Sun/shade conditions affect recruitment and local adaptation of a columnar cactus in dry forests

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• *Background and Aims* Facilitation among plants in water-limited environments (i.e. where evapotranspiration overcomes the availability of water during the growing season) has been considered a local adaptation to water and light conditions. Among cacti, early life-history stages can benefit from the facilitative effects of nurse plants that reduce solar radiation and water stress. However, whether light condition itself acts as an agent of selection through facilitation remains untested. The aim of this study was to determine (1) whether light conditions affect seedling recruitment, (2) whether the positive effect of shade on seedling recruitment is more intense under more stressful conditions and (3) whether shade condition (facilitation) reduces the magnitude of local adaptation on seedling recruitment relative to full sunlight conditions.
• *Methods* A reciprocal transplant experiment, coupled with the artificial manipulation of sun/shade conditions, was performed to test for the effects of local adaptation on germination, seedling survival and growth, using two demes of the columnar cactus *Pilosocereus leucocephalus*, representing different intensities of stressful conditions.
• *Key Results* Full sunlight conditions reduced recruitment success and supported the expectation of lower recruitment in more stressful environments. Significant local adaptation was mainly detected under full sunlight conditions, indicating that this environmental factor acts as an agent of selection at both sites.
• *Conclusions* The results supported the expectation that the magnitude of local adaptation, driven by the effects of facilitative nurse plants, is less intense under reduced stressful conditions. This study is the first to demonstrate that sun/shade conditions act as a selective agent accounting for local adaptation in water-limited environments, and that facilitation provided by nurse plants in these environments can attenuate the patterns of local adaptation among plants benefiting from the nurse effect.

Key words: Dry forest, facilitation, germination, local adaptation, Mexico, nurse-plant syndrome, *Pilosocereus leucocephalus*, reciprocal transplant experiment, seedling growth, seedling survival.

INTRODUCTION

While facilitation among plants has been recognized for a long time (Phillips, 1909), this ecological interaction has gained the attention of ecologists only in the last two decades (Holzapfel and Mahall, 1999; Bruno *et al.*, 2003; Bronstein, 2009; Holmgren and Scheffer, 2010). This type of plant–plant interaction can impose selection pressure on focal plants when the benefit from nearby neighbours increases their fitness (Bronstein, 2009), a situation that depends on environmental conditions. Within stressful environments, facilitation would be maximal as neighbours attenuate the intensity of resource limitation or tissue injury generated by abiotic stresses (Bruno *et al.*, 2003; Ariza and Tielbörger, 2011). As environmental conditions become less harmful for plants because resource limitation is less intense, negative plant–plant interactions (competition) become more common (Holmgren *et al.*, 1997). While stressful conditions can be a selective force promoting local adaptation, facilitation may reduce the intensity of selection imposed by stressful environmental conditions constraining adaptive differentiation (but see Liancourt *et al.*, 2012). To date, our knowledge of facilitation within

plant communities has mainly been developed from an ecological perspective (Brooker *et al.*, 2008; Bronstein, 2009). Although the ultimate consequences of facilitation have been discussed in recent reviews, there have been few attempts to take an evolutionary approach in the study of this plant–plant interaction (Espeland and Rice, 2007; Bronstein, 2009; Ariza and Tielbörger, 2011). As such, the hypothesis that facilitation can play a role during adaptive divergence among populations exposed to different levels of abiotic stress remains untested.

Facilitation provided by nurse plants in communities exposed to strong water limitation has been extensively demonstrated (Flores and Jurado, 2003). In these communities, plants growing beneath the canopy of taller species can take advantage of the attenuation of radiation intensity and the consequent improvement of microclimatic conditions favouring germination, survival and establishment (Smith and Huston, 1989; Holmgren *et al.*, 1997). However, in some cases, this initial positive effect can ultimately limit plant growth through light limitation (Smith and Huston, 1989; Holmgren *et al.*, 1997; Holmgren and Scheffer, 2010). Seedlings of crassulacean acid metabolism (CAM) species, which close their

stomata during the day, are expected to suffer more from high temperatures near the soil surface (Holthe and Szarek, 1985; Hernández-González and Briones, 2007). This concept is consistent with the early observation, in the Sonoran Desert, that several CAM species tend to establish beneath the shade provided by other plants (Shreve, 1931). Later, Niering *et al.* (1963) and Turner *et al.* (1966) demonstrated the effect that the shade provided by *Cercidium microphyllum* had on the successful recruitment of seedlings of the columnar cactus *Carnegiea gigantea*. Since the pioneer observations by Shreve (1931), several others have shown, through many species and communities, that nurse plants, through shading, reduce stressful conditions for recruitment (reviewed by Flores and Jurado, 2003). Specifically, the associations between columnar cacti and shrubs or trees have been reported extensively in desert regions, but only once in dry forests (Arriaga *et al.*, 1993; Larrea-Alcázar and Soriano, 2006). Despite the relevant role of germination, survival, and growth for successful recruitment and population viability in stressful environments, there have been few attempts to test for local adaptation during these critical life stages (e.g. Giménez-Benavides *et al.*, 2007; Donohue *et al.*, 2010).

Local adaptation is usually tested following local–foreign and home–away comparisons (Kawecki and Ebert, 2004). While the local–foreign contrast tests whether a gene combination in its native site, presumably affected by past selection, outperforms any gene combination belonging to other sites, the home–away contrast tests whether the adaptive evolution of higher performance at the native site imposes a cost related to dealing with novel environmental conditions. Thus, inferences of local adaptation using the home–away contrast assume that adaptive divergence between populations constrains their ability to take advantage of novel environments. However, in the absence of such costs, native plants may exhibit better performance in the away site than in the home site, because of phenotypic plasticity to take advantage of higher resource availability (e.g. Garrido *et al.*, 2012).

To determine if facilitation constitutes a relevant factor that conditions local adaptation of populations in stressful conditions, a reciprocal transplant experiment, coupled with artificial manipulation of light/shade conditions, was performed. Given that shade is one of the factors that mediate facilitation among plants in environments where evapotranspiration overcomes the availability of water during a period of the growing season (Turner *et al.*, 1966), it was expected to affect plant performance. Following this approach, it was possible to test whether light availability is a relevant micro-environmental component behind the pattern of local adaptation. If higher plant performance at the native site is conditioned by light availability, then light represents one of the selective agents that promote or constrain adaptive divergence among populations (MacColl, 2011).

