauson 1986), and we suggest that measures of importance should reflect specific adaptations of species to the most important environmental constraint. The prediction for evolutionary responses to gradients of environmental stress and productivity is straightforward when we assume that the *importance* of negative interactions decreases with stress. In that case, plants inhabiting benign environments should exhibit a larger degree of local adaptation to competition than plants from environments of high abiotic stress. The latter, in turn, should be locally adapted to abiotic stress but perform worse under competition. Although this may seem obvious, the evolutionary aspect of clinal changes in importance of interactions along gradients has rarely been studied (see Brooker *et al*. 2008, for review on facilitation).

Climatic gradients provide an excellent opportunity to simultaneously test the relevance of biotic and abiotic factors on ecological and evolutionary processes over a range of habitats (e.g. Holzapfel *et al*. 2006; Petrů *et al*. 2006). Here, we used a sharp rainfall gradient, characterized by abiotic stress (water scarcity, rainfall unpredictability) at its dry end and by biotic processes (above-ground competition due to high plant productivity) at its humid end (Holzapfel *et al*. 2006; Schiffers & Tielbörger 2006), to examine experimentally how these contrasting factors constrain plant performance and interact in determining local adaptation. Using reciprocal transplants coupled with neighbour removals, we separately examined the occurrence of local adaptation to neighbours, climate and overall (neighbour + abiotic) environmental conditions along the gradient and assessed how the relative contribution of plant–plant interactions to adaptive processes varies among sites. We studied widely distributed annual species which, under homogeneous conditions, have shown ecotypic differentiation in morphological, phenological and demographic traits (Petrů *et al*. 2006; Petrů & Tielbörger 2008). With our study, we introduce an approach that allows (i) experimentally separating the role of abiotic factors vs. plant–plant interactions in determining local adaptation, and (ii) testing for the importance of interactions along gradients based on evolutionary thinking.

Based on the above theoretical considerations and previous supporting evidence for Grime's model from our system, we predicted that (i) adaptation to stressful conditions dominates in populations local to harsh climates and adaptation to neighbour presence occurs in populations from benign climates, because (ii) the sign and importance of biotic interactions shift along climatic gradients, from positive neighbour effects at stressful sites to increasingly negative effects towards benign sites.

Materials and Methods

STUDY SITES

Our experiments were conducted at four research sites along a steep climatic gradient that runs from the Negev desert to the upper Galilee in Israel (Table 1). The overall climate in the region is Mediterranean with cold, wet winters and hot, dry summers. The growing season of annual plants is determined by local rainfall distribution, commencing usually in October–November and ending towards May at all sites except the southernmost (Arid), where the season is shorter (December–March). All sites share the same calcareous bedrock, south exposure and similar mean annual temperatures (Petrů *et al.* 2006). Thus, the main factor changing along the gradient is annual rainfall with amount and predictability increasing northwards. Adjacent sites differ by *c.* 200 mm of average annual precipitation and represent four different ecosystems (from here on: Arid, Semi-arid, Mediterranean and Mesic Mediterranean).

TARGET SPECIES

We selected two common annual species of high abundance and wide distribution along the gradient: the crucifer *Biscutella didyma* L. (Mediterranean and Irano-Turanian distribution, Zohary 1966), which naturally occurs all along the gradient (from here on *Biscutella*,

Fig. 1a), and the legume *Hymenocarpos circinnatus* (L.) Savi (Mediterranean distribution, Zohary 1972), found at the three northern sites (from here on *Hymenocarpos*, Fig. 1b). The two species are excellent models for testing our local adaptation predictions because both exhibit documented ecotypic differentiation among our populations: For *Biscutella*, Petrů (2006) reported a genetically based increase in seed dormancy (also detected by Lampei & Tielbörger 2010), seed size and reproductive allocation towards the Arid site, and larger height and later onset of flowering towards the wetter sites. These findings are indicative of adaptation to the short and unpredictable desert rainy season (i.e. more permanent soil seed bank, early flowering, high reproduction) and adaptations to a more climatically reliable but competitive environment for mesic populations (i.e. taller plants, lower reproduction, transient seed banks). Similar significant clines in reproductive allocation and flowering time exist among our *Hymenocarpos* populations (C. Ariza & K. Tielbörger, unpublished data).

SEED COLLECTION

Seeds of the target species were collected at all field sites towards the end of spring 2004 (April–May). To ensure natural breaking of summer dormancy, seeds were placed in bags of organza (transparent, permeable synthetic fabric) and left in contact with the soil for summer storage at their respective sites of origin. The seeds were then removed from the field sites before the first rains, at the end of autumn (October).

