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Corr. Author : Andrea C. Premoli

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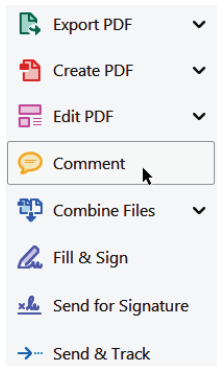
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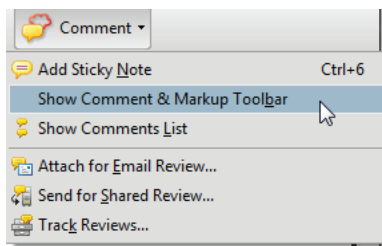


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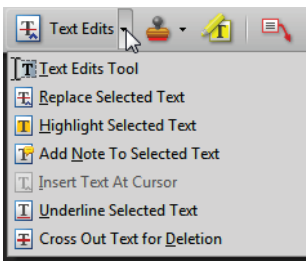


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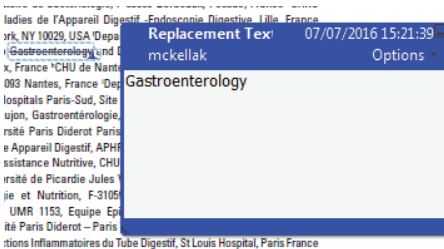
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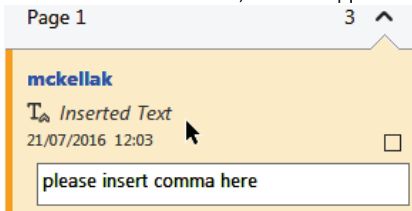


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Original Article

Population Genetic Structure of the Giant Cactus *Echinopsis terscheckii* in Northwestern Argentina Is Shaped by Patterns of Vegetation Cover

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Abstract

1.20 Species inhabiting drylands commonly depend on the surrounding vegetation for recruitment under stress, while competition may affect populations in moister environments. Our objective was to analyze how different climates and vegetation affect the fine-scale spatial genetic structure (SGS) of the columnar cactus *Echinopsis terscheckii*. At 4 sites, we estimated vegetation cover by digitized patches and the normalized difference vegetation index (NDVI). We mapped 30 individuals per population and collected tissue for isozyme electrophoresis using 15 putative loci. Spatial autocorrelation between all possible genotype pairs and the number of genetically homogeneous groups and families were calculated for each population. Greater cover (66%) and average NDVI values were detected in the most humid habitat that consisted of fewer, larger, and more dispersed vegetation patches. All populations were genetically diverse and showed significant SGS. Positive correlations were found between the distance at which maximum autocorrelation and kinship values were reached and vegetation area and patch size. Also higher NDVI values were associated with lower number of patches. Populations exposed to higher precipitation and vegetation cover consisted of sparse individuals that clustered at larger distances whereas vegetation patches in arid climates produced groups of closely related genotypes at small distances. These results support the stress-gradient genetic hypothesis. Under water stress, facilitation promotes establishment underneath patchy vegetation resulting in fine-scale family structure. In moister xerophilous forests, competition for resources, that is, light, results in sparse individuals and thus coarse-scale neighborhoods. This information can guide conservation and/or restoration efforts, such as the spatial scale to be considered in germplasm collection.

1.40 **Subject areas:** Reproductive strategies and kinship analysis; Population structure and phylogeography

Keywords: arid and semiarid environments, digitized vegetation patches, *Echinopsis terscheckii*, fine-scale genetic structure, isozymes, NDVI, spatial and family genetic structure, stress-gradient hypothesis, stress-gradient genetic hypothesis

1.45 Arid and semiarid environments impose severe restrictions upon plant establishment and survival. Unpredictable rainfall, high predation rates, and dry soils reaching high temperatures hinder seedling establishment in drylands (Peters et al. 2008). Thus, the need for safe sites for germination and early growth as well as the impact of herbivores may greatly reduce chances for the recruitment of new

