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Research article

Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species

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ABSTRACT

Positive interactions among plants have rarely been investigated with respect to their evolutionary consequences and vice versa. The outcome of facilitative interactions depends on the competitive ability and stress tolerance of the species. We tested whether this also applies to populations of conspecifics that are locally adapted to different environments and thereby differ in these traits. We hypothesised that ecotypes from less stressful environments experience a greater effect of facilitation when grown in stressful environments compared to populations adapted to these conditions.

Seeds of two ecotypes of the annual grass species, *Brachypodium distachyon*, were collected from Mediterranean and arid origins and transplanted at an arid environment within the species' distribution range. To examine the effect of biotic interactions on these ecotypes, we transplanted the individuals with and without the presence of the shrub *Gymnocarpus decander* (underneath or away from the shrub), and with and without the presence of annual vegetation (removal experiment). We examined the effect of these interactions on the two *B. distachyon* ecotypes by comparison of emergence success, biomass, and survival to reproduction.

The presence of shrubs had a positive effect on all three variables in both ecotypes. Facilitation by shrubs enabled individuals from Mediterranean origin to grow and reproduce in arid conditions. Unlike the locals, they failed to survive to reproduction away from the shrubs, because of the markedly shorter growing season in open areas. The annual vegetation did not affect emergence or survival to reproduction in either ecotype; however, the positive effect of shrubs on biomass was reduced in the presence annual vegetation in the Mediterranean ecotype.

This demonstrates that ecotypes adapted to arid conditions respond differently to these biotic interactions compared to Mediterranean populations. We argue that facilitation may have important evolutionary consequences by enabling maladapted ecotypes to invade and colonize stressful habitats.

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Introduction

Facilitative plant–plant interactions are now widely recognized as a major ecological force that shapes species distribution along ecological gradients (Choler et al., 2001; Bruno et al., 2003; Brooker et al., 2008). By extending the distributional limit at range margins and directly affecting species fitness, facilitation may have strong positive effects on local species diversity (Hacker and Gaines, 1997; Michalet et al., 2006), as well as population dynamics and demography in plant communities (Tirado and Pugnaire, 2003;

Armas and Pugnaire, 2005). Facilitation is usually the result of complex interactions in harsh environments (Michalet, 2006), and the net effect of biotic interactions is the balance between positive and negative effects that co-occur (Holmgren et al., 1997; Brooker and Callaghan, 1998; Malkinson and Tielbörger, 2010). Moreover, both species-specific effect of benefactors (Callaway, 1998; Gomez-Aparicio et al., 2004) and species-specific response of beneficiaries (Choler et al., 2001; Liancourt et al., 2005; Wang et al., 2008) have been described.

The species-specific response of beneficiaries to facilitation is often correlated with specific traits in a predictable manner. These traits are related to both their competitive response and stress tolerance ability (Liancourt et al., 2005; Gross et al., 2010; see also Maestre et al., 2009). For example, it has been demonstrated in salt marshes, that species with high salt tolerance may be less facilitated than species with low tolerance to high salinity (Pennings

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et al., 2003). Traits that allow high stress tolerance are often realised at the expense of traits enabling persistence under intense competition (Grime, 1977). Therefore, facilitation is more likely to be important for species from 'benign' environments that exhibit low tolerance to stress but have a greater competitive response ability (Liancourt et al., 2005; Gross et al., 2010). Furthermore, these differences may be enhanced if we assume that the benefactor is also a potential competitor for the beneficiary, i.e. there are also costs to being facilitated (Holmgren et al., 1997; Brooker and Callaghan, 1998).

