Can facilitation influence the spatial genetics of the beneficiary plant population?

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Summary

1. Plant facilitation is a positive interaction where a nurse or nurse plant community alters the local conditions, improving the lifetime fitness of other beneficiary plants. In stressful environments, a common consequence is the formation of discrete vegetation patches under nurse plants, surrounded by open space. The consequences of such spatial patterns have been studied mostly at the community level.

2. At the population level, facilitation causes a distribution of beneficiary individuals that could have intraspecific genetic consequences. The spatial patchiness and the increase in local aggregation can potentially affect the population fine-scale genetic structure. In addition, marked microenvironmental differences under nurses versus outside could lead to plastic phenotypic variation between facilitated and non-facilitated individuals, as for example reproductive asynchrony, potentially producing assortative mating.

3. This study tests the hypothesis that plant facilitation can have genetic consequences for the population of a beneficiary plant (*Euphorbia nicaeensis*) by affecting its spatial genetic structure and mating patterns between subpopulations of facilitated and non-facilitated individuals.

4. Facilitation in this system creates an aggregated distribution of beneficiary individuals compared to a minority of non-facilitated individuals that grow on the open ground. Facilitation also leads to slight phenological differences mediated by strong microenvironmental differences created by nurses compared to the open ground. Yet a molecular analysis showed that, although there is fine-scale spatial genetic structure in this system, there is no evidence that it is caused by facilitation. Numerical simulations further showed that spatial genetic patterns in the population are little influenced by the phenological mismatch observed in the field.

5. *Synthesis*. Facilitation leads to the strong spatial aggregation of beneficiary plants and desynchronizes their flowering phenology, but the magnitude of these effects is not enough to have local genetic consequences in our study system. Facilitation seems thus to have a homogenizing role by allowing the persistence of a diverse gene pool in populations in harsh environments, rather than promoting genetic differentiation. Further information on other systems where facilitation produces stronger spatial or phenological effects on facilitated plants is needed to fill our large knowledge gap on the genetic effects of facilitation.

Key-words: AFLP, *Euphorbia nicaeensis*, fine-scale genetic structure, flowering phenology, *Juniperus sabina*, kinship, numerical simulations, plant-plant interactions, *Sp* statistic

Introduction

Plant facilitation is a positive interaction where a nurse plant, or a nurse plant community, alters the local environmental conditions, improving the lifetime fitness of other, beneficiary plant species (Callaway 1995; Bronstein 2009). This interaction is especially relevant under stressful conditions, like those produced in resource-limited environments (Stachowicz 2001). A consequence of plant facilitation is the formation of discrete vegetation patches surrounded by open space (Prentice & Werger 1985; Eccles, Esler & Cowling 1999; Castillo, Verdú & Valiente-Banuet 2010), leading to particular spatial arrangements such as spotted, maze or banded vegetation (Aguiar & Sala 1999; Klausmeier 1999; Barbier *et al.*

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2008). Spatial patchiness at the landscape level results in further concentration of water and/or nutrients beneath the vegetation patches. Such positive feedback triggered by facilitation can lead to self-organized vegetation patterns in harsh ecosystems more often than previously thought (Rietkerk *et al.* 2002, 2004; Tirado & Pugnaire 2003).

The consequences of such spatial patterns have been studied at the community level, focusing for example on facilitation increasing species richness (Cavieres & Badano 2009) or phylogenetic diversity (Valiente-Banuet & Verdú 2007; Butterfield *et al.* 2013). However, at the population level, the facilitative interaction causes a distribution of beneficiary individuals that could also have intraspecific genetic consequences. First, both the spatial patchiness and the related increase in local aggregation produced by facilitation can potentially affect population genetic structure, in particular at small spatial scales (see McIntire & Fajardo 2011 and Till-Bottraud, Fajardo & Rioux 2012 for a case of intraspecific facilitation). Spatial structuring due to the distribution of safe sites and gradual effects within these sites, combined with limited dispersal, could lead to genetic isolation-by-distance processes (IBD) just as is expected in other contexts not related to facilitation (Wright 1943; Slatkin 1993). IBD refers to the decrease in relatedness among individuals as spatial distances increase, leading to fine-scale spatial genetic structure (SGS). The favourable conditions under nurse plants can also lead to higher clumping of benefited individuals and thus to increased SGS by enhancing the chances of mating with closely related neighbours (Doligez, Baril & Joly 1998).