	individuals within populations. A foundational study on the saguaro (<i>Carnegiea gigantea</i>), the giant cactus of the Sonoran Desert of southern United States and northern Mexico, showed that the very small proportion of seedlings that survived extreme environmental conditions and that escaped from herbivory, did so in sheltered places such as rocky surfaces or underneath other plants, that is, nurse plants (Niering 1963). The nurse-plant syndrome is a facilitation phenomenon by which plants are established under other species that may aid to buffer extreme environmental conditions, for example, reducing evapotranspiration and temperature variations, and prevent predation (Valiente-Banuet et al. 1991; Godínez-Alvarez et al. 2003; Holland and Molina Freaner 2012). In deserts, nurse-plant associations commonly occur in many groups of species including cacti, yet some globose cacti establish in association with rocky substrates other than nurse plants (Peters et al. 2008). Also rocky cavities may favor seed survival in the columnar cactus <i>Pilosocereus leucocephalus</i> , by protecting them from predators (Munguia-Rosas and Sosa 2008). Although some cacti display either uniform or random distributions, aggregated arrays are by far the most common (Godínez-Alvarez et al. 2003).	
2.5		also been demonstrated that the spatial scale of seedling establishment affects SGS. Forest stands undergoing gap-phase regeneration due to tree falls produce smaller genetic neighborhoods than those resulting from large-scale stand-devastating disturbances such as fire (Premoli and Kitzberger 2005). However, knowledge of SGS of species inhabiting drylands is scarce. One study of the columnar cactus <i>Pilosocereus tillianus</i> associated the spatial clustering of closely-related genotypes at distances <26 m and maximum coefficient of relatedness <0.05 with the local foraging behavior of pollinators (Figueredo et al. 2010).
2.10		We examined the fine-scale SGS of the columnar cactus <i>E. terscheckii</i> . We performed a spatially explicit sampling design along transects at 4 locations with variable climates and vegetation types to analyze if recruitment relates to vegetation cover. We tested the hypothesis that increased aridity, that is, greater proportion of bare soil, results in small-grained clusters of related genotypes underneath nurse plants. In contrast, areas with relatively higher resource availability, that is, yet under greater competition, consist of sparse individuals within a matrix of high vegetation cover that foster the formation of larger and genetically homogeneous neighborhoods. We ran spatial autocorrelation analyses by means of 15 isozyme loci and used satellite imagery to estimate vegetation cover. The study was conducted in arid transitional ecosystems where the giant <i>E. terscheckii</i> is the most conspicuous cactus species, that is, ecologically dominant, and can be considered a keystone species. This cactus is affected by agricultural activities in lowland areas and its wood is harvested for construction materials and crafts. Its conservation status is “Vulnerable” by IUCN, which has estimated a 30% decrease of <i>E. terscheckii</i> populations in the last 30 years due to habitat loss and exploitation (Ortega-Baes and Lowry 2013).
2.15		
2.20	Several studies have provided evidence for the positive effects of nurse vegetation on plant establishment (Callaway 1997), particularly in cacti (Franco and Nobel 1989; Valiente-Banuet and Ezcurra 1991; Mandujano et al. 2002; Godínez-Alvarez et al. 2003; Peters et al. 2008). Some of those studies followed experimental designs which may be difficult to perform in drylands due to various limitations on seed production and climate fluctuations that may greatly impact establishment. Therefore, the role of nurse plants in drylands can be inferred from correlational studies that relate the co-occurrence of adult individuals of a given species with other plants (e.g., de Viana 1996). In particular, remotely sensed images can be used to estimate positive plant co-occurrence, that is, facilitation, better than expected by chance (Xu et al. 2015). Also high spatial resolution remotely sensed imagery is widely used in automatic individual tree-crown recognition for use in forest inventories (Zhen et al. 2016). However, technical limitations exist in the delineation of some species such as columnar cacti due to the fact that their reduced basal area and lack of crown does not allow detection by the commonly used sensors.	
2.25		
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2.40	In arid and semiarid environments, harsh conditions may restrict plant survival and growth in the interplant spaces, favoring recruitment underneath the vegetation cover. However, competition for limited resources, that is, light, may also affect plant distribution particularly in more benign environmental settings. Hence, gradients in abiotic/biotic stress may affect species’ interactions in communities a concept known as the “stress-gradient hypothesis” (SGH, Bertness and Callaway 1994). According to the SGH, facilitation in communities increases and competition decreases with increasing stress. While numerous studies have focused on testing the SGH, the effects of abiotic and biotic stress on the genetic structure of natural populations has not been investigated. The influence of both positive and negative species’ interactions along resource gradients, in combination with restricted gene flow by means of pollen and seed dispersal, may produce the spatial aggregation of individuals and thus closely related genotypes (significant spatial genetic structure, SGS) in the vicinity of mother plants, that is, family genetic structure.	
2.45		
2.50		
2.55	The distribution of genotypes in space has been studied in many different plant species, and shows, in most cases, that a strong association exists between spatial genetic structuring and limited seed dispersal (Linhart and Grant 1996) particularly in small-seeded species like <i>Echinopsis terscheckii</i> (e.g., Jacquemyn et al. 2007). It has	
		Methods
		Study Species
		<i>Echinopsis terscheckii</i> (Britton and Rose) H. Friedrich and G.-D. Rowley is a columnar cactus endemic to northwestern Argentina (Kiesling 1978; Hunt 2006) where it occurs in distinct habitats (Figure 1). It is distributed within the provinces of Jujuy, Salta, Tucumán, Catamarca, La Rioja, and San Juan at the western limit of the Chaco and Monte phytogeographic provinces (Cabrera 1976). It is found between 700 and 2200 m a.s.l. with high-elevation populations located toward the northern part of the range. It inhabits dry mountain slopes mixed with xerophytic vegetation of Central Andean Puna and in the lowlands at the ecotone with transitional forests such as the dry Chaco, High Monte, and Yungas (Brown and Pacheco 2006), where 80% of precipitation falls in summer from November to February. Flowering occurs at various peaks from late spring to summer, although it may vary throughout the cactus’ distribution. Flowers have nocturnal anthesis, with an extended floral cycle until next morning. The reproductive system is self-incompatible and floral visitors are mainly nocturnal moths, and bees (Ortega-Baes et al. 2010). Fleshy fruits are dehiscent and contain numerous seeds which are small with an average mass of 0.0172 ± 0.0007 g (Ortega-Baes et al. 2010). Seed dispersal is carried out by frugivorous birds (Pablo Ortega-Baes, Universidad Nacional de Salta, Argentina, field observation). <i>Echinopsis terscheckii</i> is diploid and chromosome counts yielded $2n = 22$ (Diers 2008). The study was conducted at 4 locations under distinct environmental settings and vegetation types. Two northern populations occurred at relatively higher elevations while 2 southern ones occupy lowland areas which in turn followed