This concept has been applied on a community level to explain the coexistence of species differing in traits (see Michalet et al., 2006 and reference therein). However, such trade-offs can also occur at the intraspecific level, among populations. Therefore, a logical approach to studying the role of trait differences for responsiveness to facilitation is to look at trait differentiation within, rather than between species. Traits related to competitive response and stress tolerance may also differ between populations of the same species in a similar manner as explained above (e.g. Bijlsma and Loeschcke, 2005; Petru et al., 2006; Liancourt and Tielbörger, 2009). Namely, in species with broad distribution range, stress and competition are likely to select for different traits at the two extremes of the gradient. This may lead to within-species niche divergence (ecotypic differentiation) where stress-tolerant populations occur at the periphery, i.e. the 'harsh' end of the gradient and competitive populations occur at the core or the 'favourable' end (Aronson et al., 1992, 1993; Volis et al., 1998, 2002, 2004; Liancourt and Tielbörger, 2009). Therefore, we expect the response to facilitation to be ecotype-specific and a competitive (i.e. less stress-tolerant) ecotype should be more facilitated at the 'harsh' end of the species distribution range than a stress-tolerant ecotype (Espeland and Rice, 2007; Eränen and Kozlov, 2009). Such an ecotype-specific response to facilitation could have intriguing consequences for gene flow between populations and genetic diversity within population in marginal habitats, e.g. by enabling persistence of maladapted genotypes in stressful habitats. Unfortunately, the evolutionary dimension of facilitation has been largely overlooked (Brooker et al., 2008; Pakeman et al., 2009).

Compared to local adaptations for abiotic conditions, ecotypic differentiation with respect to biotic interactions has been studied rarely (but see Volis et al., 2004; Sambatti and Rice, 2007; Espeland and Rice, 2007; Rice and Knapp, 2008; Eränen and Kozlov, 2009; Michalet et al., 2011). In addition, the existing studies have focused on competition as the major selective constraint, and the implication of evolutionary processes on facilitative outcome is still largely unknown (but see Espeland and Rice, 2007; Eränen and Kozlov, 2009; Michalet et al., 2011).

Annual plants growing along aridity gradients in the Lower Jordan River Valley are ideally suited to address this issue. First, ecotypic differentiation has been described along these gradients, with individuals from Mediterranean populations showing higher biomass production, higher competitive response ability, delayed onset of flowering and lower drought tolerance than individuals from arid populations (Aronson et al., 1992, 1993; Volis et al., 1998, 2002; Petru et al., 2006; Liancourt and Tielbörger, 2009). Secondly, facilitation has been observed at the arid end of these aridity gradients (Holzapfel et al., 2006) and in arid environments in general (see Brooker et al., 2008 for recent review).

We provide here the result of an experimental study designed to test, under natural field conditions, the effect of local adaptation for facilitative outcome. We suggest here that the effect of facilitation described at the species level, i.e. the expansion of the realised niche for stress-intolerant species, also applies for ecotypes within a species. In this study we compare the effect of biotic interactions on individuals from a Mediterranean and arid ecotype of an annual

species distributed along an aridity gradient to test the following hypotheses:

- (1) Individuals from Mediterranean origin perform worst in arid environments compared to the local ecotype.
- (2) Facilitation by shrubs provides a benefit to both ecotypes but has a stronger effect on Mediterranean plants.

Methods

Study site and target species

The field site is located at the arid end of Wadi Shuayb (90 m a.s.l.; 31°56'24.94"N, 35°39'43.27"E), a steep valley running from east to west, perpendicular to the Jordan River, 20 km north-east of Amman, Jordan. Average annual precipitation calculated for the three consecutive seasons 2005/2006, 2006/2007 and 2007/2008 (study year) was 195 ± 72 mm (113 mm the study year) with all the precipitation occurring from November to March. The soil is desert lithosol on limestone bedrock. The experiment was conducted on a south facing slope to ensure maximal stressful conditions. Vegetation in the study site is composed of some scattered shrubs (e.g. *Fagonia mollis*, *Gymnocarpos decander*, *Teucrium polium*), few geophytes and mainly small annual grasses, forbs and legumes. The herbaceous vegetation cover is very low and patchy outside shrubs (from 0 to 10% cover), and the average biomass per area was 49 ± 18 g m⁻² the study year.