A second and unexplored set of consequences relate to the marked microenvironmental differences under nurses versus outside, which could lead to plastic phenotypic variation between facilitated and non-facilitated individuals. If the affected traits involve reproduction, there could in turn be genetic consequences (Liancourt *et al.* 2012). For instance, facilitation could lead to phenological differences between individual plants growing under nurses and those growing in the open, potentially producing genetic differentiation via assortative mating. Differences in the onset of flowering across a species' range can arise in response to variation in moisture, temperature and photoperiod (Rathcke & Lacey 1985). Liancourt & Tielbörger (2009), for example, found earlier flowering in two annual grass species growing in an arid habitat compared to their conspecifics growing in a milder, semi-arid environment a few kilometres away. More locally, in a species capable of growing both under a nurse and in the open, higher moisture and lower summer temperatures under nurses compared to the open ground could lead to delayed flowering and result in fewer mating opportunities between facilitated and non-facilitated individuals in the same population.

In spite of their potential significance, little is known on the genetic consequences of facilitation (Till-Bottraud, Fajardo & Rioux 2012; see also Ehlers & Thompson 2004 and Michalet *et al.* 2011 for the nurse genetic effects at the community level). The within-population genetic consequences of facilitation on beneficiary species can be relevant because they could

have evolutionary consequences, as predicted by Liancourt *et al.* (2012). In their model, facilitation can either diminish or increase genetic structure within populations depending on how the interaction affects gene flow among facilitated individuals. They suggest that under some specific conditions, such as in harsh environments where strong vegetation patterning is present, facilitation could reduce gene exchange between subpopulations of facilitated and non-facilitated individuals as discussed above. If the environments are sufficiently dissimilar and gene flow is limited, facilitation could eventually favour differential selection and increase phenotypic variance within populations. To our knowledge, this is the first field study exploring this possibility.

Here, we test the hypothesis that plant facilitation can have genetic consequences for the population of a beneficiary plant by affecting its spatial genetic structure and mating patterns between subpopulations of facilitated and non-facilitated individuals. We asked this question in a Mediterranean mountain ecosystem where the dominant shrub *Juniperus sabina* acts as a nurse for several perennial and annual plants (Verdú & Garcia-Fayos 2003). We focus on *Euphorbia nicaeensis*, a widespread Mediterranean spurge that in this community grows preferentially under *Juniperus* shrubs, but can also grow on the open ground. This particular distribution allows us to test for genetic structure of beneficiary plants growing under nurses and plants growing on the open ground. Specifically, we (i) measured patterns of kinship and spatial genetic structure within and among facilitated and non-facilitated *E. nicaeensis* patches in our study population using molecular markers, (ii) compared phenological patterns under and outside nurses and (iii) investigated the mechanisms behind the observed fine-scale genetic structure using numerical simulations. If facilitation leads to strong mating separation, one would expect some indication of subpopulation genetic differentiation. We therefore also calculated the molecular variation and differentiation between facilitated and non-facilitated individuals.

Materials and methods

STUDY SYSTEM

Our study was conducted at a mountain Mediterranean community (1640 m a.s.l.) known as El Verdinal (40°1′ N, 1°12′W, Valencia, in southeastern Spain), within the Javalambre karst system. After extensive human use, this community is currently characterized by a spotted landscape of scattered individual creeping *J. sabina*, the dominant shrub. Other woody species, mainly *Pinus* and other *Juniperus* species, are re-colonizing since agriculture was abandoned in the area. Climatic conditions are characteristic of Mediterranean mountains; winters are cold and long, with a freezing period > 120 d yr, while summers are warm and dry. Mean annual precipitation is 600 mm.

Juniperus sabina shrubs are prostrate and grow in a centrifugous pattern, forming 'islands' that can reach 7 m in radius (see Fig. S1 in Supporting Information) and are surrounded by open rocky space with ephemeral herbaceous vegetation. Both annual and perennial plants grow beneath them, notably *E. nicaeensis* and *Helleborus foetidus*, among others, as well as recolonizing tree species such as *Pinus nigra*

and *Juniperus communis*. *Juniperus sabina* shrubs act as nurses for these plants, likely because they ameliorate microhabitat conditions for recruiting seedlings. Specifically, they provide shade and decrease summer ground temperatures (by 9.3 °C on average), and increase water availability, organic matter and total nitrogen content (see Verdú & García-Fayos 2003 for details and evidence on the role of *J. sabina* as a nurse plant in this community).

