

Germination response of desert annuals to shrub facilitation is species specific but not ecotypic

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Abstract

Aims

Positive plant interactions can promote higher species density of beneficiary species in deserts. However, there is limited evidence examining the trait sets of seeds from beneficiary species. In this study, we examined the hypothesis that shrubs (benefactors) influence the germination of desert annuals (beneficiaries) and promote ecotypic differentiation by generating distinct microhabitats through abiotic stress amelioration. The following predictions were tested using growth chambers and field-collected seeds: (i) seed mass and viability will be greater and less variable for seeds collected from within shrub understory relative to seeds from open microhabitats, (ii) germination of seeds from shrub and open microhabitats will be greatest under simulated home (source) conditions, (iii) seeds from the shrub microhabitat will adaptively accelerate their germination rate when germinated in simulated home (source) microhabitats relative to their simulated away (reciprocal) microhabitat.

Methods

Seeds and their associated maternal plants were collected from four annual species found within a shrub understory (*Larrea tridentata*) and open microhabitats in the Mojave Desert of California (35.30°N, 117.26°W, 793 m. a.s.l.), and then reciprocally germinated in growth chambers simulating both microclimatic conditions.

Cumulative germination and germination rate was measured every 4–5 days for 42 days.

Important Findings

There was no significant difference in the mean or coefficient of variation for seed mass and viability between the shrub and open microhabitats. The source of the seeds did not significantly impact the cumulative germination, and there was no accelerated rate of germination within potential species ecotypes thereby suggesting no ecotypic differentiation. Cumulative germination was significantly higher within the shrub-simulated microhabitat for three out of the four species examined. Cumulative germination and germination rate were significantly different between species. Hence, shrubs influence the germination of desert annuals, and the outcome of this interaction is species specific, but shrub microhabitats do not necessarily alter the more conserved seed biology traits. Future studies should examine the influence of gene flow on beneficiary adaptation and the influence of species-specificity on beneficiary species response to facilitation.

Keywords: ecotypes, facilitation, germination, seeds, viability

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INTRODUCTION

Facilitation by benefactor species is well studied in community ecology (Brooker *et al.* 2008; McIntire and Fajardo 2014) and typically describes higher relative frequencies of plants and species richness within the proximity of a benefactor (He *et al.* 2013; McIntire and Fajardo 2014; Schöb and Pugnaire 2013). This spatial association is usually observed in relatively high-stress environments wherein benefactor species, such

as a dominant plant like a shrub, provide a more favourable environment for understory plant species through mechanisms such as shading, increasing soil moisture and buffering against extreme temperatures (Filazzola and Lortie 2014; Flores and Jurado 2003). These benefactors or ‘nurse-plants’ can also positively influence other biotic interactions by providing protection from herbivory (Barbosa *et al.* 2009; Smit *et al.* 2009) or increasing pollination (Molina-Montenegro *et al.* 2008; Reid and Lortie 2012). Consequently, benefactors

increase the heterogeneity of the landscape for other plant species and may influence long-term selection processes (Liancourt and Tielboerger 2011; Schöb *et al.* 2012; Valiente-Banuet and Verdu 2013; Verdu *et al.* 2009). Heterogeneity is increased as benefactors create discrete microhabitats with different environmental conditions relative to the surrounding 'open' microhabitats (Callaway 1995; Facelli and Temby 2002; Pescador *et al.* 2014; Walker *et al.* 2001). There is thus an opportunity for ecotypic differentiation to occur as individuals can respond to potential differences in conditions between the microhabitats. The potential ecotypes of the beneficiary species (canopy or open ecotype) can express different traits that are advantageous for their associated microhabitat. For example, Butterfield and Briggs (2011) showed trait differences among species more commonly found in shrub and open microhabitats and thus it is reasonable to expect that this variation could occur within a population of the same species. However, gene flow through seed dispersal and pollen movement can limit ecotypic differentiation. Although seed dispersal distances have been shown to be low in arid environments (Venable *et al.* 2008), and hence dispersal between microhabitats unlikely (Giladi *et al.* 2007, 2013), pollen movement can nonetheless influence the trait expression of plant species (Galloway 2001). There have been few studies examining the ability of shrubs to influence ecotypic differentiation (but see Liancourt and Tielboerger 2011; Sotomayor *et al.* 2014) but as local diversity responses at this scale are important and should be considered further.