G. decander Forssk. (Caryophyllaceae) was chosen as nurse species for the experiment since it is the most abundant shrub and is readily observed with a high density of annuals underneath it. It is a small shrub (10–35 cm) with small succulent leaves, typical of Saharo-Arabian regions (Zohary, 1966). Since our study was run on a site already being used in a long-term experiment, it was not possible to remove shrubs in our experimental set up (see Michalet, 2006) without disrupting the ongoing research. However, other studies performed in the region as well as our own field observations strongly suggest that facilitation explains the aggregation of annual plants underneath *G. decander* (Holzapfel et al., 2006 and references therein).

The target species chosen for this study, *Brachypodium distachyon* (L) P. Beauv. (*B. distachyon* hereafter), is a winter annual grass with a broad distribution range occurring from Mediterranean to semi arid environments, i.e. Mediterranean and Irano-Turanian regions extending into Saharo-Arabian regions (Zohary, 1966; see Opanowicz et al., 2008 for more information). Ecotypic differentiation has been described for this species with Mediterranean populations having a higher individual biomass production, higher competitive response ability, delayed onset of flowering and a lower drought tolerance than the arid populations (Aronson et al., 1992, 1993; Liancourt and Tielbörger, 2009).

The experiment

Seeds of our target species, *B. distachyon*, collected from populations originating from the Mediterranean (850 m a.s.l.; 32°1'0.42"N, 35°43'11.43"E; average annual precipitation: 417 ± 130 mm; average biomass per area was 300 ± 48 g m⁻², data are mean \pm SE) and from the study site (arid site) were used for the experiment. In both sites, seeds were collected at random from a large number of individuals (>50) growing in areas away from shrubs in May 2007.

In October 2007, 24 individuals of the shrub *G. decander* were randomly selected within a fenced area (fenced in 2005 to exclude sheep and goats) in our arid site. Under half of them, the understory vegetation (mainly annuals) was manually removed periodically

from December (beginning of germination, by removing the litter and gently brushing the soil surface to remove most of the seeds, and by clipping annual seedlings) to the end of the experiment (March 2008) to test the effect of the annual vegetation on our targets. Vegetation was also removed from 12 open areas (25 cm × 25 cm) located 1 m apart from the shrubs. Clipping of tiny seedlings did not cause any disturbance to the plots and thus was unlikely to affect performance of the remaining seedlings other than via reduced competition.

Small plastic rings (~5-cm diameter) were positioned on the ground under each shrub (one per shrub) and one meter apart, half of them with annual neighbouring vegetation and the other half without (48 in total). Six seeds of either Mediterranean or arid origin were put in each plastic ring to measure germination rates and to increase the chance of having one individual germinate. The rings were covered with organza fabric (until the first rain and subsequent emergence) and fixed to the ground with small nails. This set up served to avoid secondary seed dispersal with run-off after the first rains, helped in locating the seeds and seedlings early in the season, prevented seed predation, and allowed us to locate the remaining seeds throughout the course of the experiment. If more than one seed germinated per ring, the additional seedlings were removed after emergence. Organza and plastic rings have been successfully used in previous studies and we have not observed a detectable effect on germination (C. Ariza, personal communication). In summary, six replicates per treatment were used to study the response of the individuals from the two origins to the effect of shrubs, annual neighbouring vegetation and their interactions, using six seeds per replicate.

B. distachyon is a frequent species but grows mainly as widely spaced single individuals and not in dense stands. Therefore, it is unlikely that background germination of seeds in the soil largely affected the results for emergence in the small rings and retrospectively, there was no case where seedling numbers were larger than the maximum of six. Also, erroneous classification of seedlings from the (arid) seedbank in the experiments with Mediterranean seeds would have led to smaller differences between ecotypes, i.e. statistically significant differences should be robust against bias from background germination.

Measurements and data analyses

Nurse effect

Soil moisture and plant density were measured to shed light on the mechanism of the nurse effect of *G. decander*. Soil samples (~200 cm³) were collected 0–10 cm deep under six additional randomly selected *G. decander* and away from the shrub (1 m apart) two days and six days after a major rainfall event at the beginning of February. The soil samples were brought to the laboratory in plastic bags immediately after field collection. Soil moisture was determined by drying samples at 105 °C for 72 h, and was expressed as percentage of fresh soil mass. Plant density was measured in twelve 20 cm × 20 cm quadrats under and outside the shrub using the plastic rings locating the (control) targets as the centre of the quadrats. Data were analysed using *t*-tests with shrub as factor.