a precipitation gradient with a decrease toward the south (Table 1; see Supplementary Figure S1 for description of study sites).

Vegetation Cover Analysis

3.5 Vegetation cover was estimated within transects at each site by 2
 3.10 metrics: digitized vegetation patches and the normalized difference
 3.15 vegetation index (NDVI) which are explained below. At each site
 3.20 and within an area of 25 m × 600 m we digitized vector data consisting
 of vegetated patches directly in Google Earth Image@2016 Digital
 Globe with a submeter resolution. Patches were constructed as polygons
 and we determined the percent coverage, the number of patches, the average
 patch size, and the average distance between all possible patch pairs from
 the centroid of each patch using the Quantum GIS program 2.14. NDVI is an
 indicator that describes the greenness, the relative density, and health of
 vegetation for each picture element, or pixel, in a satellite image. This index
 takes advantage of the relatively unique spectral reflectance characteristics
 of green vegetation, calculated from the visible and near-infrared light
 reflected by vegetation. While chlorophyll absorbs visible light (from 0.4
 to 0.7 μm) for use in photosynthesis, the cell structure of plant leaves,
 on the other hand, reflects near-infrared light (from 0.7 to 1.1 μm). Thus,
 data in visible and near-infrared light spectrum

can be used to determine differences in plant reflectance and their spatial
 distribution in satellite images (Peñuelas and Filella 1998). Calculated
 values of NDVI for a given pixel range from +1.0 to -1.0: areas of barren
 rock, sand, or snow usually show very low NDVI values (e.g., 0.1 or less);
 sparse vegetation such as shrubs and grasslands may result in moderate
 NDVI values (~0.2–0.5); high NDVI values (~0.6–0.9) correspond to
 dense vegetation such as that found in temperate and tropical forests.
 However, the calculation of NDVI values is sensitive to a number of
 perturbing factors that affect the measurements made in space leading to
 misinterpretations. These include atmospheric effects (i.e., composition
 of the atmosphere in terms of water vapor and aerosols); presence of
 clouds (particularly thin and small clouds as well as cloud shadows can
 significantly contaminate the measurements); and soil effects (they tend
 to darken when wet, so that their reflectance is a direct function of water
 content). In order to minimize sources of errors in NDVI estimations,
 we used available satellite images taken during the dry season of the
 study area from 2 dates (2013 and 2014) which resulted in an average
 composite measure. These consisted of images collected during 2013 on
 3 July for sites LH, LC, and PA and 15 November for VF population; and
 during 2014 on 22 July for all sites. For each site, we downloaded
 LANDSAT-8 satellite images from:

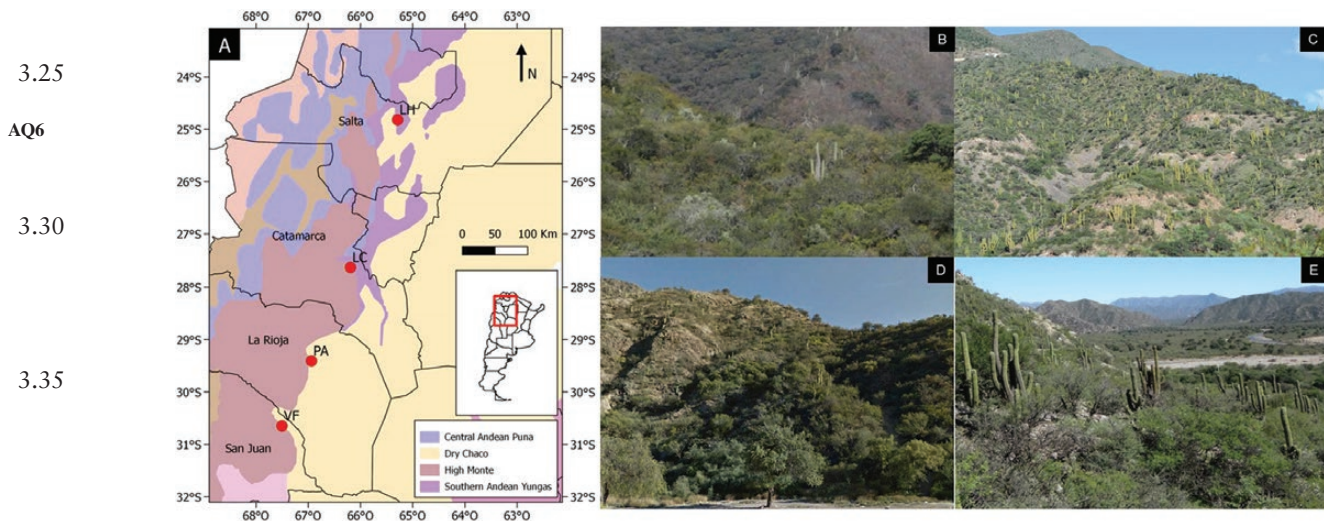


Figure 1. (A) Map showing the location of sampling sites and the ecoregions inhabited by *Echinopsis terscheckii* along its range in Argentina depicted in gray scale (see online version for full colors), and panoramic photographs taken at each site: (B) Las Higuierillas, Salta Province; (C) Oda, La Chilca, Catamarca Province; (D) Padercitas, La Rioja Province; and (E) Valle Fértil, San Juan Province.

Table 1. Description of environmental conditions for sampling sites of *Echinopsis terscheckii* from northwestern Argentina

Site, Province	Coordinates	Phytogeographic region ^a	Altitude (m a.s.l.)	Climate	Precipitation (mm/yr)	Mean annual temperature (°C)	Tmax (°C)	Tmin (°C)	Vegetation type ^a
LH-Las Higuierillas, Salta	24° 49'S 65° 17'W	Chaco Province in ecotone with Yungas	1204	Temperate humid	689	16.9	24.4	9.5	Xerophilous deciduous forest
LC-Qda La Chilca, Catamarca	27° 38'S 66° 11'W	Monte Province in transition with Prepuna	1053	Arid	326	18.4	26.2	10.8	Open xerophilous shrubland
PA-Padercitas, La Rioja	29° 24'S 66° 56'W	Monte Province in transition with Chaco	790	Continental semiarid	330	20.0	27.4	12.6	<i>Larrea</i> desert
VF-Valle Fértil, San Juan	30° 38'S 67° 30'W	Monte Province in transition with Chaco	873	Desert	243	17.9	25.4	10.5	Predominance of xeric plants

^aPhytogeographic regions after Cabrera (1971).

<https://libra.developmentseed.org/> with a spatial resolution of 30 m. The NDVI data for each site were calculated using the program Quantum GIS 2.14 and consisted of average values sampled at 30 points along 30 m × 900 m stripes equivalent to the largest transect length at site LH. The NDVI values measured at each population and year were compared using nonparametric Kruskal–Wallis test with STATISTICA v.7.0 (StarSoft 2004).