In this study, we focus on one of the perennial species facilitated by *J. sabina* shrubs, the spurge *E. nicaeensis*. This species is appropriate for our study because it is strongly associated with the nurse plant in the focal community, but can also grow in the open space, where it is less common. Around 86% of *E. nicaeensis* individuals are found under *J. sabina*, which in turn cover only about 25% of the total ground (Verdú & García-Fayos 2003). Seeds of this species can germinate in both light and dark conditions (Al-Samman, Martin & Puech 2001). In another Mediterranean locality, seedling mortality of *E. nicaeensis* is generally high but strongly reduced by facilitation under *Ulex baeticus* and *Fumana thymifolia* individuals ($X^2 = 11.33$, $P < 0.001$ when compared to survival in the open, analysed from data from Table 11.5 in Narbona 2002 for a population at 800 m a.s.l.).

To further corroborate that there is a facilitative interaction between *E. nicaeensis* and *J. sabina* at the study locality and not just spatial coincidence in response to shared microhabitat conditions, we performed a spatial analysis following the suggestion of Mcintire & Fajardo (2009) of using 'space as a surrogate' for ecological processes. This approach is especially useful for cases where experiments cannot reveal lifetime fitness differences between treatments, or at least include early life stages (germination, seedling survival and establishment), probably the most crucial stages in facilitative interactions (*e.g.* Miriti 2006). Our prediction is that *E. nicaeensis* and young (small) *J. sabina* plants should be spatially associated if they require the same microhabitat conditions for establishment. If the opposite is true, that is, that *J. sabina* and *E. nicaeensis* growing in the open do not tend to establish in the same spots, we then expect to find no close proximity between them other than expected by chance. Limiting the analysis to small *J. sabina* plants ensures that they have not yet changed environmental conditions underneath them enough to lead to facilitation. We measured the distance between 252 haphazardly chosen *E. nicaeensis* plants and the nearest small *J. sabina* shrub and compared this observed spatial distribution to two simple simulated landscapes (for details see Appendix S1 in Supporting Information). A 'random' distribution plot simulated individuals of both species in approximately their natural densities, with no biological process determining their position. A second plot simulated a clustered distribution of individuals within a radius equivalent to the mean radius of a *Juniperus* plant (2.7 m). Each of these two landscapes was simulated 100 times, and the resulting distribution of distances between *E. nicaeensis* and small *Juniperus* was compared to our observed field distribution. The distribution of our plants in the field did not differ from the random distribution, while it is highly unlikely to reflect a clustered distribution of *E. nicaeensis* and *J. sabina* (Table S1 in Supporting Information). In addition, our observed distribution did not have a peak at the smallest class distances (Fig. S2). These results suggest that the observed association between large *Juniperus* plants and *E. nicaeensis* appears as the microenvironmental conditions are modified by the growing *Juniperus* shrub and facilitate the establishment of *E. nicaeensis*. In sum, the present distribution of *Juniperus* and *E. nicaeensis* (not associated with *Juniperus* shrubs when small but associated as they grow) supports that there is a facilitative interaction. Directly measuring fitness in *E. nicaeensis* under and outside nurses would also be informative, but it is difficult in this perennial species, and it would ideally include all the early stages of the life history (germination, seedling and sapling survival), the likely stages where the positive effects of establishing under a *Juniperus* shrub take place.

At the study site, *E. nicaeensis* flowers in June and July. Inflorescences grow over the cover of *J. sabina* so that flowers are available for the few insects that can be observed visiting the flowers, mostly syrphids and other flies. *E. nicaeensis* is self-compatible (Narbona, Ortiz & Arista 2008).

FIELD SAMPLING DESIGN

In order to estimate the fine-scale genetic structure and kinship among individual *E. nicaeensis* growing under *Juniperus* and on the open ground, we selected individual *E. nicaeensis* in a spatially controlled sampling scheme that maximized the range of spatial distances among them while ensuring enough individuals were included in each distance classes. A total of 20 *J. sabina* shrubs ('facilitated' plots) were selected along two long transects that crossed each other forming an 'X'. Another 20 plots were established on the open ground ('open' plots) adjacent to the *J. sabina* plots, for a total of 40 plots. Plots on the open ground were approximately 5 × 5 m, resembling the area of a large *J. sabina* shrub, and were selected so that enough *E. nicaeensis* individuals were present. Maximum distance among plot pairs was ca. 2 km, but most plots were around the central part of the 'X'. Each plot was geo-referenced.