The reciprocal transplant experiment was performed using two sites, exposed to different levels of environmental stress, for recruitment of the columnar cactus *Pilosocereus leucocephalus*. This species grows within dry forests along the entire altitudinal span of this ecosystem (Bravo-Hollis, 1937; Guzmán *et al.*, 2003). In the study area, it grows on alluvial soils, as well as on lava flows. Plant cover in young, shallow soils, developed over lava flows is low, and *P. leucocephalus*

populations growing there have low density compared with those growing in alluvial soils. This environmental condition represents a stressful and relatively unsuitable condition for plants, because the black basaltic substrate increases soil temperatures and decreases relative humidity, thus strongly increasing water stress. In contrast, dry forests growing on alluvial soils are exposed to relatively lower levels of water limitation, they are more dense and they have higher plant cover than those growing over lava flows. As our experimental design involved two populations exposed to different degrees of water stress, the home–away contrast was also used, to determine which site represented the more stressful environment for germination, survival and growth.

We hypothesized that recruitment (germination, survival and seedling growth) in the low-density vegetation growing over the lava flow substrate would be more constrained than under the less stressful conditions found at the site with higher vegetation density located over alluvial soils. In addition, we predicted that if light/shade conditions mediate success during recruitment, and this factor plays a role during the adaptive evolution of populations, the experimental manipulation of light availability would affect the patterns of local adaptation (e.g. Ariza and Tielbörger, 2011). Following a full factorial design, the interaction among transplant site, provenance and light treatment would indicate the presence/absence of an effect of light conditions on the extent of local adaptation. In particular, we predicted that if facilitation through the attenuation of light incidence increases seedling recruitment, the magnitude of local adaptation would be higher under full sunlight than under shade light conditions. In this study, we specifically examined (1) whether the artificial manipulation of sun/shade conditions affects germination, survival and growth of *P. leucocephalus* seedlings, grown at two contrasting sites, following a reciprocal transplant experiment; (2) whether the expected positive effect of shade on germination, survival and growth (i.e. facilitation) is more intense at the site located under more stressful conditions; and (3) whether shade conditions (facilitation) reduce the magnitude of local adaptation in *P. leucocephalus* relative to full sunlight conditions.

MATERIALS AND METHODS

Species and study site

Pilosocereus leucocephalus (Cactaceae) is a bat-pollinated, hermaphroditic, self-incompatible columnar cactus (Munguía-Rosas *et al.*, 2009), distributed from Nicaragua to north-east Mexico (Guzmán *et al.*, 2003). In the state of Veracruz (Mexico), this species is distributed within dry forests from 50 to 1200 m above sea level (asl) (Bravo-Hollis, 1937). In the studied region (central Veracruz), this species is the only cactus that survives above 1100 m asl, while at lower elevations, it coexists with other cacti (*Neobuxbaumia scoparia* and *Stenocereus griseus*) (Medina Abreo and Castillo-Campos, 1993).

Plant material for our experiment was gathered from two sites that are geographically isolated from each other, thereby reducing the chances of any significant gene flow between populations (Becker *et al.*, 2006). These were

represented by the populations (hereafter deme) of San Ignacio (SI) (19°35′26″N, 96°58′38″W) and Puente Nacional (PN) (19°20′37″N, 96°28′05″W). The SI deme is located at 1150 m asl, over a rocky substrate of lava flows deposited during the Holocene, around 10 000 years ago (Negendank *et al.*, 1985). At this site, the mean annual temperature is 20·4 °C (means of the hottest and coldest months, May and January, are 29·2 and 11·5 °C, respectively), and annual precipitation is 1300·0 mm, with 79·1 % of the rains concentrated between May and October (Fernandez-Eguiarte *et al.*, 2012, period 1903–2010). Vegetation covers half of the soil surface in the area: 50·30 ± 5·86 % (data taken from each of five 50-m-long line-transects). The dominant species in this site are *Lysiloma acapulcensis*, *Dodonaea viscosa*, *Quercus oleoides*, *Cnidoscolus aconitifolius* and *Tonduzia longifolia*.

The PN deme is located within a dry forest dominated by *Tabebuia chrysantha*, *Bursera simaruba*, *Ceiba aesculifolia*, *Cordia dentata*, *Croton cortecianus*, *Esenbeckia berlandieri* and *Luehea candida*. The altitude is 87 m asl, the mean annual temperature is 25·91 °C (means of the hottest and coldest months, May and January, are 34·6 and 16·3 °C, respectively) and annual precipitation reaches 1144 mm (92·1 % of rains fall between May and October) (Fernandez-Eguiarte *et al.*, 2012, period 1903–2010). At this site, vegetation covers 93·80 ± 5·49 % of the soil surface (data taken from each of five 50-m-long line-transects).

Reciprocal transplant experiment

For each deme, one mature fruit (containing more than 800 seeds each) from 20 randomly selected plants was harvested between May and June 2009, to obtain enough seeds for the reciprocal transplant experiment. The seeds were washed to eliminate mucilage, and they were air dried and stored in paper bags at 25 °C. The experiment involved two demes (SI and PN), two sites (SI and PN) and two treatments (full sunlight and shade conditions) (hereafter light treatment). For this experiment, seeds from each deme were grown in both sites and under both treatments, under greenhouse conditions specifically constructed to simulate full sunlight and shade conditions. Because both transplant sites were located within undisturbed areas of natural vegetation, we decided to construct only one greenhouse per light treatment combination per site, to minimize environmental impacts. Although doing so caused pseudoreplication within the light treatment, we took care to avoid any other factor associated with differences between greenhouses being confounded with the light treatments. For this purpose, we obtained environmental measurements under natural conditions of the different light scenarios to compare them with those simulated within the greenhouses. Environmental conditions – photosynthetically active radiation (PAR), temperature and relative humidity – simulated within the greenhouses were equivalent to full sunlight and shade conditions observed at both studied sites within the natural vegetation. Because the plants were grown in pots filled with soil from mixed soil samples collected at both transplant sites, we controlled for possible soil effect in our results. Also, because herbivory was controlled through the application of insecticide within the greenhouses (see below), we are confident that the major environmental differences between

greenhouses were those arising from the light treatments. One of the greenhouses at each site was covered with transparent plastic to avoid natural rainfall and to simulate full sunlight conditions; the other was also covered with transparent plastic and with a mesh to reduce the availability of PAR to the plants. The north and south walls of each greenhouse were left open to reduce the possible effect of sun/shade conditions on other microclimatic variables (temperature and relative humidity). Mesh density at each transplant site was adjusted following previous estimations of PAR reaching the soil surface beneath the canopy of natural vegetation (see below).