PRODUCTION OF SEED FAMILIES

For excluding maternal effects, plants from a random sample of the seeds were grown and selfed in a common environment, i.e. a temperature-regulated glasshouse at the University of Tübingen, Germany, during the winter of 2004. Seeds obtained from the 16 maternal plants with largest reproductive output were used for a reciprocal transplanting experiment, after summer storage in organza bags at their original field sites in 2005. Although choosing the most fecund

Table 1. Field sites description

Site	Mean rainfall (mm)	Rainfall in 2005/2006 (mm)	Interannual rainfall variation (%)	Main vegetation	Soil	Location
Arid	90	52	> 30	Annuals and scattered small shrubs: *Zygophyllum dumosum* Boiss., *Artemisia sieberi* Besser and *Hammada scoparia* (Pomel) Iljin	Desert lithosol	3 km N of Sde Boker (Central Negev)
Semi-arid	300	190	25	Dwarf shrubland of *Sarcopoterium spinosum* (L.) Spach and *Thymelaea hirsuta* (L.) Endl., and annuals	Brown Rendzina	20 km N of Beer Sheva (Northern Negev)
Mediterranean	540	554	22	Dwarf shrubland of *S. spinosum* and *Calicotome villosa* (Poiret) Link, and annuals	Terra Rossa	15 km SW of Jerusalem (Judean Mountains)
Mesic Mediterranean	780	778	< 20	Mediterranean maquis/ garrigue of *S. spinosum*, *C. villosa* and *Cistus* spp., with sparse trees: *Quercus calliprinos* Webb and annuals	Terra Rossa	10 km East of Nahariyya (Northern Galilee)
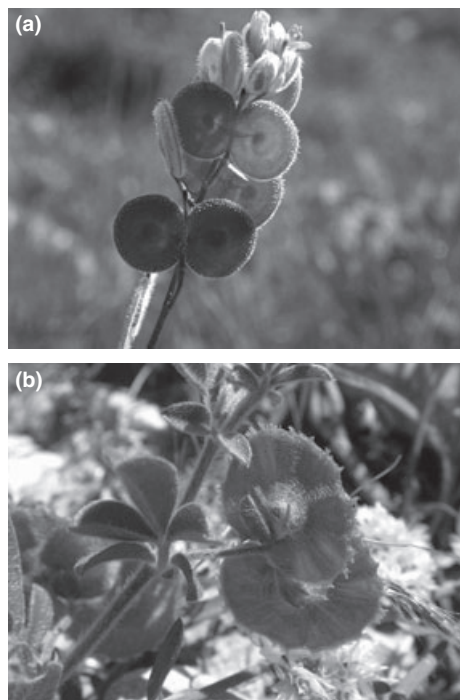
Fig. 1. Focal species: (a) *Biscutella didyma*, (b) *Hymenocarpos circinnatus*. Photo credit: Claus Holzapfel.

families may represent artificial selection, it was the only way to obtain the large seed number required for our reciprocal transplants. Also, there is no *a priori* reason to assume that large families are differentially affected by abiotic or biotic conditions than small ones.

RECIPROCAL TRANSPLANT EXPERIMENT

Towards the end of October 2005, we established a pair of plots per seed family for each of the focal species and populations at each field site. The plots were circular (15 cm in diameter) and placed in areas dominated by herbaceous vegetation. The maximum distance between two plots of a pair was 30 cm. In order to minimize shrub-annual interactions, plots were separated by at least 40 cm from the nearest shrubs. A 'no-neighbour' treatment was randomly assigned to one plot per pair, in which all emerging plants surrounding the target individual were carefully removed by hand throughout the season. The other plot, used as the control, contained all naturally growing vegetation (mostly heterospecific neighbours) in addition to our target plant. Designation of species, populations and families to each plot pair was randomized. The experiment consisted of 800 plots in total, 128 for *Biscutella* at each of the four experimental sites (4 origins × 16 families × 2 removal treatments) and 96 plots for *Hymenocarpos* at the Semi-arid, Mediterranean and Mesic Mediterranean sites (3 origins × 16 families × 2 vegetation removal treatments). In order to obtain one target individual per plot, 6–10 seeds of the designated family were placed in the middle of each plot in direct contact with the soil. The seeds were surrounded with a plastic ring *c*. 4 cm in diameter, and covered with organza. The plastic rings and the cover were used to avoid secondary dispersal of seeds prior to the growing season and to prevent contamination of the field sites with foreign seeds. With the onset of germination from the introduced seeds, the earliest germinating seedling within each ring was defined as target and marked with a coloured polyester thread. The ring, organza cover and remaining seeds were removed. Because we could only monitor

the plots on a weekly basis, this procedure allowed us to avoid possible confounding with seedlings from the seed bank and to minimize competition at early life stages for targets in the no-neighbour treatment. The fraction of targets surviving to reproduction was determined. Target plants were harvested at seed set and dried at 70 °C for 48 h. After drying, we recorded the final above-ground biomass, and reproduction (diaspore number) per germinating plant.

DATA ANALYSIS

As random mortality of families led to a highly unbalanced design, we did not consider family effects in the analyses.