Response variables for the target species

For each ecotype we measured emergence fraction and timing throughout the experiment, and total aboveground biomass and survival to reproduction at the end of the vegetation season. Multiple response variables were used because the outcome of facilitation may largely differ among life stages (e.g. Schiffers and Tielbörger, 2006).

Emergence was monitored for the six seeds per ring after each major rainfall event from December 2007 to March 2008. Percent-

Table 1

Results of the repeated-measured ANOVA for the effect of Origin, Annual neighbours and Shrub (*Gymnocarpos decander*) on percentage emergence of *Brachypodium distachyon*. Significant results are in bold.

Source	df	F	P
Origin (Ori.)	1	0.28	0.601
Annual neighbours (Ann.)	1	1.73	0.195
Shrub (Shr.)	1	19.26	<0.001
Ori. × Ann.	1	0.12	0.727
Ori. × Shr.	1	0.07	0.794
Ann. × Shr.	1	2.23	0.144
Ori. × Ann. × Shr.	1	3.40	0.073
Between subject error	40		
Time	4	36.95	<0.001
Time × Ori.	4	0.32	0.861
Time × Ann.	4	0.72	0.578
Time × Shr.	4	4.78	0.001
Time × Ori. × Ann.	4	1.50	0.205
Time × Ori. × Shr.	4	0.31	0.873
Time × Ann. × Shr.	4	0.22	0.929
Time × Ori. × Ann. × Shr.	4	1.41	0.234
Within subject error	160		

age emergence (calculated for the six seeds per ring) was arcsine square root-transformed prior to analyses and analysed over time using repeated-measures ANOVA with Origin, Annuals, Shrub and their interactions used as between subject factors, and Time and its interaction with the previous factors as within-subject factors.

Target species were harvested at the end of the vegetation season (March). Total aboveground biomass was measured by drying the samples at 70 °C for 72 h before weighing. Biomass data were log transformed and analysed using three-way ANOVA with Origin, Annuals, and Shrub considered as fixed factor.

Survival to reproduction was registered as binomial data (alive and flowering or dead/not flowering) for our target species and the effect of Origin, Annuals, Shrub and their interactions were tested using a chi-square likelihood ratio with logistic regression models.

Because of low germination and high mortality in some cases, sample size was not equal for all analyses. All analyses were carried out with JMP 5.0.1 (SAS Institute, 2002).

Results

Nurse effect

Soil moisture two days and six days after the rain was significantly higher under the shrubs than away from the shrub (18.9 ± 0.9% vs. 8.3 ± 0.5%, respectively two days after the rain, *t*-test, *t*₁₀ = 10.7, *P* < 0.001; 9 ± 0.9% vs. 4 ± 0.2%, respectively six days after the rain, *t*-test, *t*₁₀ = 5.5, *P* < 0.001). Plant density was also significantly greater under the shrubs (32.8 ± 3.6 individuals vs. 5.8 ± 1.7 individuals in 20 cm × 20 cm quadrats, *t*-test, *t*₂₂ = 6.8, *P* < 0.001).

Response variables for the target species

Overall, shrubs increased total emergence for the seedlings from the two origins irrespective of the presence of annual vegetation (Table 1). In addition, shrubs also affected the timing of emergence (significant Time × Shrub interaction, Table 1 and Fig. 1). Emergence under shrubs started earlier (December 2007) while the main emergence wave was delayed to the beginning of February 2008 outside the shrubs.