In both transplant sites, PAR was measured at the soil surface, in open areas with full sunlight and under the canopy, during one sunny day in August 2009. These measurements were recorded every hour between 0600 and 1800 h, using two Li-190 sensors connected to a Li-1000-32 data logger (LI-COR Bioscience, Lincoln, NE, USA), under each environmental condition. Daily PAR patterns were used to calculate total radiation received as the integral of each curve. Canopy effect on PAR was analysed using two-way ANOVA for fixed effects. As expected, PAR reduction beneath the canopy at the site with higher vegetation cover was higher (86·3 %) (1·65 ± 0·056 vs. 12·05 ± 0·64 kmol m^{-2} h^{-1}, $P <$ 0·05) than in the more stressful site with lower vegetation cover (76·5 %: 2·89 ± 0·48 vs. 12·29 ± 0·4 kmol m^{-2} h^{-1}, $P <$ 0·05; Supplementary Data Table S1, Fig. 1). Based on these results, shade simulation at SI consisted of a reduction of 75 % of PAR, while a reduction of 85 % was applied at PN.

Artificial light manipulation

After the greenhouses were constructed, PAR, temperature and relative humidity were measured in both sun/shade treatments and in the outdoors, to validate our simulation of natural conditions and to verify that the greenhouse effects represented those related to simulated full sunlight/shade conditions. PAR measurements were obtained within each greenhouse every hour between 0700 and 18:00 h, using two light sensors (Li-190). The same procedure was followed to estimate relative humidity (HRP45A/D; Vaisala, Helsinki, Finland) and temperature (LI-1000-16). All sensors were connected to the Li-1000-32 data logger, and measurements were taken on a sunny day (2 June 2010). Each variable was analysed with a two-way ANOVA for fixed effects. Our results indicated that our artificial manipulation of light conditions was similar to what happens under natural conditions. In PN, artificial shading reduced sunlight availability by 80·6 %, while in SI, sunlight reduction was 73·2 % (Table 1). No differences between sites were detected, but significant differences between treatments were found (Table 1, Supplementary Data Table S2, Fig. 1). The interaction site × treatment was also non-significant (Table S2). PAR and temperature estimates outside the greenhouses did not differ from those obtained for the full sunlight treatment in the greenhouses (Table 1, Supplementary Data Table S2, Fig. 1).

Shade conditions in the greenhouses reduced the temperature by 5·8 °C in SI and 3·2 °C in PN and increased relative humidity by 10·45 % in SI and 4·96 % in PN (Table 1, Supplementary Data Table S2, Fig. 1). Not only were the mean values more stressful in SI than in PN, but the stressful
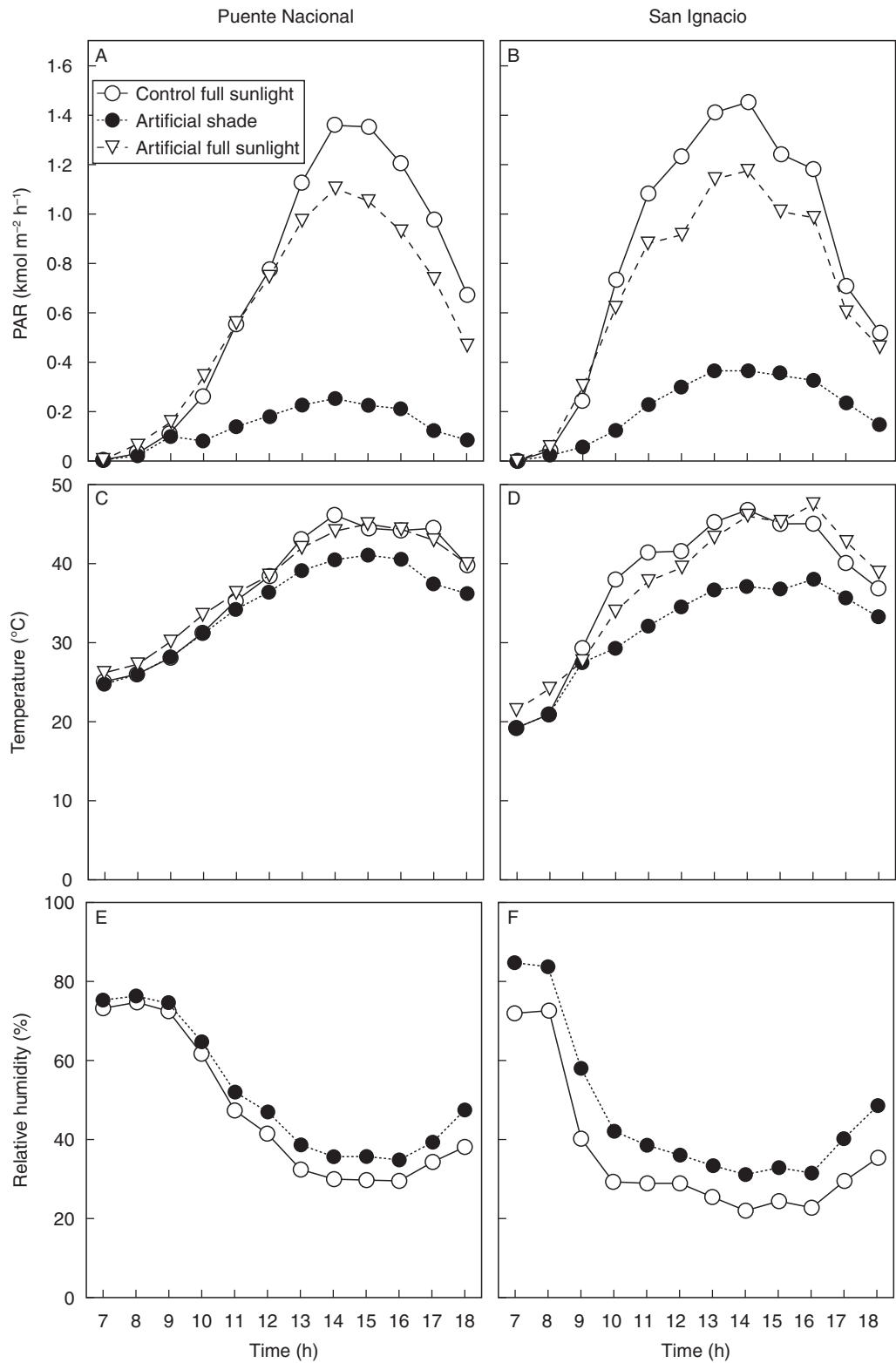
FIG. 1. (A, B) Daily pattern of photosynthetically active radiation (PAR), (C, D) temperature and (E, F) relative humidity for sun/shade treatments within the greenhouses and under control full sunlight in both transplant sites (Puente Nacional and San Ignacio, as indicated). Values of relative humidity for the control full sunlight condition at both transplant sites were not recorded.