Survival to reproduction

As survival data were binomially distributed, we constructed a frequency table that included counts of targets surviving to seed production in each treatment and identified major associations between survival rates and planting site ('climate' in the following), neighbour presence and population origin. For this analysis, we used the automatic selection of best model option in STATISTICA, which involves a stepwise hierarchical fitting of log-linear models to the observed cell frequencies in the contingency table. The goodness-of-fit of each model is evaluated with Chi-square. The analysis starts fitting a first-order model with all variables but no interactions, followed by a second-order model with all two-way interactions, a third-order model with all three-way interactions and so on, until one of the models significantly fits the frequency table (i.e. its respective Chi-square statistics is non-significant). Then terms that do not significantly contribute to the fit of the higher-level models are removed iteratively until the most parsimonious solution is reached. STATISTICA uses tests of marginal and partial associations (Brown 1976) to define the terms to delete in the backward elimination procedure. To avoid low-frequency values in the log-linear analysis, the automatic procedure used adds a 0·5 constant to all cells.

Plant–plant interaction importance

The contribution of neighbour presence to target performance, relative to the impact of all other environmental factors, was quantified with the interaction importance index, I_{imp} (Seifan *et al.* 2010). $I_{imp} = N_{imp}/(|N_{imp}| + |E_{imp}|)$; where N_{imp} and E_{imp}, respectively indicate the contribution of interactions with neighbours and other environmental effects to plant performance. $N_{imp} = P_{+N} - P_{-N}$; $E_{imp} = P_{-N} - \text{Max } P_{\pm N}$. Max $P_{\pm N}$ is the maximum performance value recorded (in the presence or absence of neighbours) along the gradient. I_{imp} is continuous, symmetric under most experimental conditions and ranges from −1 to +1. I_{imp} is negative under competition, positive when facilitation occurs and approaches zero when plant–plant interactions are unimportant compared with other factors. We calculated I_{imp} using the Max $P_{\pm N}$ for each population.

Using two-way ANOVAs, we tested the effects of climate and population origin on I_{imp} – calculated on biomass at harvest and reproductive output. Differences between populations are denoted by a significant origin effect, differences due to climatic conditions are indicated by a significant climate effect, and differential responses of target populations to biotic interactions at different climates are represented by a significant origin × climate interaction. The latter implies local adaptation to neighbours when biotic interactions at a certain climatic environment are less important for native populations than for foreign populations. Thus, analyses of I_{imp} permit testing for

patterns of adaptation to neighbours between populations. Based on the theory, we predicted that interaction importance should increase along the gradient for all populations and that, due to local adaptation to competition, competitive interactions should be less important for plants of wetter origin than for xeric plants at all sites.

Untransformed index values best fulfilled the assumptions of ANOVA. Therefore, all analyses presented are based on untransformed I_{imp} values.

Local adaptation to climate and overall (biotic and abiotic) environmental conditions

We searched for patterns of local adaptation to abiotic factors using data of target performance (biomass at harvest and reproductive output) in plots without neighbours by means of a two-way ANOVA with origin and climate as main factors. We assumed that by removing the effect of surrounding vegetation, target performance is mostly constrained by abiotic factors (mainly rainfall), although we acknowledge that other non-competitive biotic interactions (herbivory, seed predation, etc.) may differ among sites. A similar ANOVA for the response of targets in control plots was used to test for adaptation to overall native environments (climate + biotic factors). In both cases, local adaptation was inferred if populations performed better at their site of origin than foreign populations accompanied by a significant population origin × climate interaction (Knight & Miller 2004). Our prediction was that competition amplifies patterns of local adaptation, i.e. arid populations perform best at stressful climates when neighbours are removed, and humid populations perform better at their home site, particularly when neighbours are present.

All data analyses were performed separately for each species and response variable using STATISTICA (version 7 for Windows, Statsoft, 2004, Tulsa, Oklahoma, USA,). We included data from reproductive and non-reproductive targets in all ANOVAs because using measures from reproductive targets only led to highly unbalanced designs and the results were not different from those presented here. Significant effects of main factors or interactions in ANOVAs were evaluated with Tukey's *post hoc* comparisons ($P < 0.05$). Biomass and reproduction data were square root transformed to improve the normality and homogeneity of variances in two-way ANOVAs.

Results

SURVIVAL TO REPRODUCTION

Biscutella's survival was only closely associated with climate (i.e. planting site) ($\chi^2 = 40.57$, d.f. = 56, $P = 0.9398$). An inspection of the survival fractions (Table S1, Supporting information) indicated that differences between planting sites were entirely caused by the failure to survive at the Arid site, i.e. survival among the other sites did not differ. The results for *Hymenocarpos* were similar and did not reveal an association between survival to reproduction and climate, population origin, or neighbour presence (best fit model = survival, $\chi^2 = 34.14$, d.f. = 34, $P = 0.461$).

IMPORTANCE OF PLANT–PLANT INTERACTIONS

The ANOVAs performed on I_{imp} did not detect significant effects of the factors or interactions tested for any species or dependent variable (see Table S2, Fig. 2). Due to lack of

reproduction, I_{imp} values for *Biscutella*'s seed production were zero at the Arid site, and the index was not significantly different from zero at any other site along the gradient, according to separate *t*-tests conducted on *Biscutella*'s biomass and reproduction mean I_{imp} values ($P > 0.05$). Contrastingly, the negative mean I_{imp} values observed in *Hymenocarpos*' biomass at each climate differed from zero (*t*-tests $P < 0.05$) and, except for the Semi-arid climate, the negative values of interaction importance measured on this species' diaspore production also differed from zero at all sites (*t*-tests $P < 0.05$).