Genetic Analysis

Samples for genetic analysis were collected in December 2013 (LH) and April 2014 (other sites). At each location, we sampled 30 individuals following a spatially explicit sampling design across 400–900 m long transects. The position of each sampled individual along each transect was mapped to an estimated accuracy of 1 m (Supplementary Figure S2). Particularly for the LH site ($N = 28$), only 21 individuals were considered for the analyses; the other 7 individuals were excluded since the distances among them exceeded 900 m. As a result, LH site had a smaller number of paired individuals per distance class, and thus consisted of sparse individuals (Supplementary Figure S3). From each individual, we collected fresh tissue of the main trunk or secondary branches. Samples consisted of a cube of approximately 5 cm side, which was labeled and stored in a portable cooler for posterior analysis at Laboratorio Ecotono, Universidad Nacional del Comahue, Bariloche, Argentina. Once in the laboratory, proteins were extracted by crushing with liquid nitrogen 2 g of fresh parenchymatic tissue (after mechanical removal of the cuticle), using mortar and pestle and adding 1.5 mL of grinding buffer (Mitton et al. 1979). Protein homogenates were stored at $-70\text{ }^{\circ}\text{C}$, both as soaked wicks of Whatman No. 3 chromatography paper and in 0.5 mL Eppendorf tubes as backups. Fifteen isozyme loci were resolved using a combination of 3 buffer and 9 enzyme systems: aldolase (*Ald*; EC 4.1.2.13), glycerate-2-dehydrogenase (*G2d*; EC 1.1.1.29), isocitrate dehydrogenase (*Idb*; EC 1.1.1.42), and malate dehydrogenase (*Mdh-1*, *Mdh-2*, *Mdh-3*; EC 1.1.1.37) in the histidine system by King and Dancik (1983); menadione reductase (*Mnr-1*, *Mnr-2*; EC 1.6.99.2), peroxidase (*Per*; EC 1.11.1.7), phosphoglucosmutase (*Pgm-1*, *Pgm-2*; EC 5.4.2.2), and shikimate dehydrogenase (*Skdh*; EC 1.1.1.25) in the morpholine-citrate system (Ranker et al. 1989); and phosphoglucosomerase (*Pgi-1*, *Pgi-2*, *Pgi-3*; EC 5.3.1.9) in the B system by Conkle et al. (1982). Electrophoresis was performed for 6 h using 12% w/v starch gels (Starch Art, Starch Art Corporation, Smithville, TX). Stains for specific enzymes were suspended in 1% agar and individually poured on gel slices. Banding patterns were visualized using a light table (Porta-Trace, Gagne, Inc., New York, NY). Loci and alleles were scored by relative mobility assigning the lowest numeral to the most anodal (fast) ones. Given that no genetic control was performed on the analyzed isozymes, loci were considered putative. Nevertheless, banding patterns were similar to those found for the same enzymes analyzed in other plant species for which formal analyses were performed (Murphy et al. 1996). Isozyme electrophoresis was used for the analysis of a great number of cactus species (reviewed by Figueredo et al. 2010) and fine-scale genetic studies in plants (Vekemans and Hardy 2004) which proves the utility of this technique, particularly in cases, such as our study, where no funding was available for other DNA-based methods as SNPs.

Data Analysis

Isozyme genotypes were used to calculate genetic diversity parameters: the mean number of alleles per locus (N_A), the mean effective

number of alleles per locus that measures the evenness of allelic frequencies (N_E), the proportion of polymorphic loci under the *sensu stricto* criterion (P), and observed (H_O) and expected (H_E) heterozygosity using the program PopGene v 1.32 (Yeh et al. 1999). The multilocus fixation index ($F = 1 - H_O/H_E$) (Wright 1931) was calculated for each population, and deviations from random-mating expectations were tested by chi-squared tests for each polymorphic locus. In addition, the proportion of distinguishable genotypes was measured as G/N , where G is the number of different genotypes and N is the number of individuals at each population/distance class (Ellstrand and Roose 1987). The multilocus genotype diversity (D) was measured following Clark-Tapia et al. (2005). D measures the probability that 2 individuals selected at random from a population of N individuals will be from different multilocus genotypes. D ranges from 0 to 1, with 1 being the maximum diversity.

The fine-scale SGS at each population was evaluated by means of spatial autocorrelation analyses among all possible paired genotypes of mapped individuals. These analyses were performed using 2 approaches: pairwise kinship coefficients (Loiselle et al. 1995) and the autocorrelation coefficient r between geographic and genetic distances (Smouse and Peakall 1999) using the programs SPAGeDi v 1.2 (Hardy and Vekemans 2002) and GenALEx v 6.5 (Peakall and Smouse 2006, 2012), respectively. For each site, we estimated mean coefficients among all pairs of individuals within each of 16 distance classes at 25 m intervals, with geographic distances not exceeding 400 m. We used the S_p statistic to evaluate the amount of SGS, calculated as $-b/(1 - F_{(1)})$, where b is the slope of the linear regression and $F_{(1)}$ is the mean kinship coefficient in the first distance class, following Vekemans and Hardy (2004). Because the populations were rather elongated (transects), kinship coefficients were regressed against linear distances and not logarithmic (Rousset 2000; Vekemans and Hardy 2004). Statistical significance of the autocorrelation coefficient r was tested using 2 methods: random permutations of individuals and bootstrapping across loci with 1000 random permutations and bootstrap replicates, respectively. We also calculated other parameters indicative of SGS: k_{\max} as the maximum value of the kinship coefficient; Dk_{\max} as the distance at which the maximum value of kinship coefficient occurs, r_{\max} as the maximum value of spatial autocorrelation; and Dr_{\max} as the distance at which the spatial autocorrelation reaches the maximum r value.