Within each one of the 40 plots we labelled up to 12 *E. nicaeensis* individuals to monitor flowering phenology (see below). In a few cases, there were fewer than 12 individuals. We collected green leaf tissue of each individual *E. nicaeensis* and stored it in silica gel for DNA extraction. In total, 458 plants were sampled.

AFLP GENOTYPING

We studied the patterns of kinship and molecular variation in our study population using the amplified fragment length polymorphism (AFLP) procedure. Although this technique detects anonymous 'loci' (or more properly genomic regions), it has the advantage of providing a large amount of markers spread across the whole genome and is particularly useful for non-model species. DNA of each individual *E. nicaeensis* was extracted from 50 mg of dried leaf material using the Speedtools Plant DNA Extraction Kit (Biotools, Madrid, Spain). We followed the protocol described by Vos *et al.* (1995), with variations including the use of fluorescent primers (detailed protocol in Appendix S2). Around 65 primer pair combinations were assayed in a pilot study with 12 random individuals, and seven primer pairs with the clearest profiles were selected for fingerprinting of the 458 individuals (see Table S2). Selective amplification products were pool-plexed and detected using an ABI PRISM 3730 automated DNA sequencer.

Final scoring of peak presence or absence was done manually using GeneMarker V 1.85 software (SoftGenetics, State College, PA, USA) with stringent criteria. Bin positions were automatically established but adjusted manually. Bins within groups of peaks with higher intensity or besides peaks with notorious stutter band were rejected. In general, only fragments within the range of 90–500 bp were considered, to avoid peaks in the smaller range, where there is a higher chance of homoplasy and electropherograms have high background noise. Peaks > 500 bp were also avoided because of their weak intensity and potential for false negatives. Only polymorphic peaks that overlapped homogeneously when all samples were superimposed

were accepted. We also discarded loci that were present (or absent) in < 5 (about 1%) of the sampled individuals. The resulting data set included 112 polymorphic loci. All individuals had unique multilocus profiles.

For a subset of 24 plants (about 5% of the total sample size), AFLP analyses were performed twice to evaluate scoring error rates (Bonin *et al.* 2004). Replicate scoring included all steps in the genotyping process, starting from the restriction and ligation reactions. The final peak scoring step was performed blindly. Mean error rate per locus was estimated as the ratio of the number of contradictory scores (band presence or absence) in the two independent analyses to the total number of replicated phenotypes. These rates varied among primer combinations (Table S2) and averaged 1.6% ($\pm$0.50) across all loci.

SPATIAL ANALYSIS

To test the hypothesis that facilitation can lead to differences in fine-scale spatial genetic structure (SGS) within the *E. nicaeensis* population, we calculated pairwise kinship coefficients using SpaGeDi 1.3 (Hardy & Vekemans 2002), based on the multilocus AFLP genotypes. Pairwise kinship coefficients were computed for: (i) facilitated individuals (i.e. between all *E. nicaeensis* growing under *J. sabina),* (ii) non-facilitated individuals (i.e. between all *E. nicaeensis* growing in the open) and (iii) globally for all individuals irrespective of facilitation, for comparison. Kinship coefficients were based on the estimator developed by Hardy (2003) for dominant markers, which requires the input of the inbreeding coefficient. Our estimates assumed an inbreeding coefficient of 0.1, a low value consistent with a species with several strategies to prevent selfing: plants are markedly protogynous and andromonoecious with strong asynchrony between flower types associated with different inflorescence levels (Narbona, Ortiz & Arista 2008).