Predominantly, plant facilitation studies focus on the relationship between a benefactor and the mature beneficiary plant species (Brooker *et al.* 2008) with limited emphasis on benefactor and beneficiary-seed interactions (but see Liancourt and Tielboerger 2011; Sotomayor *et al.* 2014). The seed to seedling life-stage has been proposed can be the most critical life-stage for annuals (Facelli *et al.* 2005; Lortie and Turkington 2002; Pake and Venable 1996), thus, it is important to consider for plant-plant interactions. Benefactors likely facilitate seed germination because seeds are highly receptive to environmental cues and will only germinate in favourable environmental conditions and soils (Armas *et al.* 2008; Donaldson-Matasci *et al.* 2013; Finch-Savage and Leubner-Metzger 2006; Hortal *et al.* 2013). Thus, it may be expected that seed traits including germination may show evidence of ecotypic differentiation between microhabitats. Seeds would be expected to show greater germination within their home microhabitats as the seeds have an adaptive history with those conditions. However, maternal effects likely also influence germination in a similar manner to ecotypic differentiation and this should be taken into consideration (Bischoff and Muller-Scharer 2010; Donohue 2009; Galloway and Etterson 2007). Benefactors have been shown to increase competition among understory species (Callaway and Walker 1997; Garcia-Cervigon *et al.* 2013) thus the germination rate among potential ecotypes is expected to differ with potential canopy ecotypes demonstrating adaptive-accelerated

germination in response to the perceived competitive environment (Donohue *et al.* 2010; Dyer *et al.* 2000). Seed quality can also be influenced by benefactors not only due to differences in selection pressures but also as it is a more favourable environment for plant growth thereby producing larger plants that subsequently produce higher quality seeds (Kos and Poschlod 2008; Moles *et al.* 2004). Benefactors can also directly impact canopy species by changing the microenvironment that the seeds experience and indirectly through maternal effects. This has important implications for beneficiary plant evolution as germination behaviour influences the selection pressures experienced by beneficiaries at later life stages (Donohue *et al.* 2010). Therefore, if benefactors influence the germination of seeds, directly or indirectly, they can promote ecotypic differentiation between populations in the open and populations within the benefactor understory. In theory, this can further lead to long-term divergent evolutionary trajectories in these species (Donohue *et al.* 2010) and has implications for the maintenance of diversity within stressed systems with important benefactor assembly effects.

To conduct an initial test for evidence of shrub-driven ecotypic differentiation in deserts, we replicated the experimental design of a recent study by Sotomayor *et al.* (2014). The authors found no effect of shrubs on germination or ecotypic differentiation for seeds collected from under shrubs and in the open. Herein, we utilized this protocol (a modified reciprocal common garden method) using four annual species from the Mojave Desert to examine whether shrubs in another desert ecosystem can influence the seed biology of beneficiary species and to determine if there is preliminary evidence for the ability of shrubs to promote ecotypic differentiation of seed traits. The current design represents an initial test for ecotypic differentiation as it does not separate potential parental effects on the germination response of the beneficiary species. We hypothesize that the microclimatic component of facilitation by shrubs leads to ecotypic differentiation among species of desert annuals. The following predictions were tested under controlled conditions: (i) seed mass and viability will be greater and less variable for seeds collected from within shrub canopies relative to seeds from open microhabitats, (ii) germination of seeds from shrub and open microhabitats will be greatest under home (source) conditions, (iii) seeds from the shrub microhabitat will express accelerated germination rates when germinated in simulated shrub (home) microhabitats relative to the simulated open (away) microhabitats.

METHODS

Study site

Surveys and collections of annual plants and their associated seeds were conducted at Kelso Dunes in the Mojave Desert, California (35.30°N, 117.26°W, 793 m.a.s.l.). The site is an arid sandy basin dominated by shrubs and winter annuals. Two common shrub species were *Larrea tridentata*

(DC.) Coville (Zygophyllaceae) and *Ambrosia dumosa* (A. Gray) Payne (Asteraceae). *Larrea tridentata* is an evergreen shrub that ranges from 1 to 3 m in height and has a sparse canopy with small waxy green leaves and small yellow flowers. Common annual plant species within the region include *Aliciella leptomeria*, *Camissonia campestris*, *Chaenactis fremontii*, *Cryptantha angustifolia*, *Eriophyllum lanosum*, *Eriophyllum wallacei*, *Eschscholzia minutiflora*, *Gilia latiflora*, *Malacothrix glabrata* and *Rafinesquia neomexicana*. The Mojave Desert receives between 34 and 310 mm of precipitation yearly (112.8 mm on average) with 60–90% occurring during the winter season (Urban *et al.* 2009). The mean annual temperature is 23.4°C with an average July maximum temperature of 42.1°C and an average January minimum temperature of 5.6°C (WRCC 2010). The growing and reproductive season of winter annuals is heavily dependent on the timing of fall/winter rains and it can be as short as 5 months (from December to May) or as long as 8 months (from September to May) (Beatley 1974). Germination of the annuals is triggered by rainfall, and vegetative growth typically occurs from late March to early April (Beatley 1974). California was in a severe drought from 2012 to 2013 (drought level D2; USDM 2013). The 2012–2013 growing season (October 2012–May 2013) saw only 30 mm of precipitation, which is well below the regional average (ASOS Needles, CA weather station).