Overall, biomass production of *B. distachyon* was greater under shrubs than away from the shrubs. The effect of the shrubs was stronger for individuals from Mediterranean origin than for those

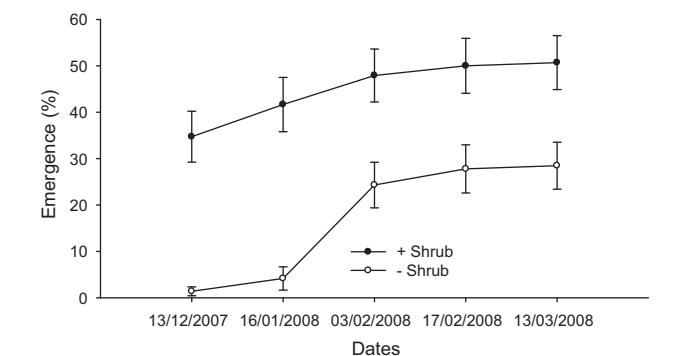


Fig. 1. Percentage emergence for *Brachypodium distachyon* under and outside the shrub *Gymnocarpus decander* during the vegetation season 2007/2008 (mean \pm 1 SE, n = 24). Data were pooled across treatments for presentation because there were no significant effects of origin and annual neighbours.

Table 2
Results of the three-way ANOVA for the effect of Origin, Annual neighbours and Shrub (*Gymnocarpus decander*) on biomass of *Brachypodium distachyon*. Significant results are in bold.

Source	df	F	P
Origin (Ori.)	1	6	0.414
Annual neighbours (Ann.)	1	1.29	0.266
Shrub (Shr.)	1	78.65	<0.001
Ori. \times Ann.	1	1.88	0.182
Ori. \times Shr.	1	10.02	0.004
Ann. \times Shr.	1	4.14	0.052
Ori. \times Ann. \times Shr.	1	12.56	0.002
Error	26		

from arid origin. However, the significant Origin \times Annual neighbours \times Shrub interaction in combination with Tukey HSD post hoc test indicates that the stronger biomass production for individuals from Mediterranean origin under the shrub was mainly observed without annual neighbouring vegetation (Table 2 and Fig. 2).

Survival to reproduction was greater for individuals from arid origin than for individuals from Mediterranean origin and it was

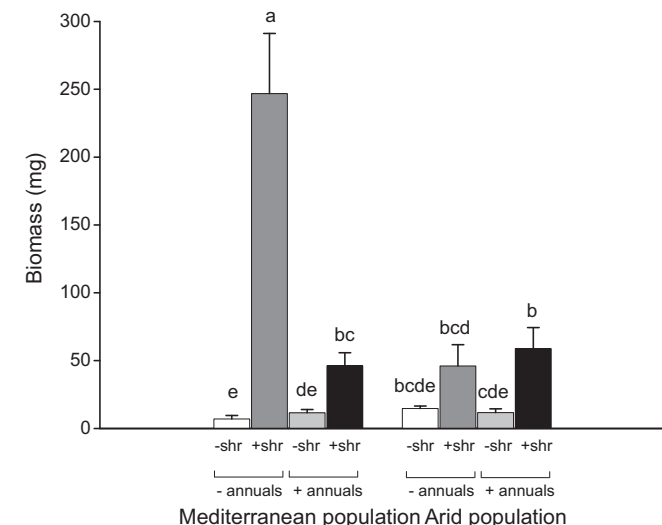


Fig. 2. Effect of the shrub *Gymnocarpus decander* and annual vegetation on biomass of *Brachypodium distachyon* from Mediterranean and arid origin (mean \pm 1 SE, $3 < n < 6$). Letters indicate significant means contrasts among species. Bars that share letters were not different at $P < 0.05$ (Tukey HSDS post hoc test).

Table 3
Results of the logistic regression for the effect of Origin, Annual neighbours and Shrub (*Gymnocarpus decander*) on number of individuals of *Brachypodium distachyon* surviving to reproduction at the end of the vegetation season. Significant results are in bold.

Source	df	χ^2	P
Origin (Ori.)	1	7.26	0.007
Annual neighbours (Ann.)	1	0.01	0.924
Shrub (Shr.)	1	12.45	0.0004
Ori. \times Ann.	1	0.02	0.895
Ori. \times Shr.	1	0.01	0.942
Ann. \times Shr.	1	0.01	0.925
Ori. \times Ann. \times Shr.	1	0.02	0.902

greater under shrubs than away from the shrubs (Table 3 and Fig. 3). Moreover, no individual from Mediterranean origin managed to survive to reproduction outside shrubs whereas individuals from arid origin were able to do so (Fig. 3). Annual neighbouring vegetation had no significant effect on this pattern.