Kinship coefficients were then associated with paired spatial distance in autocorrelograms with 11 distance classes (up to 10, 25, 50, 100, 200, 400, 600, 800, 1000, 1400 and 1662 m). We did not measure the exact position of individual *E. nicaeensis* plants, or the distance between them, but only the plot's central position (i.e. all *E. nicaeensis* under the same nurse are assigned to the first distance class). For a careful interpretation of the correlograms, we calculated the *Sp* statistic (Vekemans & Hardy 2004). The *Sp* statistic is calculated by the ratio $Sp = -(b-\log)/1-F_1$, where *b-log* is the slope of the regression of pairwise kinship coefficients on the natural logarithm of the spatial distance. If significant, it indicates isolation by distance in a two-dimensional space. F_1 is the kinship of the first distance class, which approximates the genetic distance between competing individuals. To test for significance of *Sp* values, SpaGeDi uses jackknifing over loci to provide approximate standard errors for the multilocus estimates of *b-log*. We used the SE to calculate 95% confidence intervals of the *b-log* and the *Sp* statistics.

Before running SGS analyses, we checked whether individual AFLP loci showed evidence of being under selection. We used Baye-Scan 2.1 (Foll & Gaggiotti 2008) considering plants growing in the open and plants growing under *Juniperus* as separate subpopulations. BayeScan uses differentiation between populations to highlight loci with exceptional genetic differentiation when compared to the neutral expectation. The default chain parameters worked well for our data, so the sample size was 5000 with a thinning interval of 10. We set the prior odds to the neutral model to 10 and used a beta prior for the uncertainty of the inbreeding coefficient (F_{is}), with mean = 0.1 and SD = 0.05. This analysis detected no loci under potential selection even when using very liberal threshold q-values to control for multiple testing (results not shown).

FLOWERING PHENOLOGY

During the summer of 2011, we monitored the flowering phenology of all marked *E. nicaeensis* individuals in our field population, except for one pair of plots that were difficult to access. From mid-May until mid-July, we made weekly visits and registered the presence of functional cyathia in 349 *E. nicaeensis* individuals. We compared the flowering pattern of plants under *J. sabina* and in the open with a mixed effects model analysis for repeated measures, using plot as a random factor, with packages nlme (Pinheiro *et al.* 2012) and multcomp (Hothorn, Bretz & Westfall 2008) in R.

NUMERICAL SIMULATIONS

Numerical simulations were carried out to test for the potential effect of phenological mismatches on the *Sp* values and on local differentiation, in simulated populations with shorter or longer dispersal. First, a spatially randomized 200 m × 200 m landscape mimicking the study population was constructed considering, among other factors, size distribution of nurses (see Fig. S1) and the actual density of *E. nicaeensis* within and outside the nurses. AFLP phenotypes were assigned randomly to each individual according to allele frequencies in the real population. Secondly, in each generation, mating probability and seed dispersal were determined using variable pollen and seed power-exponential dispersal kernels, depending on the simulation set (see below). Power-exponential dispersal kernels are commonly used in plants as they are able to accommodate a wide range of functions and are characterized by only two parameters, the scale (α) and shape (β) parameter. For example, the kernel reduces to the exponential distribution when $\beta = 1$, and to the normal distribution when $\beta = 2$, with more leptokurtic functions obtained for $\beta < 1$. Third, population size was considered stable, with mortality adjusted to obtain a full population turnover every 10 generations, following realistic life-history observations in *E. nicaeensis* (generation time of ~3 years and longevity of ~30 years). Facilitation was included in the model by controlling carrying capacity (i.e. density) under nurses or in the open, as no explicit information on performance differences due to facilitation (e.g. in survival or reproduction) is available in our model system. Simulations were run for 10 overlapping generations, enough for SGS to build, as has been observed in similar simulation studies (e.g. de-Lucas *et al.* 2009). Finally, individuals under nurses and outside them were sampled following precisely the sampling scheme used in the real population, both in terms of number of individuals and their spatial position.

We ran 8 sets of 20 simulations each, with the following parameters:

set 1: this set considered short pollen ($\alpha = 1.5$ and $\beta = 0.65$, with average dispersal distance of 9.54 m) and seed ($\alpha = 0.7$ and $\beta = 1$, with average dispersal distance of 1.40 m) dispersal, using published estimates for other *E. nicaeensis* species or populations (Gómez & Espadaler 1994; Narbona, Arista & Ortiz 2005).

set 2: same with longer dispersal (for pollen: $\alpha = 2.3$ and $\beta = 0.44$, with average dispersal distance of 95.31 m; and for seeds: $\alpha = 5.2$ and $\beta = 0.1$, with average dispersal distance of 10.40 m), based on published estimates for other herbs (e.g. Hardy *et al.* 2004). For seeds, this distance is probably close to explosive dispersal plus secondary dispersal by ants.

sets 3 and 4: same as *set 1* and *set 2*, respectively, but adding the effect of the observed mismatch in the peak of floral phenology of *E. nicaeensis* growing under *J. sabina* compared to those in the open.

sets 5 and 6: same as *set 3* (long dispersal), but increasing the mismatch in the flowering peak to 12 and 36 days, respectively, to explore the effect of diverging phenology on potential local differentiation.