ADAPTATION TO CLIMATE

In the absence of neighbours, *Biscutella*'s biomass and reproduction were affected by climate (Table 2, Fig. 3a) but not by population origin or by the climate × origin interaction (Fig. 4a). There was an apparent but non-significant pattern of best performance of plants at adjacent planting sites with wetter climate. *Biscutella*'s final biomass increased from the Arid to the Mediterranean sites but declined at the Mesic Mediterranean site. Significant differences in biomass were detected between the Mediterranean and all other sites and between Mesic Mediterranean and Arid sites (Tukey's test $P < 0.05$, Fig. 3a). Diaspore production followed the same trend as biomass, however observed differences occurred between the Arid site and the Semi-arid and Mediterranean sites (Tukey's test $P < 0.05$, Fig. 3a). In contrast, *Hymenocarpos*' biomass did not differ between climates in the absence of neighbours, and differences in fruit production were only marginally significant (Table 2, Fig. 3b). There was a consistent but non-significant trend for a home-site advantage for all *Hymenocarpos* populations (no significant origin or climate × origin effect; Table 2, Fig. 4b).

ADAPTATION TO THE OVERALL (BIOTIC + ABIOTIC) ENVIRONMENT

Qualitative patterns of local adaptation were amplified for Mediterranean populations in both species and for Mesic Mediterranean populations in *Hymenocarpos* (compare Fig. 4 with Fig. 5) but these patterns were not statistically significant (Table 2). *Biscutella*'s biomass and reproduction under natural neighbour conditions increased steadily with improving climatic conditions, and among-site differences in both performance parameters were highly significant (Table 2, Fig. 3a). The high accumulated biomass of plants at the two wet sites differed from that at the drier climates, but differences in biomass were also found between the Arid and Semi-arid sites (Tukey's test $P < 0.05$; Fig. 3a). Differences in seed production were detected between the Arid and all other environments and between Semi-arid and Mesic Mediterranean sites. Population origin and the origin × climate interaction were not significant for these variables (Table 2, Fig. 5a). Population origin was the only tested factor with a significant effect on the final biomass and fruit production of
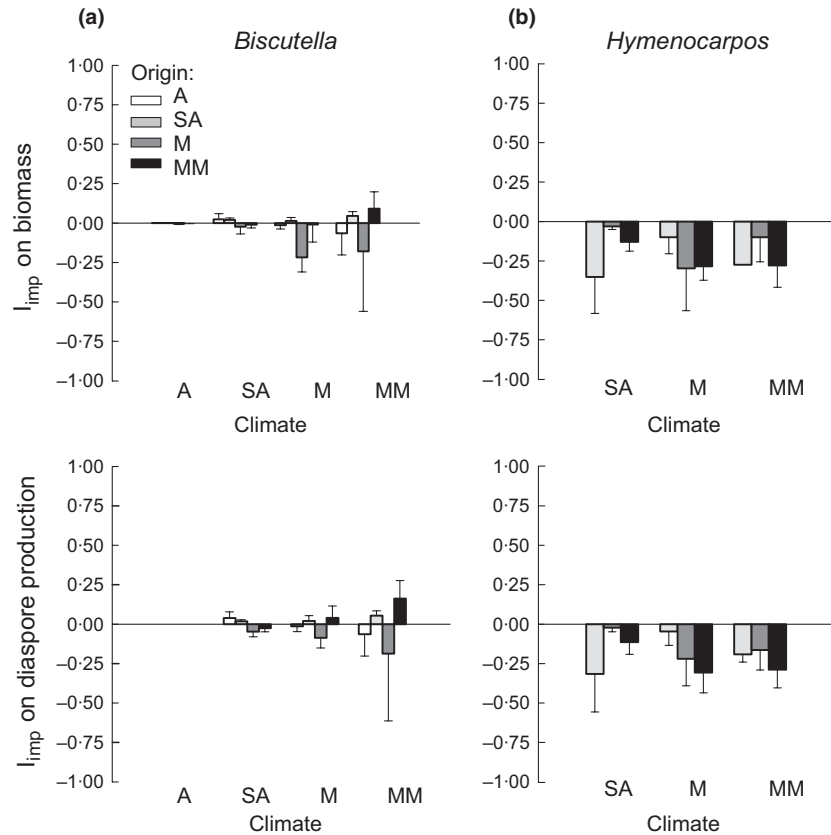
Fig. 2. Importance of biotic interactions along the climatic gradient for target populations of (a) *Biscutella didyma* and (b) *Hymenocarpos circinnatus*. Biotic interaction importance is expressed with the interaction importance index (I_{imp}), calculated for biomass (at harvest) and diaspore production for each species and population. Bars indicate mean population I_{imp} values + SE at sites where the species naturally occur but which differ in climate: Arid (A), Semi-arid (SA), Mediterranean (M) and Mesic Mediterranean (MM).