Field plant survey and seed collection

The following four common annual plant species were selected within this system: *Chaenactis fremontii* A. Gray (Asteraceae), *Malacothrix glabrata* (A. Gray ex D.C. Eaton) A. Gray (Asteraceae), *Aliciella leptomeria* (A. Gray) J.M. Porter (Polemoniaceae) and *Eriophyllum wallacei* (A. Gray) A. Gray (Asteraceae). To assess whether these annual plant species were facilitated by *Larrea tridentata* in the field, the relative density of each annual species was measured using 0.25 m² quadrats. The quadrats were placed on the north side of 15 randomly selected *Larrea tridentata* shrubs and in adjacent, paired open microhabitats within 2 m of each shrub's drip line. The north side of shrub canopies was sampled as greater facilitative effects have been observed at this microhabitat for shrubs in North-American desert systems (Schafer *et al.* 2012).

Mature annual plants and their associated seeds were collected from within 50 randomly selected *Larrea tridentata* shrub canopies (north side) and adjacent open microhabitats in May 2013. A single plant and its associated seeds from each species (if present) were randomly collected from each microhabitat (hereafter referred to as its 'source' in statistical models). The height of each mature plant was measured and included as a covariate in statistical models. Seeds from each plant were weighed and included as a covariate in statistical models as seed mass has been commonly shown to influence germination (Leishman and Westoby 1994). The length (at longest axis), width (perpendicular to length) and height to tallest vegetation stem were measured on every *Larrea tridentata* canopy where seeds were collected ($n = 50$). Shrub

size was then calculated using the equation for volume of a hemisphere $\left(v = \frac{2}{3}\pi abc\right)$. The average shrub volume and canopy area (i.e. under and within dripline) where seeds were collected was 14.17 ± 1.61 m³ and 6.30 ± 0.55 m², respectively.

Study species seed description

Chaenactis fremontii seeds are black, 6–8 mm in length, and are club-shaped. The pappus is made up of four scales that are 6–8.5 mm (Morefield 2012). *Malacothrix glabrata* seeds are brown, 2–3 mm, and are fusiform in shape with a truncated tip. The pappus is made up of 1–5 bristles (Davis 2012). *Aliciella leptomeria* seeds are yellow-brown, 1–2 mm in length and have a narrow ellipsoid shape (Porter 2012). *Eriophyllum wallacei* seeds are black, 2–3 mm and narrowly club-shaped. The pappus is between 0.4 and 0.8 mm (Mooring and Johnson 2012). Many desert annual forb species show dormancy (Baskin *et al.* 1993; Harel *et al.* 2011; Pake and Venable 1996) so we expect our study species to show some degree of dormancy but to our knowledge the dormancy cycle of these species has not been demonstrated.

Seed viability assessment

Seed viability was assessed using 2, 3, 5-triphenyl-2H-tetrazolium chloride (TTC) tests (Baskin and Baskin 2001) for three of the four annual species (there were too few seeds of *Eriophyllum wallacei* available and seeds from this species were solely used for germination trials). We randomly selected 25 seeds from each species and source with four replicates (total of 100 seeds per species per source) for viability testing. Seeds were soaked in deionized water for 24 h, then dissected to expose the embryo, and placed in a 1% of TTC at 25°C for 24 h (Baskin and Baskin 2001). Seeds were scored as viable if their embryos stained pink or red (Baskin and Baskin 2001).

Germination trials

Germination trials were conducted in Sanyo MLR-351-H growth chambers simulating shrub and open conditions. Air temperature and humidity were recorded with four HOBO U-23 Pro-V2 probes, and light intensity and air temperature were recorded with four HOBO UA-002-64 probes. Each probe was placed at a different microhabitat (two *Larrea tridentata* shrubs and two adjacent open areas). The two shrubs selected were representative in size for the *Larrea tridentata* population at the site loggers were buried at each microhabitat with the sensor placed 3–5 cm above the soil surface but protected from direct irradiance. Microclimatic conditions were recorded from early January until mid-February 2012. The HOBO data was then averaged into 2-h intervals during daylight hours and 4-h intervals overnight to program the environmental conditions of the growth chamber (supplementary Table S1). A 2-h interval was used from the daylight hours as the variability in temperature and humidity was greater in comparison to overnight hours thus to effectively estimate a wide-range in variability we used shorter time intervals.

Six growth chambers were used with three simulating shrub conditions and three for open conditions using a full-factorial reciprocal design to test the following factors: simulated microhabitat (shrub or open), source (shrub or open) and species (the four annual species). Due to a limited number of seeds available per species, the number of replicates and seeds included in the germination trial varied per species (Table 1). Seeds (10 or 20 depending on the species, see Table 1) were placed in Petri dishes lined with moistened filter paper. Placement of Petri dishes within chambers was randomized. Seeds were watered every 2–3 days to saturation. Germination was recorded every 4–5 days for 42 days. Seeds were considered germinated when the radicle or coleoptile was visible (Finch-Savage et al. 2006) and were then removed from the Petri dish. To get an estimate of germination dynamics of each species we calculated the number of days to 50% germination (days_{50}), which was the number of days it took for half of the seeds in each replicate to germinate.