MOLECULAR DIVERSITY AND GENETIC
DIFFERENTIATION

Within-population (plots) genetic diversity Hj was calculated in AFLP-surv (Vekemans 2002) for the observed field data. We also estimated band richness (Br), an analogue of allelic richness, using the rarefaction approach of Coart *et al.* (2005) implemented in AFLPdiv for a sample of seven individuals. To estimate differentiation among groups, we used hierarchical AMOVA in GenAlEx 6.5 (Peakall & Smouse 2006, 2012) to partition the molecular variance among facilitated versus in the open (Φ_{RT}), sampling plots (Φ_{PT}) and within plots. The significance of the model was tested with 9999 permutations over the whole data set. The AMOVA analysis was also used to test for the potential effect of high phenological differentiation between facilitated and non-facilitated plots in the simulated data.
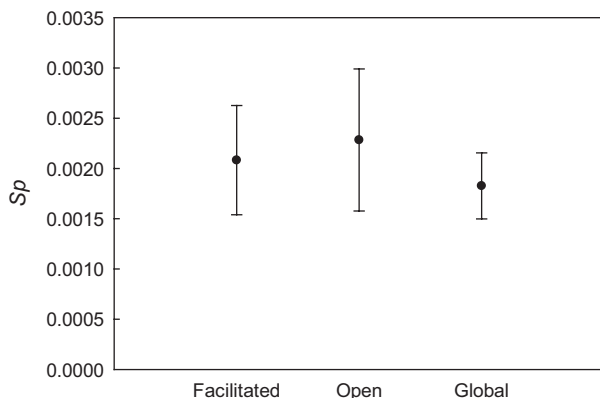
Results

SPATIAL GENETIC PATTERNS

In our study population, kinship among individual *E. nicaeensis* decreased with geographical distance, indicating the presence of isolation by distance (IBD). This was confirmed by low, yet significant Sp values. However, Sp values calculated for subgroups of facilitated and non-facilitated *E. nicaeensis* ('open') did not differ from each other or from a global estimate including all individuals (Fig. 1). This suggests that facilitation is unlikely to be the cause of the fine-scale spatial structure found in this population.

FLOWERING PHENOLOGY

Our field observations showed a slight offset in the flowering phenology of facilitated *E. nicaeensis* compared to those growing on the open ground (Fig. 2). Plants outside of *J. sabina* were more likely to start flowering earlier than those facilitated and to finish the season earlier. The chance of flowering was higher in plants growing in the open for the first 2 weeks and lower at the end of the season than in facilitated plants (post hoc comparisons for each sampling date in

repeated measures analysis, $P = 0.04$, 0.06, and 0.03, respectively, after Benjamini & Hochberg (1995)'s correction for multiple comparisons). The mid-season peak flowering on the study year, however, occurred at about the same time in both conditions.

SIMULATIONS

Our simulated data sets produced Sp values similar to our field observations (truncated at 200 m for direct comparison; Table 1). Simulations that implement longer average dispersal distances (seed: 95.31 m, pollen: 10.40 m; *set 2*) yielded Sp estimates that were closer to our observed values than those using shorter dispersal distances (seed: 9.54 m, pollen: 1.40 m). Slight discrepancies in the actual Sp values might result from the use of inaccurate or incomplete parameters of the simulated model, but the similarity in relative differences between observed and simulated Sp values suggests that our models capture the main factors determining mating in the field. Incorporating the flower phenology mismatch observed in the field in the simulations (i.e. changing the mating probability among facilitated and non-facilitated individuals to account for these phenological differences) did not strongly affect the estimates of Sp (see *sets 3* and *4* in Table 1).