Table 2. Results of two-way ANOVAs testing the effect of planting site (= climate) and population origin on biomass and diaspore production of the studied species

| | | Neighbours present | | | | | | | | Neighbours absent | | | | | | | |
| | | Biomass | | | | Reproduction | | | | Biomass | | | | Reproduction | | | |
Species	Source of variation	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P
Biscutella didyma	Origin	3	0·00	0·33	ns	3	6·91	1·40	ns	3	0·02	0·94	ns	3	4·44	0·73	ns
	Climate	3	0·18	12·14	***	3	57·19	11·61	***	3	0·23	11·38	***	3	50·29	8·25	***
	Origin × Climate	9	0·01	0·82	ns	9	4·96	1·01	ns	9	0·02	0·94	ns	9	5·90	0·97	ns
	Error	140	0·01			143	4·93			159	0·02			165	6·09		
Hymenocarpos circinnatus	Origin	2	0·26	5·58	**	2	4·73	3·99	*	2	0·03	0·31	ns	2	0·22	0·11	ns
	Climate	2	0·01	0·14	ns	2	1·60	1·35	ns	2	0·18	2·19	ns	2	6·31	3·12	0·049
	Origin × Climate	4	0·06	1·28	ns	4	1·47	1·24	ns	4	0·05	0·61	ns	4	0·66	0·33	ns
	Error	61	0·05			74	1·18			71	0·08			80	2·02		

Separate analyses were conducted for targets growing with and without neighbours using square-root-transformed data (*P < 0·05, **P < 0·01, ***P < 0·001).

Hymenocarpos in the presence of neighbours (Table 2). Differences in performance were detected between plants of the two humid origins for the two variables (Tukey's test P < 0·05). In both cases, maximum performance was observed in plants of Mediterranean origin and minimum in Mesic Mediterranean plants.