Discussion

B. distachyon plants from Mediterranean and arid origin differed substantially in their response to neighbours. Our most salient finding was that Mediterranean ecotypes, unlike the local ecotypes, were not able to reproduce away from nurse plants. Therefore, our hypothesis about the importance of nurse plants for reproduction of the Mediterranean ecotype was confirmed to a surprisingly extreme degree. We attribute our findings to local adaptation for differing abiotic and biotic conditions at the two extremes of the distribution range (Aronson et al., 1992, 1993; Liancourt and Tielbörger, 2009). This pattern may have important consequences for microevolutionary processes.

Because we used seeds collected in the field, we cannot exclude the possibility that maternal environment effects modified the phenotype of our plants. However, these effects are unlikely to bias our results for two main reasons: First, several parallel studies in W. Shuayb and along a similar gradient in Israel have indicated that for many species (>50), including our study species, trait variation among different origins (e.g. Petru et al., 2006; Lampei and Tielbörger, 2010) is maintained in a second and third generation of inbred lines raised under common conditions, indicating

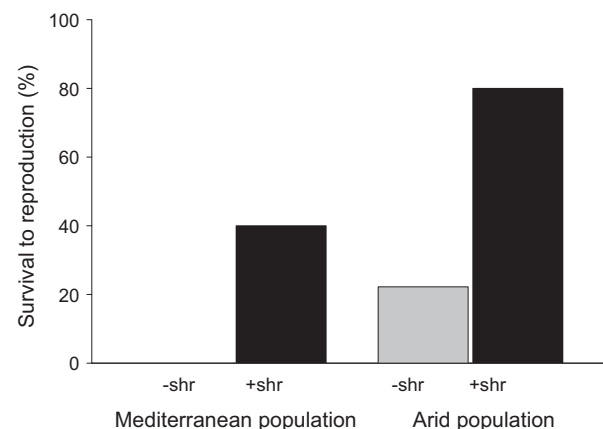


Fig. 3. Effect of the shrub *Gymnocarpus decander* and annual vegetation on the percentage of individuals of *Brachypodium distachyon* from Mediterranean and arid origin surviving to reproduction at the end of the vegetation season. Annual neighbours had no significant effect in our model. Therefore, data with and without annual neighbours were pooled for presentation.

genetically determined differences between populations from different climates. Secondly, we could demonstrate for a coexisting species, that while arid and Mediterranean ecotypes differ in their expression of maternal effects, the qualitative trait differences between these two origins (e.g. flowering time) were unaffected by the maternal environment (C. Ariza et al., unpublished manuscript).

The idea that local adaptation of plants from 'benign' environments leads to higher responsiveness to facilitation has been raised but has never been documented in the field. Espeland and Rice (2007) found that plants from benign habitat patches were more facilitated than plants from stressful patches, but the experiments were done in a greenhouse and with intraspecific neighbourhoods. Another recent study was done in the field but did not show ecotypic variation in responsiveness to positive interactions (Eränen and Kozlov, 2009). Most previous studies demonstrating specific responses to facilitation have been conducted on the species level (e.g. Choler et al., 2001). For example, it has been shown that the relationship between stress tolerance and competitive response ability may explain why some species (those with low stress tolerance and high competitive response ability) are more likely to be facilitated than stress-tolerant species (Choler et al., 2001; Liancourt et al., 2005; Wang et al., 2008; Gross et al., 2010). The same applies at the ecotype level, the lower drought tolerance of the individuals from Mediterranean origin allowed maximising the benefits of having a nurse. The better competitive response of the *B. distachyon* of Mediterranean origin, observed in dense Mediterranean vegetation in our previous study (Liancourt and Tielbörger, 2009), may also have minimised the negative effects of competition for light and other resources by the nurse plant itself.