TABLE 1. *Mean ($\pm$ s.e.) of photosynthetically active radiation (PAR), temperature and relative humidity at both transplant sites (Puente Nacional and San Ignacio) for simulated sun/shade conditions within the greenhouses and in an open habitat (control full sunlight outside the greeenhouse)*

	PAR (kmol m^{-2} h^{-1})		Temperature (°C)		Relative humidity (%)	
	PN	SI	PN	SI	PN	SI
Simulated shade	2·208 $\pm$ 0·0082[a]	3·092 $\pm$ 0·518[a]	34·56 $\pm$ 1·68[ab]	31·68 $\pm$ 1·83[a]	51·66 $\pm$ 4·78[b]	47·05 $\pm$ 5·53[ab]
Simulated full sunlight	10·715 $\pm$ 0·420[b]	10·743 $\pm$ 1·183[b]	37·75 $\pm$ 1·97[b]	37·50 $\pm$ 2·53[b]	46·70 $\pm$ 5·27[ab]	36·60 $\pm$ 5·09[a]
Control full sunlight	11·378 $\pm$ 0·293[b]	11·557 $\pm$ 0·785[b]	37·70 $\pm$ 1·51[b]	37·79 $\pm$ 1·91[b]	n.a.	n.a.

Different letters indicate significant differences among treatments within sites following a Tukey–Kramer test ($P < 0.05$). na, data not available.

conditions also lasted longer in SI than in PN: temperatures in the shade were higher than 40 °C from 1300 h until 1600 h in SI, while in PN they never reached 40 °C, and the period of time with relative humidity below 40 % spanned from 1000 h until 1700 h in SI and from 1300 h until 1700 h in PN (Fig. 1). Overall, our artificial manipulation of light conditions ensured that greenhouse effects corresponded mainly to differences in light availability rather than to temperature or relative humidity.

Measurements and data analyses

The number of seeds to be used in the experiments was determined by taking into account that the germination percentage of non-scarified seeds geminated in agar (2 %) under controlled laboratory conditions (12 : 12 h photoperiod under 25 and 20 °C, respectively) was 81·5 $\pm$ 1·02 % ($n = 450$ Petri dishes with 20 seeds each). Twenty randomly selected parental plants from each transplant site were used to obtain the sample of seeds for the experiment. A total of 16 000 seeds were used to estimate the proportion of germinated seeds per pot [2 sites $\times$ 2 demes $\times$ 2 light treatments $\times$ 100 pots (800 cm^3) $\times$ 20 seeds per pot]. Within each treatment combination, 100 seeds from each fruit were divided into five pots (20 seeds per pot) and located within the greenhouse following a completely randomized design. Seeds were located within the pots at the surface level, and thus emergence is equivalent to germination. In each transplant site, five randomly selected soil samples (0–40 cm depth) were taken and mixed before filling the experimental pots. To avoid seed predation by insects, an insecticide (parathion-methyl at a concentration of 2 %) was applied to the seeds before sowing them, and thereafter the same insecticide was applied every 2 d to avoid seedling herbivory. In October 2009, 49 d after sowing, the proportion of germinated seeds (those with cotyledons) in each pot was recorded.

To estimate seedling survival, an independent pool of seeds was used, following a similar procedure [2 sites $\times$ 2 demes $\times$ 2 light treatments $\times$ 100 pots (4300 cm^3) $\times$ 40 seeds per pot]. After germination, 15 randomly selected seedlings were left in each pot (12 000 seedlings). These pots were used to estimate the proportion of seedling survival and growth. After almost one year (326 days), the surviving seedlings were counted, and one seedling per pot was harvested to estimate dry weight as a proxy of growth. The seedlings were oven dried at 80 °C for 48 h before weighing them. During the

experiment, the pots were watered at field capacity, and an insecticide (parathion-methyl 2 %) was applied every 2 d to avoid seedling herbivory. Herbivory by small vertebrates was controlled as all greenhouses were surrounded by a fence. Seedlings of other species were eliminated every week to reduce interspecific competition within the pots.

Although seed mass might account for some variation in seedling recruitment (Jakobsson and Eriksson, 2000; Susko and Lovett-Doust, 2000), no evidence of a genetic correlation between seed mass and germination was detected for PN ($r = 0.125$, $P > 0.05$, $n = 30$) and SI ($r = 0.135$, $P > 0.05$, $n = 30$) (A. Miranda-Jácome and C. Montaña, unpubl. data). The proportion of germinated seeds, survival and growth were analysed using a three-way ANOVA. Site, deme and light treatments were considered fixed effects. Multiple contrasts were performed using the Tukey–Kramer test. Following this model, a significant site $\times$ deme interaction represents a necessary condition to test for local adaptation. We used the local–foreign contrast to test whether the native deme, at its home site, outperformed the foreign deme. The home–away contrast was also examined, to test whether a given deme had a better performance at its home site than at the away site (Kawecki and Ebert, 2004; Garrido *et al.*, 2012). Following both approaches to test for local adaptation, the three-way interaction site $\times$ deme $\times$ light was used to demonstrate if light conditions affected the pattern of local adaptation. In other words, this interaction tests if the better performance of native plants at their home site is affected by light conditions. Based on our hypothesis, we specifically examined whether local adaptation is attenuated under lower PAR, and whether this pattern is more intense in the less stressful site (PN).