The population genetic structure at each site was inferred using spatial Bayesian methods and Markov chain Monte Carlo (MCMC) simulations as implemented in the R-package Geneland version 4.0.5 (Guillot et al. 2005a). The geographical coordinates of each individual were included in the MCMC simulations to determine the number of genetic clusters (Guillot et al. 2005b). We set the parameters to run the uncorrelated frequency spatial model at 100 000 MCMC iterations, thin rate 100, and burn-in 200 for 10 runs at K (number of clusters) values from 1 to 10. The run with the highest posterior probability was chosen to infer the best K .

In order to identify family groups within sampled individuals we used sibship reconstruction methods, which are used when no information on parental genotypes or on family groups is available for collected samples. We used the full likelihood approach implemented in the software Colony version 2.0.6.3 (Jones and Wang 2010) to estimate the probability that any 2 individuals were likely to be either full or half-sibs.

To investigate the relationship between spatial genetic metrics and vegetation cover of the studied populations we ran partial Spearman's rank correlations between genetic parameters (S_p , k_{\max} , Dk_{\max} , r_{\max} , Dr_{\max}) and vegetation cover metrics. The later were NDVI

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4.100
4.105
4.110
4.114

2013 and 2014, average NDVI, number of patches, patch distance as the average distance among patches, patch size as the average patch size, and total area as the percentage of vegetated area. In addition, vegetation cover metrics and spatial genetic parameters were correlated to climatic variables of studied populations. These were: annual precipitation (P) and mean (T), maximum (T_{\max}) and minimum (T_{\min}) temperatures that were obtained from <https://es.climate-data.org> (Supplementary Figure S4).

Results

The results of mapping analysis show that vegetation cover is denser (66%) in the northern and most humid LH site than in the remaining sites LC 42%, PA 31%, and VF 41%. Study sites varied greatly in the number of vegetated patches. Vegetation under lower precipitation regimes, that is, LC and VF, had the highest number of patches (LC 105 and VF 96), whereas those under relatively more humid conditions yielded relatively fewer patches (LH 21 and PA 36). Similarly, populations under higher precipitation consisted of more and relatively bigger patches. Also, those populations yielded greater average NDVI values (LH 0.134 and PA 0.129) than those under more arid and desert climates (LC 0.042 and VF 0.079) (Table 2).

All sites had high levels of heterozygosity and polymorphism, greater than 0.3 and 87%, respectively (Table 3). At shorter distances, within 50 m, one encounters 81% of all of the genotypes found at a site. This pattern resulted in high multilocus genotype diversity of at least 0.976 (Supplementary Table S1); similar results were found at larger spatial scales (<100 m) (Supplementary Table S2).

Fine-scale genetic analyses yielded a significant SGS in all *E. terscheckii* populations (Figures 2 and 3). Maximum kinship coefficients were attained at small spatial distances (~25–50 m). Maximum values varied between 0.15 and 0.17 at most sites, except for Valle Fértil, where this value was as high as 0.398. Similarly, the Valle Fértil site attained the greatest r_{\max} of 0.269. Higher Sp were obtained for the 2 low-elevation southern sites Padercitas and Valle Fértil with similar values of 0.094; whereas the lowest $Sp = 0.015$ was found

in the relatively high-elevation wettest northern site Las Higuierillas, and an intermediate value of 0.048 was obtained for the mid-elevation northern site La Chilca (Table 4, Supplementary Figure S1). Similarly, Valle Fértil site yielded the greatest average multilocus within-population inbreeding of $F = 0.418$. Significant departures from Hardy–Weinberg equilibrium were measured in several populations. At Valle Fértil, 64% of total tests yielded positive and significant estimates suggesting heterozygous deficiency, that is, inbreeding (Supplementary Table S3). La Chilca and Padercitas sites had similar mean F of 0.181 and 0.103, respectively, as well as 40% and 38% of loci showed departure from equilibrium conditions indicating significant inbreeding. In contrast, Las Higuierillas had a mean negative F of -0.202 . While 69% of loci yielded nonsignificant departure from random mating, the remaining 31% consisted of negative values which may suggest heterozygous excess at this site (Supplementary Table S3). Populations from drier climates contained more clusters (as estimated by Geneland) and a greater number of families (as estimated by sibship reconstruction). In populations from wetter locations a greater percentage of sampled individuals could be assigned to full- or half-sib families (Table 5).