Statistical analyses

Differences in field plant density were compared using a generalized linear model (GLM) fit to a Poisson distribution with a log link function with species and source included as fixed effects and shrub size as a covariate. To further estimate the ecological significance of the shrub–plant interactions, the relative interaction index (RII; Armas et al. 2004) was calculated using the following equation: $\text{RII} = \frac{(s-o)}{(s+o)}$ where s is the plant density in the shrub and o is the plant density in the open. RII varies from +1 to –1 wherein positive values indicate facilitation and negative values suggest competition.

Differences in seed mass between species and sources as fixed factors were tested using a general linear model using JMP 10 (SAS Institute Inc. 2012). Pair-wise *post hoc* comparisons using Chi-square tests were performed when model effects were $P < 0.05$ (Littell et al. 2006; SAS Institute Inc. 2012). Shrub volume (shrub size) and maternal plant height were included in the analyses as covariates. Viability and cumulative germination differences were tested using GLMs fit to a quasi-binomial distribution and the logit link function in R (R Development Core Team 2015). For viability analyses, species and source were included as fixed factors and seed mass as a covariate. For cumulative germination, species, source and simulated microhabitat were

included as fixed factors with shrub size, maternal plant height and seed mass included as covariates. Pair-wise *post hoc* comparisons using Chi-square tests were performed when model effects were $P < 0.05$ using the *glht* function (*multcomp* package) in R. Differences between species, source and simulated-microhabitat for days_{50} with maternal plant height, seed mass and shrub size included as covariates were determined using a GLM fit to a negative binomial distribution using the log link function and the *glm.nb* function (MASS package) in R. A negative binomial distribution was used as the response variable is discrete but over-dispersed (i.e. variance exceeds the mean). If a covariate was significant from any of the above-mentioned models, linear regressions were performed to examine the relationship between the response variable and covariate. To test for potential scaled variability between sources, the coefficient of variation (CV) was calculated for seed mass and viability and compared using *t*-tests with species as replicates.

RESULTS

Subordinate plant species association patterns

Chaenactis fremontii plant density was greatest in the shrub understory, plant density for *Aliciella leptomeria* density was greatest in the open and *Malacothrix glabrata* and *Eriophyllum wallacei* densities did not differ by microhabitat (Table 2; Fig. 1). The size of the shrub did not significantly influence this model or any other model wherein shrub size was included as a covariate.

Seed trait variation contrasts

Seed viability and seed mass varied significantly among plant species but not by seed source (Table 2). Seed mass did not influence seed viability (Table 2). Within species, larger maternal plants produced significantly heavier seeds (Table 2; Fig. 2). There was no significant difference between the Coefficient of Variation for viability or seed mass among seed collected from both microhabitats (viability: $t = 0.57$, $P = 0.5963$; seed mass: $t = -0.02$, $P = 0.9828$).

Germination responses to simulated microhabitats

Cumulative germination differed among species, between seed sources and by simulated microhabitats. *Chaenactis*

Table 1: experimental design for the growth chamber germination trials including the number of seeds, shrub-open pairs and replications used by species and source microhabitat

Source	<i>Chaenactis fremontii</i>		<i>Malacothrix glabrata</i>		<i>Aliciella leptomeria</i>		<i>Eriophyllum wallacei</i>	
	Shrub	Open	Shrub	Open	Shrub	Open	Shrub	Open
Number of seeds	20	20	20	20	20	20	10	20
Shrub-open pairs	10	10	10	10	7	9	3	10
Replications	4	4	4	4	4	4	5	4

The number of seeds is the number of seeds included in each experimental unit. The number of shrub-open pairs is the number of paired microhabitats where seeds were collected.

Table 2: results of the general linear model and generalized linear models testing for differences in plant density, seed mass and seed viability among species and seed source (shrub or open)

Effect	DF	Density		Seed mass		Seed viability	
		Chi-square	P value	Chi-square	P value	Chi-square	P value
Species	3	57.41	<0.0001	523.1	<0.0001	4.2801	0.0001
Source	1	2.562	0.1094	0.0358	0.8499	0.5123	0.1437
Shrub size	1	1.959	0.1616	0.7874	0.3749		
Seed mass	1					0.1804	0.3857
Parent height	1			7.587	0.0059		
Species*source	3	57.924	<0.0001	2.483	0.4784	0.1242	0.7717

Shrub size, parental plant height and seed mass were included as covariates in some of the models. Significance at $\alpha < 0.05$ is denoted by bolded values.