Discussion

Our overall findings contradict our initial hypothesis. There was neither evidence for local adaptation to neighbour presence, climatic or overall (biotic + abiotic) environmental conditions, nor support for a shift in the main factors promoting local adaptation along the gradient. Similarly, the predicted change in the importance of negative plant–plant interactions with increasing productivity was not detected. This is surprising, given the contrasting climatic and neighbour conditions between sites and the fact that previous studies described significant differences in plant responses to neighbours along our gradient (Holzapfel *et al*. 2006; Schiffers & Tielbörger 2006;) and along similar gradients in the region (Liancourt & Tielbörger 2009). In general, non-significant results allow two possible conclusions: effect size was small, i.e. the hypoth-
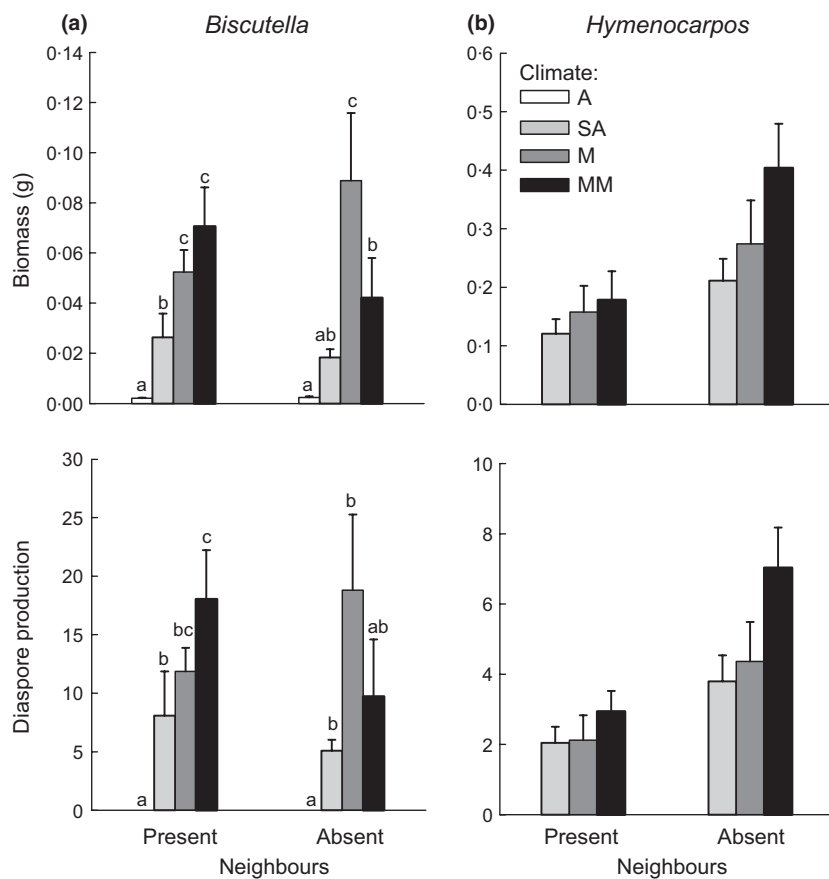
Fig. 3. Biomass and diaspore production of (a) *Biscutella didyma* and (b) *Hymenocarpos circinnatus* along the study gradient. Climatic effects on target performance are indicated by differences between experimental sites in the neighbour removal treatment (neighbours absent). Effects of the overall environment are expressed as differences between sites in the control treatment (neighbours present). Bars represent mean values + SE of pooled population performance at the climatically different experimental sites: Arid (A), Semi-arid (SA), Mediterranean (M) and Mesic Mediterranean (MM). Letters above bars indicate differences between sites within treatments for each response variable ($P < 0.05$).
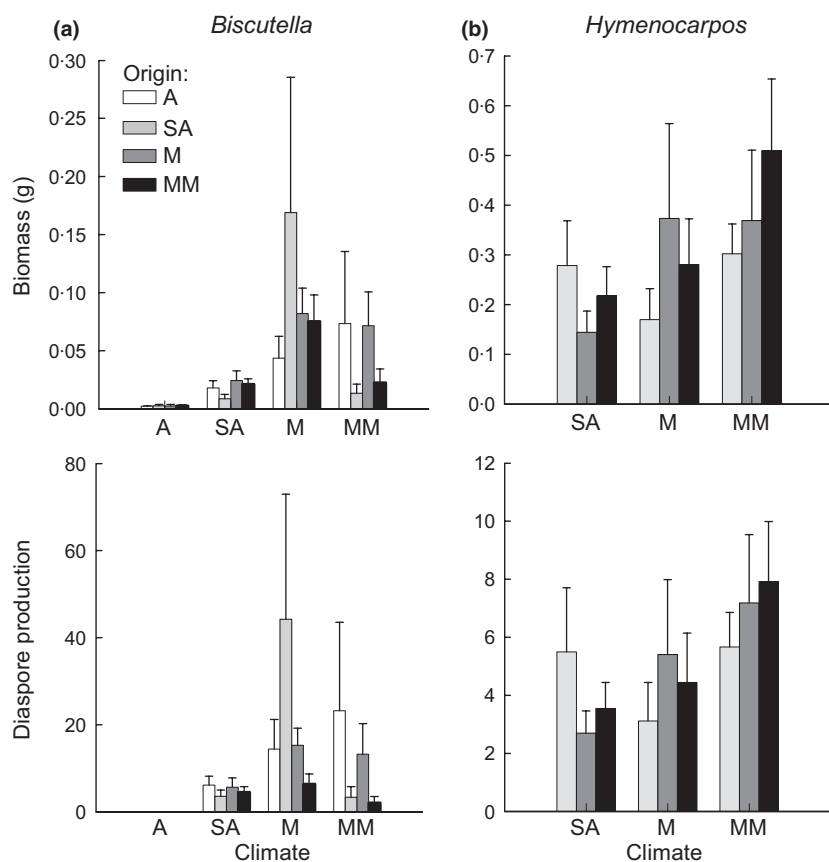
Fig. 4. Climatic effects on biomass and diaspore production of target populations of (a) *Biscutella didyma* and (b) *Hymenocarpos circinnatus*. Response variables were recorded in target individuals growing in the absence of neighbours at sites where the species naturally occur but which differ in climate: Arid (A), Semi-arid (SA), Mediterranean (M) and Mesic Mediterranean (MM). Bars represent population means + SE.
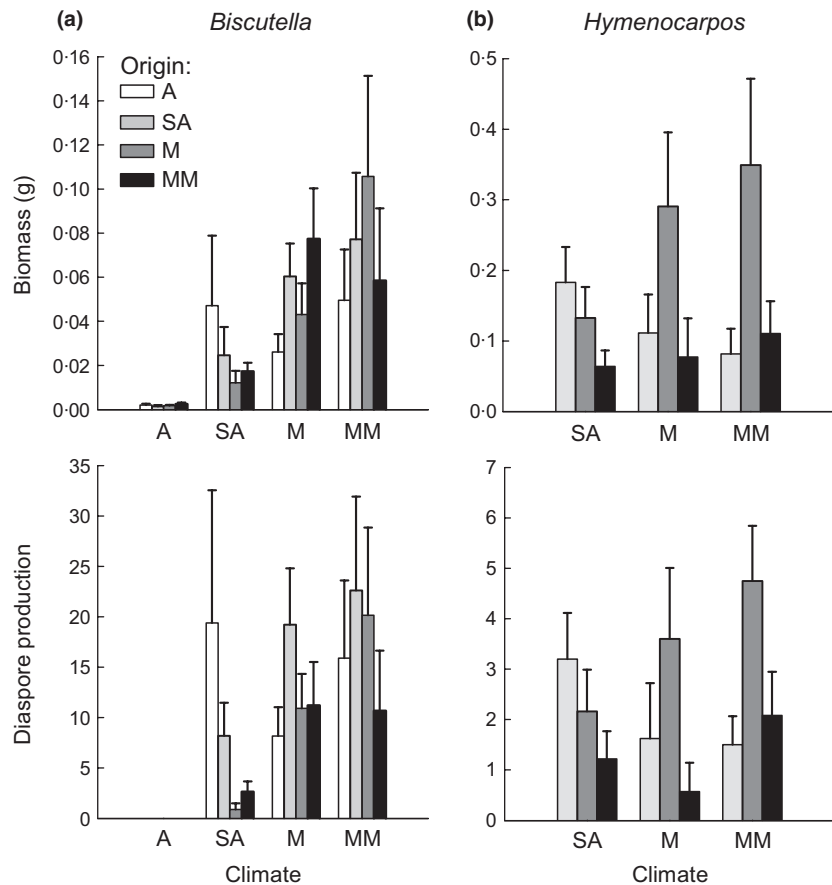
Fig. 5. Overall environmental effect on biomass and diaspore production of target populations of (a) *Biscutella didyma* and (b) *Hymenocarpos circinnatus*. Response variables were recorded in target individuals growing in control plots (in the presence of neighbours) at sites where the species naturally occur but which differ in climate: Arid (A), Semi-arid (SA), Mediterranean (M) and Mesic Mediterranean (MM). Bars represent population means + SE.