Vegetation cover metrics were significantly correlated among each other. The number of patches was negatively related to average NDVI and to average between-patch distance (Supplementary Table S4). This indicates that sites with lower NDVI (i.e., sparse vegetation) consisted of a greater number of patches which in turn were less distant from one another. Significant correlations were obtained between vegetation cover metrics and spatial genetic parameters. In particular, the distance at which maximum autocorrelation (Dr_{\max}) and kinship coefficients (Dk_{\max}) were attained had a positive relation with the percentage of vegetated area and average patch size, respectively (Supplementary Table S4). Also, maximum autocorrelation values (Dr_{\max}) were significantly correlated with greater SGS (Sp) (Supplementary Table S4). Out of the 4 climatic variables analyzed (annual precipitation, and mean, maximum and minimum temperatures) only precipitation yielded a positive association with Dr_{\max} , Dk_{\max} , and patch size (Supplementary Table S5).

Table 2. Vegetation cover measured as the normalized difference vegetation index (NDVI) and digitized spatial metrics for sampled sites of *Echinopsis terscheckii* from northwestern Argentina

Site, province	NDVI 2013	NDVI 2014	NDVI average	No. of patches	Patch distance (m)	Patch size (m ²)	Total area (%)
LH-Las Higuierillas, Salta	0.139 ^a	0.128 ^a	0.134	21	230 ^a	473 ^a	66
LC-Qda La Chilca, Catamarca	0.038 ^b	0.046 ^b	0.042	105	147 ^b	59 ^b	42
PA-Padercitas, La Rioja	0.111 ^c	0.147 ^a	0.129	36	193 ^a	129 ^a	31
VF-Valle Fértil, San Juan	0.078 ^b	0.08 ^b	0.079	96	158 ^c	65 ^b	41

Superscript letters indicate significant differences $P < 0.05$ Kruskal–Wallis mean test.

Table 3. Genetic diversity parameters of *Echinopsis terscheckii* at the population and patch-scale levels at 4 locations in northwestern Argentina

Site	N	N_A	N_E	P	H_O	H_E	G	G/N	D
LH	21	2.4 (0.3)	1.7 (0.2)	87	0.419 (0.086)	0.337 (0.060)	17	0.81	0.976
LC	30	2.8 (0.3)	2.1 (0.2)	87	0.396 (0.088)	0.460 (0.058)	29	0.97	0.998
PA	29	3.1 (0.2)	2.0 (0.2)	100	0.416 (0.080)	0.462 (0.049)	27	0.93	0.995
VF	30	3.0 (0.2)	2.3 (0.2)	93	0.322 (0.081)	0.543 (0.047)	29	0.97	0.998

N , number of individuals; N_A , number of alleles; N_E , effective number of alleles; P , percentage of polymorphic loci; H_O , observed heterozygosity; H_E , expected heterozygosity; G , number of different multilocus genotypes; G/N , proportion of different multilocus genotypes; D , multilocus genotype diversity. LH: Las Higuierillas, Salta Province; LC: Qda. La Chilca, Catamarca Province; PA: Padercitas, La Rioja Province; VF: Valle Fértil, San Juan Province.