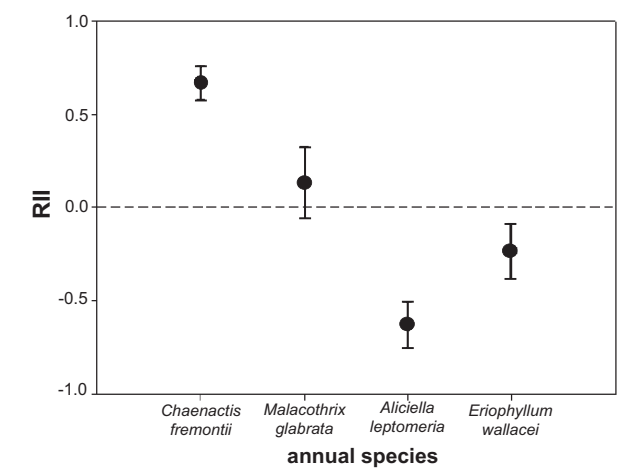


Figure 1: relative interaction indices (RII) for the plant densities of four annual desert species from the Mojave Desert. Values shown are means \pm SE. Plant density was recorded in shrub and open microhabitats in the field.

fremontii (shrub-associated plant in field), *Malacothrix glabrata* (neutral shrub association) and *Eriophyllum wallacei* (neutral shrub association) showed higher cumulative germination in the simulated shrub microhabitat. Conversely, cumulative germination was higher in the open for *Aliciella leptomeria* (open-associated species) (Table 3; Fig. 3). Consequently, the open and shrub-associated species maintained a consistent signal in germination matching to microenvironment whilst the neutral species preferred shrub microenvironment. Maternal plant height significantly influenced cumulative germination (Table 3) as germination declined with increasing mature plant height (Fig. 4), however, this effect was due to differences in height among species. Although there was a significant interaction detected between species and seed source this was driven only by *Eriophyllum wallacei* with germination occurring significantly higher for seeds from the shrub (Chi-square = 3.90, DF = 1, $P = 0.048$). There were no other significant source interactions detected.

The annual plant species and simulated microhabitat had a significant effect on germination rate estimated by days₅₀.

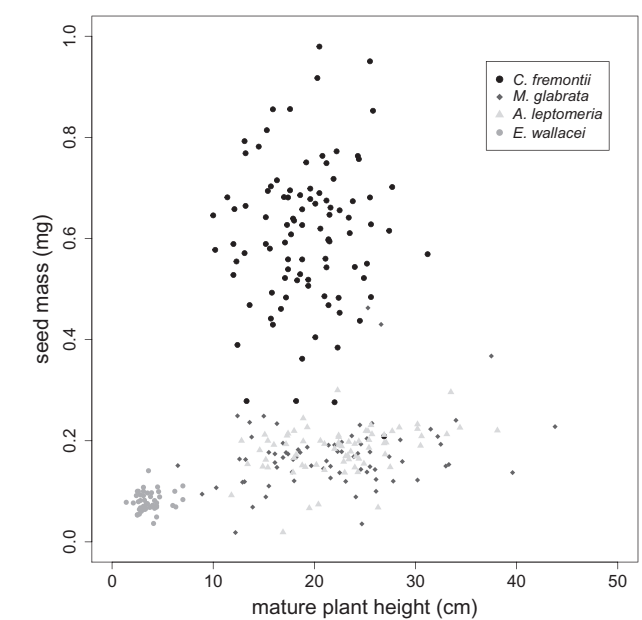


Figure 2 : the relationship between maternal plant height and seed mass for desert annuals collected in the Mojave Desert (linear regression $R^2_{adj} = 0.0557$, $P < 0.001$).

However, the effect of the simulated microhabitat on germination rate was driven by *Chaenactis fremontii* and this was the only species that germinated significantly earlier in the shrub-simulated microhabitat (Table 3; Fig. 5). None of the interactions tested in the model were significant and no significant source effects were detected.