GENETIC DIFFERENTIATION BETWEEN FACILITATED
AND NON-FACILITATED PLANTS

We compared gene diversity between *E. nicaeensis* growing under *J. sabina* and in the open. Within-group diversity statistics were similar in the two groups. Considering *E. nicaeensis* in plots under *J. sabina* and in the open as separate 'sub-populations' ($N = 20$ for each group), we found no differences in gene diversity (mean Nei's Hj = 0.237 and 0.239, respectively) or band richness (mean Br = 1.60 and 1.61, respectively). The AMOVA analysis found that most of the molecular variance occurred within plots (about 96%). The remaining 4% of the variance happens mostly among plots ($\Phi_{PT} = 0.036$, $P < 0.001$), but not between facilitated and open plots ($\Phi_{RT} = -0.0001$, $P = 0.5$). In short, there is low

Fig. 1. Sp values ($\pm$95% confidence intervals) for three sets of *Euphorbia nicaeensis* individuals. 'Facilitated': within those growing under *Juniperus sabina*, 'Open': within those growing in the open, and 'Global' for all individuals irrespective of facilitation.
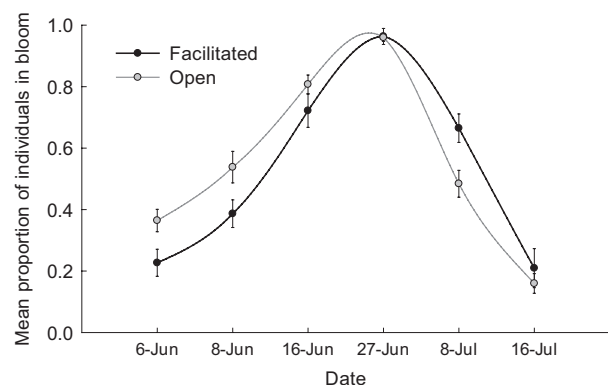

Fig. 2. Flowering phenology of *Euphorbia nicaeensis*. Curves show the mean ($\pm$SE) proportion of individuals in bloom in plots under nurses (facilitated) and in the open ($N = 19$ plots, respectively).

Table 1. *Sp* values (±SE) obtained from field data truncated at 200 m, and in simulated data sets generated assuming different dispersal distances, and with the observed phenological mismatch between facilitated and individuals growing in the open

	Facilitated	Open	Global
Observed	0.0043 (±0.0005)	0.0040 (±0.0006)	0.0040 (±0.0003)
Short dispersal (*set 1*)	0.0181 (±0.0006)	0.0164 (±0.0006)	0.0118 (±0.0003)
Short dispersal and observed phenology (*set 3*)	0.0175 (±0.0006)	0.0149 (±0.0006)	0.0114 (±0.0003)
Long dispersal (*set 2*)	0.0056 (±0.0002)	0.0048 (±0.0003)	0.0043 (±0.0002)
Long dispersal and observed phenology (*set 4*)	0.0058 (±0.0004)	0.0048 (±0.0004)	0.0044 (±0.0002)

but significant differentiation between *E. nicaeensis* plots, but the differences do not appear to be associated with being under *J. sabina* or in the open. In fact, not even the simulated high phenological mismatch between facilitated and non-facilitated plots implemented in simulation *sets 5* and *6* resulted in consistent local differentiation; for 12 days of phenological separation, mean $\Phi_{RT} = -0.0004$ (±0.0004 SE) and for 36 days of separation, mean $\Phi_{RT} = 0.0008$ (±0.0003 SE), for 20 simulations in each case. These low values of Φ_{RT} were not significantly different from zero in almost all cases.

Discussion

This within-population study of facilitation effects in *E. nicaeensis* tested two potential genetic consequences of facilitation previously unexplored in the field. First, we measured if the local spatial and demographic effects of facilitation could in turn generate fine-scale genetic structure and found this was not the case in our study population. Secondly, we detected small phenological differences mediated by the microenvironment created by nurses compared to the open ground around them. We know of no previous evidence of facilitation affecting the flowering phenology in beneficiary plants. However, the phenological mismatch did not translate into genetic separation between facilitated and non-facilitated individuals. Below we discuss these two types of effects, spatial and phenological changes, and their potential implications in systems where facilitation is important.