RESULTS

Germination

On average, mean ($\pm$ s.e.) percentage of germinated seeds was higher in the shade (58·4 $\pm$ 1·4 %) than under full sunlight (36·3 $\pm$ 1·5 %) at PN (56·9 $\pm$ 1·4 %) compared with SI (37·9 $\pm$ 1·6 %), and for the PN deme (57·3 $\pm$ 1·5 %) compared with the SI deme (37·5 $\pm$ 1·4 %) (Table 2, Figs 2A, 3A, 4A). The significant site $\times$ light treatment interaction revealed that germination did not differ between light treatments in PN, while there was an important reduction in germination under full sunlight in SI (Fig. 2A). Site $\times$ deme interaction indicated

TABLE 2. *Effects of transplant site (S), sun/shade conditions (L), provenance (P) and their interactions on germination, survival and seedling growth of the columnar cactus* Pilosocereus leucocephalus

Source of variation	d.f.	Germination (%)			Survival (%)			Growth (g)		
		Sum of squares	F	P	Sum of squares	F	P	Sum of squares	F	P
Site (S)	1	72 200·0	144·93	***	213·5	1·31		2·7	11·92	***
Sun/shade (L)	1	97 903·1	196·53	***	4170·9	25·66	***	97·0	424·58	***
Provenance (P)	1	77 815·1	156·21	***	1494·2	9·19	**	3·3	14·45	***
S × L	1	138 864·5	278·76	***	117·6	0·72		0·01	0·02	
S × P	1	8320·5	16·70	***	1880·9	11·57	***	0·01	0·02	
L × P	1	5253·1	10·55	***	854·22	5·25	*	3·4	14·99	*
S × L × P	1	97·9	0·20		856·2	5·26	*	0·1	0·55	
Error	792	394 539·5			12 875			181·05		
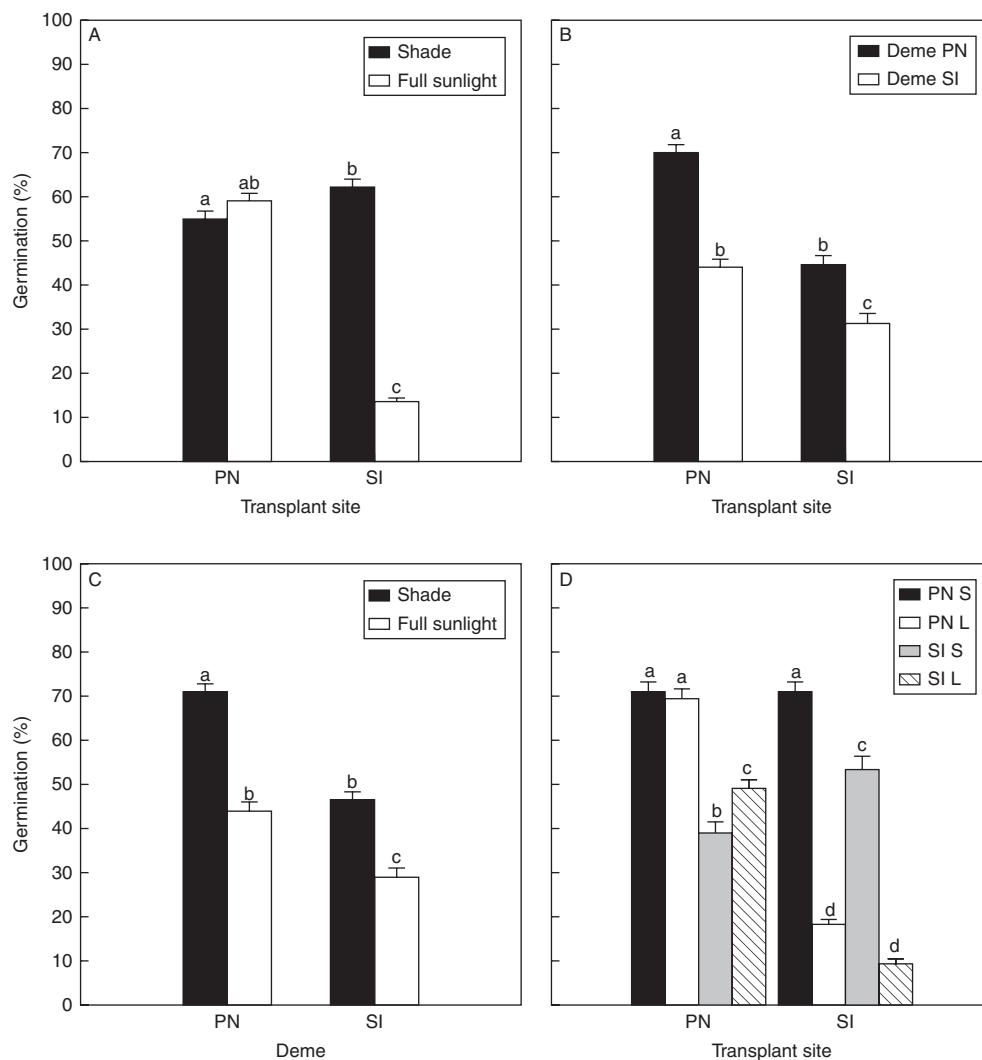
FIG. 2. Mean ($\pm$ s.e.) germination (%) of the columnar cactus *Pilosocereus leucocephalus* in a dry forest in eastern Mexico. (A) Mean values at both transplant sites (Puente Nacional and San Ignacio) under experimental shade and full sunlight conditions. (B) Mean values at both transplant sites for each deme (Puente Nacional, San Ignacio). (C) Mean values for both demes at each light treatment (shade, full sunlight). (D) Mean values for both transplant sites and deme under shade and full sunlight conditions. Different letters above bars indicate differences between treatments and sites for each response variable ($P < 0.05$).