Our findings for different response variables and soil moisture provide us with a parsimonious explanation for the main environmental constraints that could be ameliorated by the shrubs and perceived differentially by the ecotypes. First, similar to other arid environments in the region and elsewhere, the shrub *G. decander* increased soil moisture and plant densities locally, most likely due to decreasing evapotranspiration (Franco and Nobel, 1988; Flores and Jurado, 2003; Holzapfel et al., 2006). Therefore, limited drought resistance of the Mediterranean ecotype may be the first explanation for the differential response of the two ecotypes to the shrubs. Even more interesting was our finding related to the phenological development: emergence started markedly earlier for all plants growing under shrubs and resulted in an extension of the growing season by more than one month compared to outside shrubs. This effect is of primary importance since the length of the vegetation season is one of the most limiting factors in arid environments, and a key adaptation to arid conditions includes faster phenological development (Aronson et al., 1992; Petru et al., 2006 and references therein). This was also confirmed for *B. distachyon* in our previous study (Liancourt and Tielbörger, 2009), where Mediterranean ecotypes grown in a standard environment required three to four more weeks from germination to flower production than arid plants (see also Aronson et al., 1992). This indicates that a main reason for the reproductive failure of Mediterranean plants without nurses was probably their inability of completing their life cycle in the short growing season in open areas. To our knowledge, such a facilitative mechanism, i.e. increasing the length of the vegetation season, has never been recorded before.

Another interesting aspect of our experiment is the difference in the effect of the annual vegetation on the two *B. distachyon* ecotypes. The increased competition among annuals under the shrubs, with increasing annuals density is consistent with observations of density-dependent biotic interactions in arid environments (Goldberg et al., 2001; Chesson et al., 2004) and with the proposed increase in competition intensity with increasing favorability and productivity of the environment (Grime, 1973). However, the

annual vegetation apparently limited biomass under the shrubs for the individuals from Mediterranean origin only, even though competition by the annuals was not strong enough to overcome the positive effect of the shrubs. This result apparently contradicts our observation of a superior competitive response in Mediterranean *B. distachyon* to annual vegetation in a Mediterranean community (Liancourt and Tielbörger, 2009).

We propose two non-mutually exclusive hypotheses to explain the absence of response of the individuals from arid origin to the annual vegetation. On the one hand, the negative effect of the shrubs could have overwhelmed the negative effect of the annuals on the individuals from arid origin, explaining no additional effect of the annual vegetation. On the other hand, different resources may drive the outcome of competitive interactions among annuals in Mediterranean vs. arid environments. Indeed, competitive interactions in pulse-driven environment such as arid environments happen during pulse events (Goldberg and Novoplansky, 1997). During these pulse events, annuals are competing for water, i.e. the limiting resource (Chesson et al., 2004). Consequently, the arid ecotype may have a better competitive response to annuals in arid environments due to a higher drought tolerance. Additional experiments manipulating explicitly the limiting resources would be needed to tease apart these two hypotheses.

Our study demonstrates that local adaptation leads to differential response to facilitation in the field. This suggests that not only is facilitation affected by evolutionary processes (i.e. ecotypic differentiation), but that facilitation may in turn have important evolutionary consequences. For example, if migration among populations is possible and common, facilitation may open a pathway for immigrants/maladapted genotypes from 'benign'/core environments into 'harsh'/marginal conditions. As such, positive interactions could be an important mechanism by which genetic variability is maintained in populations inhabiting stressful environments. For example, shrubs may serve as a refuge for wet adapted ecotypes during drought years and thus allow coexistence of a larger number of genotypes in a given environment. This genetic diversity, which has been observed previously in peripheral (i.e. arid) annual grass populations in the region (Volis et al., 1998), may buffer the consequences of large spatio-temporal variations in environmental conditions, which are characteristic for deserts (Frank and Inouye, 1994). On the other hand, there may also be negative consequences because maladapted ecotypes are capable to persist in a stressful environment. In summary, our findings and that of a previous study (Espeland and Rice, 2007) demonstrate that more attention on the role of facilitation at a population level is needed to investigate its consequence on the distribution of genotypes/ecotypes along environmental gradients within the distribution range of a species and on maintenance of genetic diversity in marginal habitats.

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