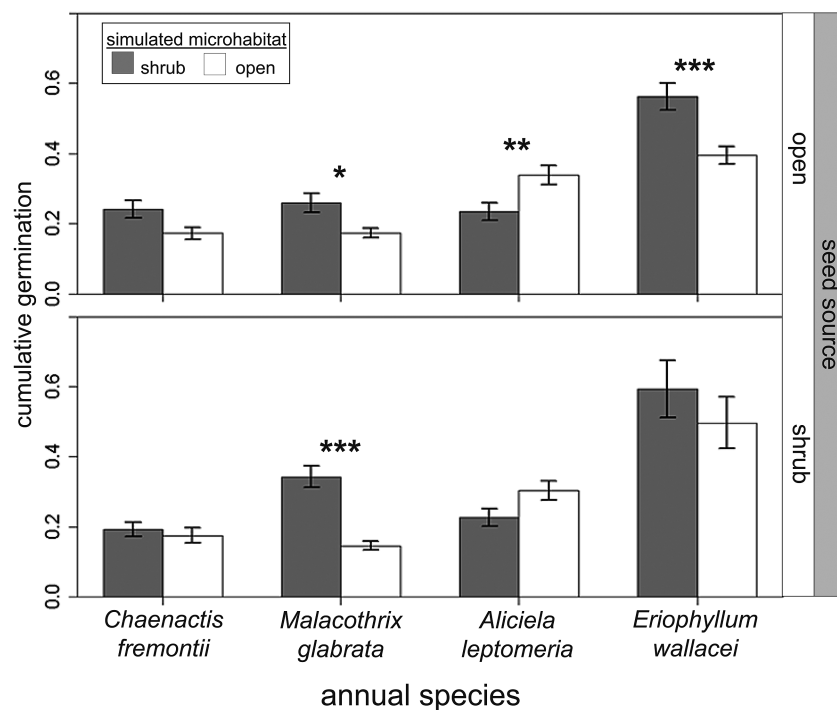
DISCUSSION

Plant facilitation is a dominant and frequently reported process in shrub-annual deserts. This study examined whether there was preliminary evidence that these shrub facilitation effects also influence the seed traits of beneficiary species. We predicted that micro-environmental facilitation by shrubs in deserts could lead to ecotypic differentiation in beneficiary species. In this desert ecosystem, there was no evidence for

Table 3: results of GLMs testing for differences in germination and days₅₀ among species, seed source (shrub or open) and simulated microhabitat (shrub or open chamber conditions)

Effect	DF	Germination		Days ₅₀	
		Chi-square	P value	Chi-square	P value
Species	3	32.06	<0.0001	300.0	<0.0001
Source	1	0.050	0.5440	2.212	0.1369
Simulated microhabitat	1	2.676	<0.0001	15.24	<0.0001
Species*source	3	1.209	0.0305	2.856	0.4143
Species*simulated microhabitat	3	6.815	<0.0001	4.426	0.2190
Source*simulated microhabitat	1	0.020	0.7008	0.208	0.6484
Species*source*simulated microhabitat	3	0.907	0.0827	1.586	0.6625
Seed mass	1	0.003	0.8742	0.0560	0.8134
Shrub size	1	0.1310	0.3256	1.005	0.3162
Parent height	1	1.286	0.0021	0.058	0.8104

Seed mass, shrub size and parental height were included as covariates. Significance at $\alpha < 0.05$ is denoted by bolded values.

**Figure 3:** the cumulative germination for seed source and simulated microsite for four desert species from the Mojave Desert. Vales shown are means \pm SE. Significance at $\alpha < 0.05$ denoted by *** \leq 0.001, ** \leq 0.01, * \leq 0.05.

ecotypic differentiation for the seed traits or germination patterns for four desert annuals that occur under shrubs and in the open. There was, however, clear evidence for species-specific responses to facilitation and for the capacity of shrubs to ameliorate conditions for species that are distributed evenly between shrub and open microhabitats in the field. This positive effect on germination was species-specific as three out of the four species displayed significantly higher germination within the shrub-simulated microhabitat. The species more frequently associated with open microhabitats, *Aliciella*

leptomeria, had higher germination in open-simulated conditions. The same pattern was detected for one of the three species whose germination was facilitated (*Chaenactis fremontii*). Although the other two species experienced higher germination in the shrub, in the field, the frequency of mature plants was equivalent between shrub and open microhabitats. This may be related to ontogenetic shifts in facilitative interactions between shrubs and some species of annuals (Miriti 2006; Soliveres et al. 2010) which may occur due to changes in functional traits for both shrubs and annuals

(Zhang and Zhao 2015). These results suggest species-specific micro-environmental matching or filtering in the field through a combination of stress amelioration from shrub

facilitation and changes in competitive interactions between annuals at different life stages (Schiffers and Tielborger 2006; Soliveres *et al.* 2010; Soliveres *et al.* 2011). Seed mass and viability did not differ between microhabitats likely because seed traits are typically highly conserved and genetically regulated (Moles *et al.* 2005; Pake and Venable 1996; Qi *et al.* 2014). Thus, shrub facilitation of beneficiary species may begin as early as the germination life-stage, but shrubs do not promote ecotypic differentiation.