that in both transplant sites, seeds from PN presented higher germination (%) than those from SI, but the difference was stronger in PN (Fig. 2B). The PN deme had a better performance at its home site than at its away site, while the SI deme had a lower performance at its home site than at its away site (Fig. 2B). The significant deme × light treatment
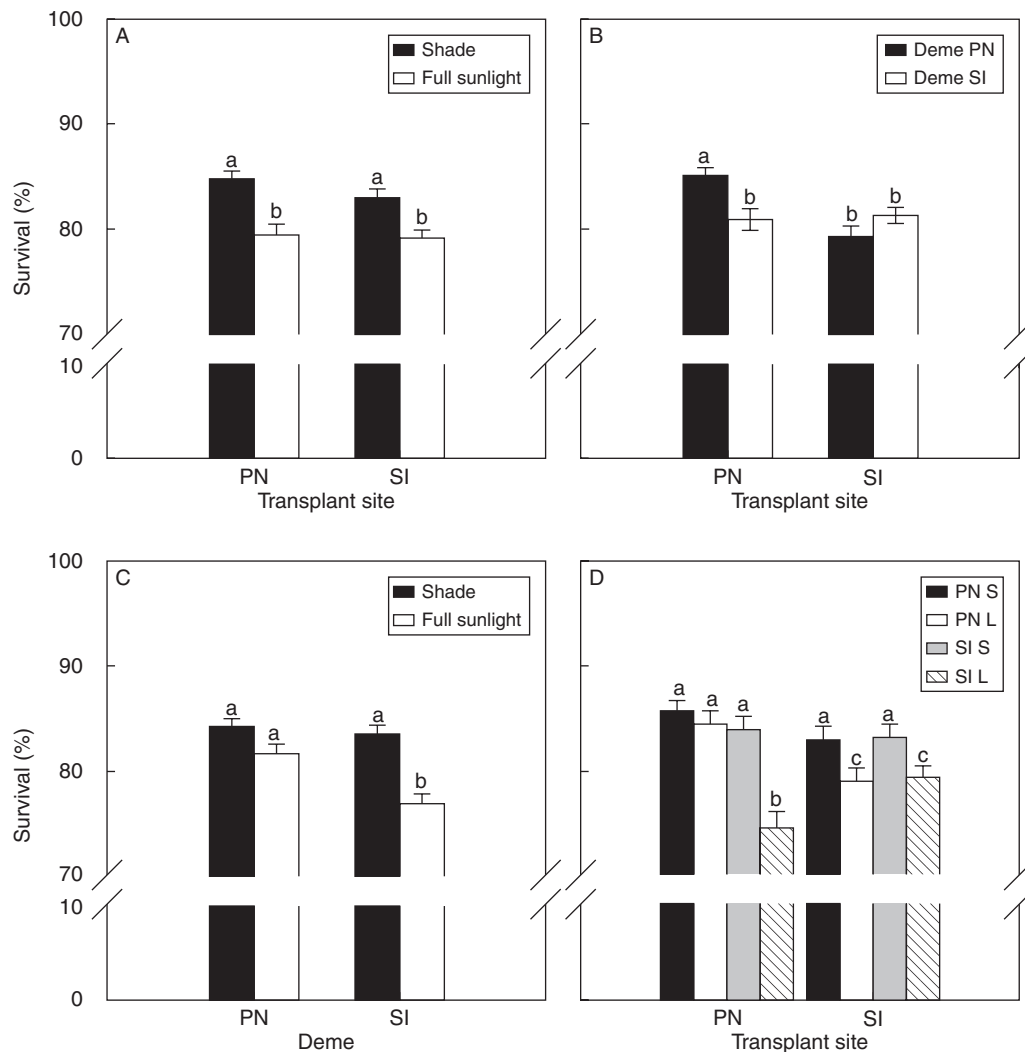
FIG. 3. Mean ($\pm$ s.e.) survival (%) of the columnar cactus *Pilosocereus leucocephalus* in a dry forest in eastern Mexico. (A) Mean values at both transplant sites (Puente Nacional and San Ignacio) under experimental shade and full sunlight conditions. (B) Mean values at both transplant sites for each deme (Puente Nacional, San Ignacio). (C) Mean values for both demes at each light treatment (shade, full sunlight). (D) Mean values for both transplant sites and deme under shade and full sunlight conditions. Different letters above bars indicate differences between treatments and sites for each response variable ($P < 0.05$).

interaction indicated that although germination was higher in the shade, this difference was stronger for the PN deme (Fig. 2C). No significant interaction among site × deme × light treatment was detected (Fig. 2D). Overall, the results for germination following the local–foreign and the home–away contrasts indicated that, unlike the SI deme, the PN deme was locally adapted.

Survival

Mean ($\pm$ s.e.) seedling survival was 81.5 ± 0.5 %. Although mean survival between transplant sites did not differ (Fig. 3A), significant differences were detected between demes (Fig. 3B) and treatments (Table 2, Fig. 3C). Shading increased survival (83.80 ± 0.61 %) compared with full sunlight (78.23 ± 0.67 %), and the PN deme demonstrated higher survival (84.88 ± 0.8 %) than the SI deme (80.15 ± 0.9 %) (Fig. 3C). Both site × deme and deme × light treatment interactions were significant

(Table 2). The native PN deme outperformed the foreign SI deme, and it had a better performance at its home site than at its away site, providing evidence that the PN deme was locally adapted (Fig. 3B). The positive effect of shading on survival was only detected for the SI deme, as it had a lower performance under full sunlight than in the shade (Fig. 3C). The interaction site × deme × light treatment revealed that the performance of the demes at both transplant sites depended on sun/shade conditions (Table 2, Fig. 3D). At PN, the native deme (PN) had a similar performance under full sunlight and shade conditions, while the foreign deme (SI) demonstrated a lower performance only under full sunlight. At SI, both demes had similar, higher performances in shade than under full sunlight. Following the local–foreign contrast, only the PN deme had a higher performance in its native site than in the foreign site, but only under full sunlight conditions. In addition, the home–away contrast indicated that the better performance of both demes at their home sites than at the away sites was
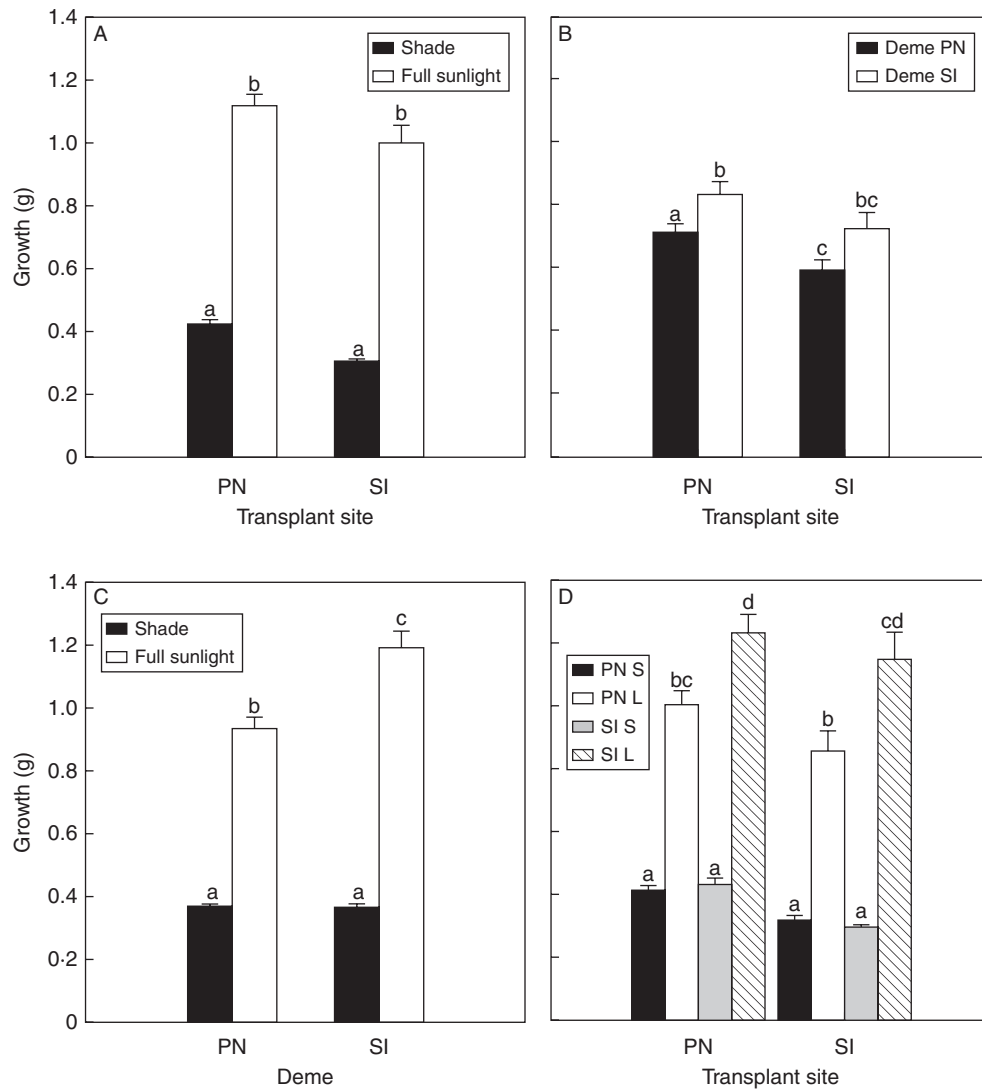
Fɪɢ. 4. Mean (± s.e.) growth (accumulated biomass) of the columnar cactus *Pilosocereus leucocephalus* in a dry forest in eastern Mexico. (A) Mean values at both transplant sites (Puente Nacional and San Ignacio) under experimental shade and full sunlight conditions. (B) Mean values at both transplant sites for each deme (Puente Nacional, San Ignacio). (C) Mean values for both demes at each light treatment (shade, full sunlight). (D) Mean values for both transplant sites and deme under shade and full sunlight conditions. Different letters above bars indicate differences between treatments and sites for each response variable ($P <$ 0·05).