esis needs to be revised, or the power of the tests was not sufficiently large, i.e. natural variation was too large compared with sample size to detect patterns. In the following, we address both possibilities in detail.

LOCAL ADAPTATION

Our sharp environmental gradient provides an ideal setting for testing shifts in patterns of local adaptation within species, as opposite ends are dominated by two different but co-occurring selective forces: scarcity and unpredictability of rainfall and competition (Petrů *et al.* 2006). Therefore, we predicted a shift from local adaptation to harsh climatic conditions at the xeric part of the gradient to adaptation to competition at the humid end. Our results do not support our predictions for either of the target species on any of the measured traits. First, no significant climate × origin interaction effect was detected in I_{imp}, indicating that the importance of biotic interactions, relative to other environmental factors, did not differ between populations across sites. Therefore, it was unlikely to observe site-specific adaptation to competition. Secondly, no significant climate × origin interaction effect on target performance was detected for plots without neighbours, suggesting that responses to changing abiotic conditions did not differ between populations, e.g. populations native to arid environments are not better adapted

to drought. In addition, we did not detect local adaptation to overall (biotic + abiotic) environmental conditions.

It is known that adaptation processes are dynamic, as selective factors (e.g. rainfall) may fluctuate with time and affect both intensity and direction of natural selection (e.g. Kalisz 1986; Kelly 1992). Such effects may cause temporal divergence or convergence of selective regimes for different habitats (Kindell, Winn & Miller 1996) and thus observed local adaptation patterns may differ with time (e.g. Rice & Mack 1991; Galloway & Fenster 2000; Hereford & Winn 2008). Due to raising plant families prior to the field experiment (i.e. 2 years of preparation), we could not conduct the experiment for more than 1 year. Therefore, we cannot unequivocally exclude the occurrence of local adaptation in our system. For example, consistent with our prediction, evidence exists for ecotypic variation in traits related to drought resistance, environmental variation and competition in our focal species along our gradient (Petrů *et al.* 2006; Petrů & Tielbörger 2008; Tielbörger & Petrů 2010) and for other annual species in a nearby gradient (Liancourt & Tielbörger 2009). In a reciprocal transplant experiment with Mediterranean and Semi-arid populations of two annual grasses, Liancourt & Tielbörger (2009) demonstrated that Mediterranean populations are adapted to local biotic conditions. This was further supported by detecting in their Mediterranean plants ecotypic differentiation in traits that confer greater competitive response

ability. In their study, however, there was no indication of local adaptation to Semi-arid climatic conditions. Interestingly, the authors attribute their failure to detect adaptation to abiotic stress to the fact that their study was conducted in an exceptionally wet year, where water availability was not limiting either end of their gradient. In our case, the opposite may have occurred with differences in neighbour effects along the gradient being overridden by extreme drought, i.e. plant performance was strongly affected by abiotic stress rather than by biotic stress. Taken together, these findings indicate that the relative importance of biotic vs. abiotic factors may shift between years in a manner consistent with the stress-gradient hypothesis or Grime's (1977) model. If so, the probability of detecting strong local adaptation should be generally small in environments that vary unpredictably in time (i.e. deserts). Still, plants from less variable environments (i.e. Mediterranean sites) should exhibit some degree of local adaptation, especially to competition, and these are more likely to be detected in wetter years (Liancourt & Tielbörger 2009).

It is difficult to relate our findings to the existing literature because studies separating competitive from abiotic effects on local adaptation are scarce and their results contradictory. For example, Rice & Knapp (2008) and Knight & Miller (2004) have shown that plant–plant interactions amplify the expression of local adaptation, whereas others did not find this pattern at all or only for few species (Kindell, Winn & Miller 1996; Bischoff *et al.* 2006; Sambatti & Rice 2006). Also, only one of these studies can be put in the context of predictable changes in interaction importance (Knight & Miller 2004), and their findings indicate a larger importance of competition in the less productive habitat. Our results do show some indication of an amplification of local adaptation patterns when neighbours are present, but this was only detected for the populations from the wetter sites and it was not significant. In summary, we must conclude that the available evidence is insufficient, and many more studies across a range of environments are needed to decouple the different factors determining local adaptation.