We predicted that shrub micro-environmental facilitation could promote the development of higher quality seeds for individual plants growing within the shrub understory. However, we did not find evidence for seed trait variation between shrub and open microhabitats. Seeds collected from larger plants at maturity had greater mass, but had lower cumulative germination, however, this trend was due to differences in species heights and not due to maternal effects. Seed mass and viability are strongly influenced by regulatory factors to stabilize seed size for dispersal and is a commonly conserved plant trait (Moles *et al.* 2005). Seed size has also been shown to influence dormancy with larger seeds exhibiting lower dormancy (Pake and Venable 1996). Thus, as seed mass and viability are highly conserved traits constrained by strong regulatory processes it is not likely that these traits will be influenced by climatic factors (Qi *et al.* 2014). This provides a viable explanation for the lack of difference in seed mass between microhabitats. These strong

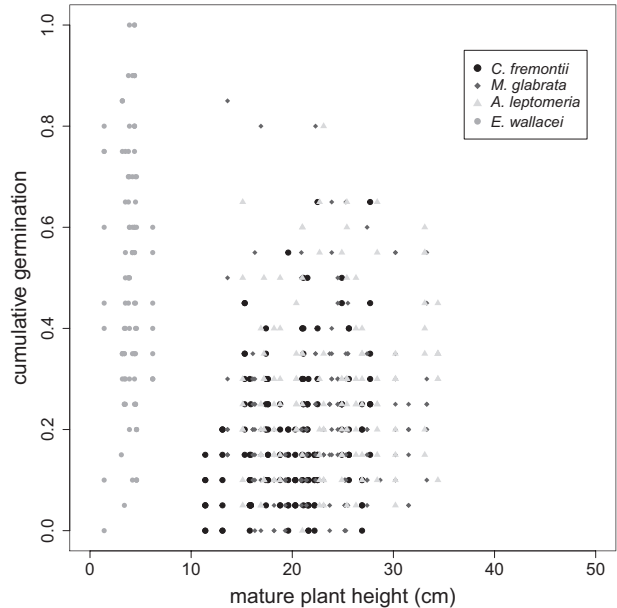


Figure 4: relationship between mature plant height and cumulative germination for four desert annuals from the Mojave Desert ($R^2_{adj} = 0.118$, $P < 0.001$).

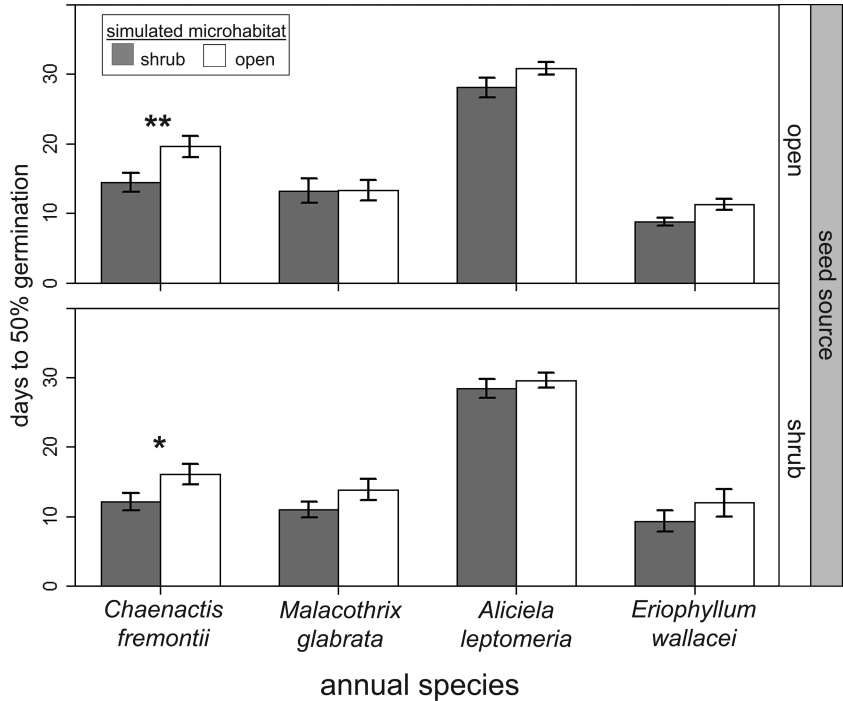


Figure 5: the $days_{50}$ for seed source and simulated microsite for four desert species from the Mojave Desert. Vales shown are means \pm SE. Significance at $\alpha < 0.05$ denoted by $*** \leq 0.001$, $** \leq 0.01$, $* \leq 0.05$.

regulatory factors reduce the likelihood in detecting differences in seed mass within a species between shrub and open microhabitats.