In the study population, *E. nicaeensis* plants are more abundant under nurse *J. sabina* shrubs than in the open (Verdú & Garcia-Fayos 2003). The spotted distribution of the nurses leads to a patchy distribution of *E. nicaeensis* and creates high aggregation of *E. nicaeensis* plants under nurses, so that distances among individuals are shorter than among those in the open ground. This is because most *E. nicaeensis* individuals live under nurses, but nurses cover only a quarter of the soil surface in this locality. However, none of these spatial effects condition the mating patterns and gene flow between facilitated and non-facilitated individuals. Facilitation could promote the spatial conditions that lead to a mating separation of facilitated individuals from non-facilitated congeners, but the effects in our study population, if present, occur at such short spatial scales, that they are likely overridden by gene flow.

Consistent with this, our simulations suggest that both seed and pollen dispersal in this population are higher than expected from informal field observations of floral visitors

(low visitation rates by syrphid and muscid flies) and seed dispersal (explosive, with secondary removal by ants). This is not surprising, as dispersal distances calculated from molecular markers, in particular for pollen, are frequently larger than those previously suspected (Godoy & Jordano 2001; Albadalejo *et al.* 2009). Our results suggest that *E. nicaeensis* dispersal values are similar to those in other insect-pollinated perennial herbs that grow to similar sizes (Hardy *et al.* 2004; Matter *et al.* 2013). Dispersal distances are thus larger than the average patch size, and this of course diminishes the chances for local differentiation in our study system, as was predicted by Liancourt *et al.* (2012) in simulations addressing this question. This is further confirmed by our results showing no indication of genetic differentiation between subpopulations of facilitated and non-facilitated plants in *E. nicaeensis*.

Microenvironmental conditions are known to vary drastically under nurses compared to the surroundings in many habitats where facilitation is common, and this is also the case in our study population. In *E. nicaeensis*, lower temperatures (by around 9 °C) and higher moisture under nurses are likely the cause for a delay in flowering onset compared to non-facilitated plants. This phenological differentiation was not high during the year of study, and simulations showed that it played a negligible role in preventing mating between facilitated and non-facilitated *E. nicaeensis*, likely because peak flowering occurred at about the same time in both groups. We expected that if the phenological mismatch were higher (for example in years with more extreme climate), it could presumably contribute to assortative mating. However, our simulations with high (12 days) and even unrealistic (36 days) phenological separation showed that in our system gene flow can prevent genetic differentiation under all circumstances. Still, temperature differences of a few degrees are known to determine flowering onset differences in climatic clines (e.g. Montesinos-Navarro *et al.* 2011), and strong phenological separation can even lead to reproductive isolation (Savolainen *et al.* 2006). The effects of phenological separation are still worth exploring in plants with a mixed mating system or with a strong tendency to self-pollinate. Other reproductive traits potentially affected by facilitation, such as a higher attraction of pollinators, could exacerbate assortative mating and give opportunity for differential selection between facilitated and non-facilitated plants.

In summary, although facilitation leads to strong spatial grouping of beneficiary plants and desynchronizes their flowering phenology, we found no local genetic effects of facilitation in our study system. Our results thus indirectly support

Liancourt *et al.* (2012) view that facilitation can act as a cohesive force, allowing plants to persist in environments outside of their normal ranges of tolerance without preventing gene flow with central populations. The possibility that facilitation could instead generate within-population differentiation is not sustained in our study system, yet this hypothesis is perhaps worth further exploration, for example in other facilitated plants with stronger dispersal limitations or low outcrossing, because facilitated individuals commonly coexist with non-facilitated ones in the same populations. Further field studies on the within-population effects of facilitation would add up to the recent surge of evidence on the evolutionary consequences of plant–plant interactions, including the preservation of traits over evolutionary time, the reduction of extinction probability in complex communities, and the increase of species phylogenetic diversity (reviewed in Thorpe *et al.* 2011 and Valiente-Banuet & Verdú 2013).

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Data accessibility

AFLP data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.81840 (Castellanos *et al.* 2014).

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Supporting Information

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

Figure S1. Size distribution of *Juniperus sabina* shrubs at the study area.

Figure S2. Field distances between *Euphorbia nicaeensis* plants and the nearest small *Juniperus sabina.*

Table S1. Skewness and kurtosis of the distributions of distances between *Euphorbia nicaeensis* plants and the nearest small *Juniperus sabina* shrubs in the field and simulated landscapes.

Table S2. AFLP primer information and scoring error rates.

Appendix S1. Methods of spatial test of shared microhabitat of *Euphorbia nicaeensis* and *Juniperus sabina.*

Appendix S2. AFLP laboratory protocol.