PLANT–PLANT INTERACTIONS ALONG THE GRADIENT

Our results did not statistically confirm any of the existing models of plant–plant interactions along environmental gradients (Grime 1977; Bertness & Callaway 1994). Interaction importance was similar across climates for our focal species, despite species-specific patterns of response to neighbour presence. Although biomass and reproduction of *Hymenocarpos* were negatively affected by neighbours at all sites, interactions shifted from slightly negative to positive (albeit not significant) along the gradient for *Biscutella*. Similar non-significant results and neighbour effects were observed when analysing interaction intensity (data not shown). This is surprising, given that two separate studies (Holzapfel *et al.* 2006; Schiffers & Tielbörger 2006) found that competition intensifies towards the wetter sites of our gradient. Also, a recent study in two climatically different sites nearby indicated that Mediterranean plants of an annual grass were better adapted

to competition than plants from semi-arid habitats (Liancourt & Tielbörger 2009). However, all these studies were conducted during years of above-average annual rainfall. As shifts in species interactions occur not only in spatial but also along temporal environmental gradients, different interaction patterns may be observed among years (Greenlee & Callaway 1996; Tielbörger & Kadmon 2000; Forey, Touzard & Michalet 2010), or even within the same season (Holzapfel & Mahall 1999; Kikvidze *et al.* 2006; Schiffers & Tielbörger 2006). Therefore, lack of congruence between our predictions and results could be related to the strong drought recorded in our study winter at the Arid and Semi-arid sites, where only 58% and 63% of the average annual precipitation, respectively, were recorded.

To explain differences in the pattern of interactions recorded for *Biscutella* between our and previous studies, we propose that, during dry years, positive effects of neighbouring annual plants are overridden by water scarcity at the xeric end of the gradient, but become apparent at wetter sites. Such a breakdown of positive interactions with extreme drought has been observed before (Tielbörger & Kadmon 2000; Maestre & Cortina 2004) and has been explained by the fact that benefactor species are constrained by lack of resources too (Michalet *et al.* 2006). Therefore, facilitation is most likely to occur under stressful but 'not too stressful' conditions. Applied to our system, we would expect to find facilitative neighbour effects during wet years (e.g. Schiffers & Tielbörger 2006), but only at the dry end of the gradient where water availability is still low. Under drought, we would expect no facilitation at the arid end but overriding positive interactions towards the wetter end. This model may explain that we found no strong positive effect of neighbours on plant performance at the drier stations, but a trend for facilitation at the wettest station. Apparently, even under average rainfall conditions at the productive sites (our study), facilitative effects of neighbours (e.g. shading) may balance competitive effects. In fact, the importance of plant–plant interactions did not differ from zero along the gradient for *Biscutella*, implying that other environmental factors (water stress, for instance) were more important for the performance of this species at all sites. The patterns of interaction with neighbours recorded for *Hymenocarpos* also reflect limited water availability in our study, as the importance of interactions for this species was different from zero at all sites but low. Finally, survival was not related to increasing neighbour density along the gradient for any population, corroborating the idea that net negative neighbour effects are less likely to limit survival than growth during periods of water stress (Goldberg & Novoplansky 1997; Miriti, Wright & Howe 2001; Schiffers & Tielbörger 2006).

Although there is ample theoretical background about how the importance of interactions among plants may change along environmental gradients, evidence stems mostly from community ecology where evolutionary aspects are often overlooked. Vice versa, studies on local adaptation of single plant species are rarely designed to test theories of community organization. Here, we combined the two approaches for sep-

arating the role of abiotic vs. biotic factors for local adaptation. In addition, this is also the first time to approach local adaptation to interactions with local neighbours using a measure of interaction importance, i.e. the recently derived I_{imp} index (Seifan *et al.* 2010).

Plant–plant interactions in our study were not as relevant as we expected. The power of our statistical analysis was low and, given the large variability in performance among plant individuals observed in this and previous studies, the number of surviving plants in our drought year was too small to detect significant differences among treatments. Though it was logistically not feasible to expand the experiment beyond one season, the 2 years that followed our study were also very dry and, retrospectively, we would most likely have obtained similar results if we had conducted the experiment for 3 consecutive years. Therefore, longer-term studies are desirable to test whether plant interactions and the likelihood of detecting them indeed fluctuate as described above. We further advocate the use of experiments that combine manipulations of neighbour conditions with cross-transplants in future studies of local adaptation. This will also help understanding how local adaptation to climate and other factors could affect extinction risk under future climate change.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Fraction of *Biscutella didyma* and *Hymenocarpos circinnatus* individuals surviving to seed production.

Table S2. Two-way ANOVAS testing the effect of climate and population origin on plant–plant interaction importance (I_{imp}) along the gradient.

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