only detected under full sunlight conditions, indicating that the light environment represented a selective agent at both sites.

Growth

Unlike germination and survival, seedling growth, estimated as accumulated dry biomass by the end of the experiment, was higher under full sunlight (1·06 ± 0·03 g, mean ± s.e.) than under shading (0·36 ± 0·01 g) (Table 2, Fig. 4). Seedlings from SI acquired more biomass (0·78 ± 0·03 g) than those of PN (0·64 ± 0·02 g), and growth was higher in the less stressful PN environment (0·77 ± 0·02 g) than in SI (0·65 ± 0·03 g). Although we were not able to detect a significant site × deme interaction (Table 2), following the local–foreign contrast, plants from SI at their native site showed more growth than the PN plants. Following the home–away contrast,

plants from PN had a better performance at their home site than at the away site (Fig. 4B). The significant deme × light treatment interaction indicated that the SI deme performed better than the PN deme under full sunlight (Fig. 4C). The site × deme × light treatment interaction was not significant, indicating that the response of both demes at each transplant site and light environment was similar (Fig. 4D).

DISCUSSION

Artificial manipulation of light/shade conditions altered the pattern of local adaptation for seedling survival of both demes, as the better performance of native seedlings at their home versus away site was only expressed under full sunlight conditions. This evidence indicates that sun/shade conditions represent a selective agent promoting adaptive divergence

between demes of *P. leucocephalus*, and suggests that facilitation through changes in the light environment can constrain adaptive divergence between populations. Unlike the deme of the more stressful site (SI), the deme of the less stressful site (PN) would not be invaded by non-native genotypes from SI, suggesting that the PN deme has a longer history of interaction with native conditions than the SI deme. Our results demonstrated that full sunlight negatively affected germination and survival, but it increased growth. Environmental conditions were less stressful for seedling recruitment at PN than at SI. The components of seedling recruitment expressed significant levels of population genetic differentiation and local adaptation. In particular, the deme of the less stressful site (PN) was found to be locally adapted for germination, survival and growth, while the deme of the more stressful site (SI) was locally adapted only for survival under full sunlight conditions.

Previous studies have proposed that positive interactions among plants, such as facilitation, would be higher as the intensity of environmental stress increases (e.g. Holmgren *et al.*, 1997). Our results support this expectation; at the site with higher vegetation cover (PN, 93 %), sun/shade treatments did not affect germination, while at the site with lower vegetation cover (SI, 50 %), germination was significantly increased by shading. Although SI is 1000 m above PN (1150 vs. 87 m asl), the higher temperatures at the soil surface and lower relative humidity generated by the black, young basaltic substrate resulted in more stressful conditions for recruitment in SI than in PN. Our data indicated that in PN, under shading conditions, 55 % of the seeds germinated, of which 85 % survived; therefore, 47 % of the seeds still survived 1 year after germination. At this site, the overall success rate under full sunlight conditions is similar (59 % germination × 79 % survival). In contrast, under shading conditions in SI, 51 % of the seeds survived as seedlings (62 % germination × 83 % survival), while under full sunlight, only 11 % of the seeds survived (14 % germination × 79 % survival). Thus, while at the less stressful site (PN), with higher vegetation cover, no benefit of shading was detected during recruitment after the first year since germination, a significant benefit was detected at the more stressful site (SI). To our knowledge, the present study is the first to dissect the effect of sun/shade conditions on the three processes related to seedling recruitment; thus, further comparisons with other studies are not possible at this time.