Facilitation by shrubs did not influence ecotypic differentiation within species of desert annuals at the germination life-stage. Gene flow between microhabitats may reduce the chance of detecting ecotypic differentiation in annuals between shrub and open microhabitats. Although seed dispersal distances among desert annuals are low, dispersal between microhabitats may still occur (Venable *et al.* 2008; Volis *et al.* 2010). Additionally, pollen transfer distances likely exceed seed dispersal and paternal effects can influence offspring traits including seed mass and germination (Galloway 2001). Previous studies have shown that offspring perform better when they occur in the same environmental conditions as the maternal plant conditions (Galloway 2005; Galloway and Etterson 2007; Herman and Sultan 2011). However, high gene flow between microhabitats can result in offspring that are not well adapted to these environmental conditions as selective pressures would vary among generations. This could be tested in the future by controlling shrub and open sourced pollen and ovules and reciprocally crossing these gametes in a full factorial design and germinating the offspring in both home (source) and away conditions. If high gene flow between microhabitats is producing suboptimal adaptations, performance will be lower in 'away' conditions relative to 'home' conditions. Ecotypic differentiation driven by positive interactions is an important opportunity to further plant facilitation and this protocol can be modified and applied more broadly to test for the influence of gene flow, and adaptation.

Shrubs mediate both above and below ground effects to determine seed germination and plant establishment, however, our experiment did not test *in situ* these soil effects and focused instead on the above-ground facilitation effects of shrubs on annual plants (Duponnois *et al.* 2011; Li *et al.* 2010; Valles *et al.* 2011). Although the microclimatic conditions included in this study influence seed germination and plant establishment, soil modification by shrubs also contributes to this facilitative effect (Armas and Pugnaire 2005; Armas *et al.* 2008; Walker *et al.* 2001). Shrubs increase soil moisture, nutrients and microbes in comparison to open areas and these soil modifications contribute to the facilitative effect of shrubs on understory plants (Holzapfel and Mahall 1999; Hortal *et al.* 2013; Wang *et al.* 2011). Increased soil moisture within the shrub has been shown to promote germination and establishment of seedlings (Bonanomi *et al.* 2008; Clauss and Venable 2000; Kos and Poschlod 2008). Nutrient content is also higher due to greater litter accumulation within shrubs resulting in fertile islands that increase the survival of seedlings (Abraham *et al.* 2009; Rao and Allen 2010; Schafer *et al.* 2012). Microbe biomass and community compositions improve the establishment and survival of beneficiary species within shrub microhabitats (Gange *et al.* 1993; Hortal *et al.* 2015; Rodriguez-Echeverria *et al.* 2013). Thus, both above

and below-ground influences on facilitative dynamics contribute to the seed biology of desert annuals.

The response of seeds from annual plant species to shrub facilitation was species specific and likely life-stage specific with three out of the four species under study showing higher germination in shrub-simulated conditions. These findings are similar to previous studies that have shown the response of beneficiary species to benefactors to be species-specific and that positive effects were most pronounced at early life-stages (Catorci *et al.* 2011; Landero and Valiente-Banuet 2010; Lortie and Turkington 2008). This species specificity can be due to different functional traits of both the benefactor and beneficiary species that can influence the sign of this plant-plant interaction (Schöb *et al.* 2012; Schöb *et al.* 2014). For example, species that require a low light environment may be facilitated by a benefactor with a dense canopy, however, light demanding species may not benefit from this association (Zhang and Zhao 2015). Beneficiaries are predicted to experience a positive effect of associating with a benefactor only when they are outside of their optimal environmental conditions and thus experiencing stress (Gross *et al.* 2010). Therefore, species-specific differences in facilitation could result from differences in environmental requirements among beneficiary species. Additionally, these environmental requirements and the conditions experienced by beneficiary species can change throughout their lifespan and facilitative effects are life-stage dependent (Armas and Pugnaire 2009; Miriti 2006; Schiffrers and Tielborger 2006; Soliveres *et al.* 2010). This ontogenetic shift in facilitative interactions explains why only one of the three study species benefited from the shrub effect at the mature plant life-stage while the other species had a neutral shrub association. These two species could have functional traits at later life-stages that are disadvantageous within the shrub microhabitat. A similar study conducted by Sotomayor *et al.* (2014) found all species including two species facilitated at the mature plant life-stage germinated preferentially in open microhabitats. This contrasts to our results which showed higher germination under the shrub for most species. A possible explanation is that ontogenetic shifts in net interactions from competitive effects at early life-stages to positive effects at later life stages (Miriti 2006) and the converse for this California desert. The life-stage and species specificity is an important opportunity to classify annual plants based on their relative 'facilitation potential' with respect to shrubs using this protocol.

CONCLUSIONS

There was no evidence for seed or seedling ecotypes in desert annuals between shrub and open microhabitats. However, facilitative interactions were nonetheless highly species-specific and suggest that this specificity can begin at the earliest life stages for beneficiary species. There was also clear evidence of microhabitat matching by seeds with strong association patterns of the plants in the field. This study has important implications

for ecological management as shrubs are increasingly being tested as tools to help re-establish native plants in degraded ecosystems. Managers need to consider both the association patterns of the extant plants within a region and the sowing microhabitat for seeds in their restoration planning. Species-specific interactions at all life-stages with shrubs should also be identified preintervention in desert ecosystems in particular.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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