Whereas the benefits of nurse plant facilitation have been widely recognized (reviewed by Flores and Jurado, 2003), they are expressed only when the improvement of plant–water relations under the canopy exceeds the costs caused by lower light levels (Holmgren *et al.*, 1997). For example, Franco and Nobel (1988, 1989) showed that the lower surface temperature and enhanced nutrient availability under the canopy of the grass *Hilaria rigida* facilitates seedling establishment of *Agave deserti* and *Ferocactus acanthodes* but shading and competition for water reduces seedling growth. A similar pattern was inferred for *Carnegia gigantea* and the nurse plants *Ambrosia deltoidea* and *Cercidium microphyllum* (Franco and Nobel, 1989). In our study, seedlings grown in the shade accumulated less biomass than those grown under full sunlight, indicating a decreasing effect of facilitation on

growth as shading increases. In particular, seedlings in PN under shading acquired one-third of the biomass of those sown under full sunlight (0·38 vs. 1·20 g). In SI, this difference was much lower (0·38 vs. 0·94 g), suggesting that the cost of shading increases under non-limiting conditions, where plants come closer to attaining maximal growth under full sunlight, while in a more stressful environment, such as SI, maximal growth is likely to be limited, even under full sunlight conditions (Hilbert *et al.*, 1981; Wise and Abrahamson, 2005).

Since the initial observation of the beneficial effect of shading on recruitment in arid environments (Shreve, 1931; Turner *et al.*, 1966), there have been few attempts to determine which of the components of seedling recruitment (germination, survival, growth) is more vulnerable to stressful conditions (but see Giménez-Benavides *et al.*, 2007; Rodríguez-Pérez and Traveset, 2007). For instance, in the platyopuntia (*Opuntia rastrera*), both survival and growth benefited from shade (Mandujano *et al.*, 1998), while in the cilindropuntia (*Cylidropuntia leptocaulis*), shade increased growth, but not germination or survival (Flores-Torres and Montaña, 2012). Working with two columnar cacti from Tehuacan Valley, Flores *et al.* (2004) found that *Neobuxbaumia tetetzo* and *Pachycereus hollianus* had similar germination (seedling emergence) in open places than under nurse plants where they also had a longer survival time. In the present study, shade increased germination and survival, but reduced growth, suggesting that the beneficial effect of shading did not operate as a general mechanism, acting in the same way in all components of seedling recruitment. It is possible that the differential effect of shade reflects different strategies of recruitment among cacti; however, further examination is necessary before a general pattern can be outlined. In turn, there should be obvious demographic consequences derived from variations in environmentally induced differences in the proportion of survivors. Overall, demographic analyses in several cacti species suggest that the sensitivity of seedling transition to the following demographic stages is larger than that of permanence at the seedling stage. This has been confirmed by calculating the sensibilities of the transition matrices of the columnar cacti *Neobuxbaumia macrocephala*, *N. mezcalaensis* and *N. tetetzo*, given by Esparza-Olguín *et al.* (2005), and *Escontria chiotilla*, given by Ortega-Baes (2001).

Local adaptation

Both demes were genetically differentiated for the three components of seedling recruitment. Germination and survival were 1·51 and 1·04 times higher, respectively, for the deme inhabiting the less stressful environment (PN), while seedling growth was 0·86 times lower than that of the deme of the more stressful environment (SI). The higher rate of growth of SI seedlings suggests that natural selection may have increased the ability to take advantage of even scarce resources within a stressful environment characterized by the near absence of a developed soil layer. In turn, the higher rates of germination and survival of the PN deme suggest that under less stressful microclimatic conditions and better soil conditions, selection on early establishment processes may be more intense.

Despite the importance of seedling recruitment as a demographic process, local adaptation studies in plants have rarely examined this component of fitness, and never before have they dissected the three converging processes (germination, survival and growth) (Leimu and Fischer, 2008; Donohue *et al.*, 2010). Our results indicate that environmental conditions at both studied sites promoted adaptive divergence, although the pattern of local adaptation was not symmetrical. While the PN deme was locally adapted for germination, survival and growth, the SI deme was locally adapted for survival. This pattern suggests that (1) the PN deme had a longer history of selection, and/or (2) it was less exposed to other evolutionary processes that could reduce the effect of natural selection, such as genetic drift and extensive gene flow (Gandon and Michalakis, 2002). Although the natural history of both sites indicated that PN was more likely to represent an older environmental condition, it is not possible to discount the possibility that genetic drift and gene flow played a role in the actual pattern of local adaptation. Further studies on the distribution of genetic variation among populations would provide more definitive evidence. The fact that reciprocal local adaptation was detected for seedling survival under full sunlight conditions indicates that both demes were constrained to succeed under novel conditions, reflecting a cost of specialization (Williams, 1966; Kawecki and Ebert, 2004).

Although several studies have demonstrated local adaptation of plants to physical environmental conditions (Linhart and Grant, 1996; Leimu and Fischer, 2008), there have been few attempts to determine which environmental factors represent the agents of selection behind local adaptation. A recent study examining two annual species (*Biscutella didyma* and *Hymenocarpus circinnatus*) along a gradient of water availability combined a reciprocal transplant experiment with the artificial manipulation of plant–plant interactions by hand-removal of neighbours. Their results provided no evidence that plant facilitation under stressful conditions accounts for the pattern of local adaptation (Ariza and Tielbörger, 2011). Our manipulation of light/shade conditions within the reciprocal transplant experiment revealed that both demes had higher survival rates at their home sites than at the away sites, but only under full sunlight, indicating that light conditions played a role in the evolution of adaptive divergence among populations. In addition, only the PN deme outperformed the foreign deme (SI), indicating that the PN deme was less likely to be invaded by foreign genotypes native to SI. Our results showed no differences between sites regarding the extent of local adaptation driven by sun/shade conditions; therefore, it is not possible to conclude that local adaptation mediated by facilitation is more intense under stressful conditions. Because SI represents a recently colonized site, it might suffer from reduced genetic variation and time for a rapid response to selection (Blows and Hoffmann, 2005).

Since Darwin, the role of natural selection in delineating life-history strategies has accumulated an important empirical background (Stearns, 1992; Roff, 1992). Nevertheless, we are still far from identifying the physical and biotic agents of selection behind the adaptive divergence among populations (MacColl, 2011). Reciprocal transplant experiments, coupled with the experimental manipulation of relevant environmental factors, can provide conclusive evidence of the role of specific selective agents on patterns of local adaptation (Ariza and Tielbörger, 2011; Garrido *et al.*, 2012; this study). Our results suggest that facilitation through a reduction in the selection pressure imposed by full sunlight conditions could reduce the loss of genetic variation within populations under stressful conditions.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: ANOVA for the effect of site (Puente Nacional/San Ignacio), microhabitat (outside/beneath the understorey) and their interaction on photosynthetically active radiation. Table S2: ANOVA for the effects of site (Puente Nacional/San Ignacio), treatment (control full sunlight, simulated full sunlight, simulated shade) and their interaction on photosynthetically active radiation, temperature and relative humidity.

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