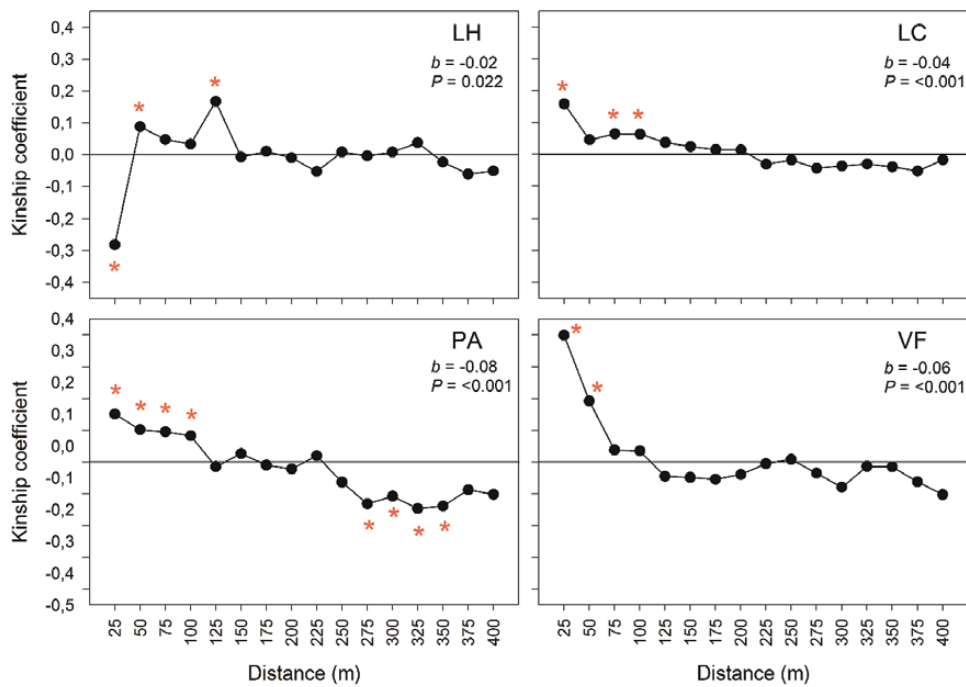


Figure 2. Spatial autocorrelograms based on Loiselle's kinship coefficients calculated using SPAGeDi on fifteen isozyme loci for *Echinopsis terscheckii*. Sixteen distance classes were defined at continuous 25 m intervals. Horizontal solid line indicates absence of spatial autocorrelation. Asterisks indicate kinship coefficients significantly different from zero ($P < 0.05$). Significant values of the slope b of the regression of kinship coefficients on the linear geographic distance are given. LH: Las Higuierillas, Salta Province; LC: Qda. La Chilca, Catamarca Province; PA: Padercitas, La Rioja Province; VF: Valle Fértil, San Juan Province.

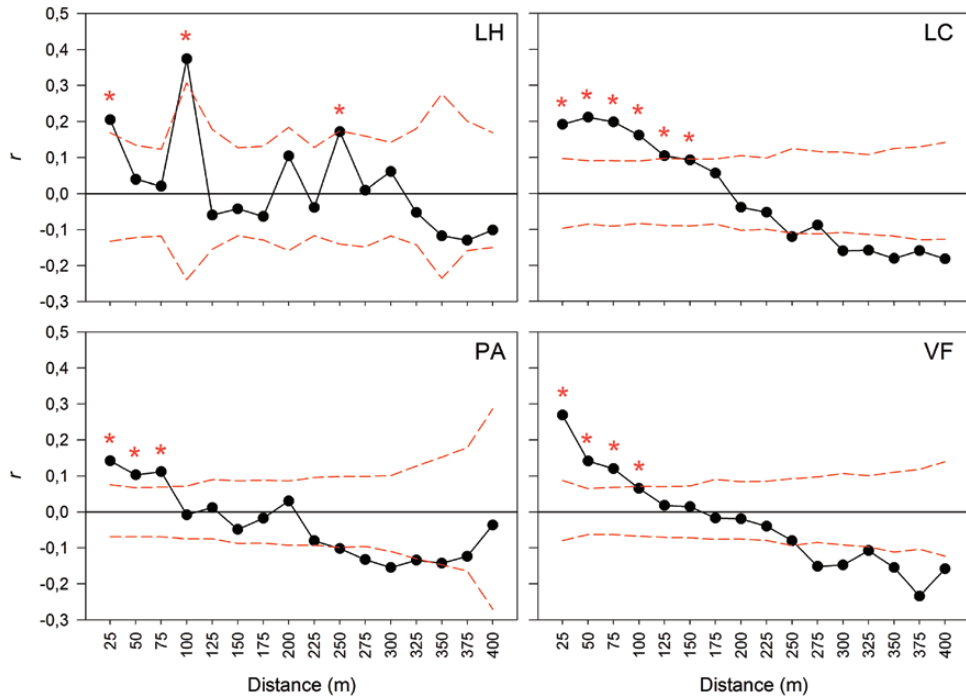


Figure 3. Spatial autocorrelograms based on autocorrelation coefficient r calculated using GenAlEx on 15 isozyme loci for *Echinopsis terscheckii*. Sixteen distance classes were defined at continuous 25 m intervals. Horizontal solid line indicates absence of spatial autocorrelation. Dashed lines represent upper and lower limits of the 95% confidence intervals determined by permutation. Asterisks indicate autocorrelation coefficients significantly different from zero ($P < 0.05$) determined by bootstrap resampling. LH: Las Higuierillas, Salta Province; LC: Qda. La Chilca, Catamarca Province; PA: Padercitas, La Rioja Province; VF: Valle Fértil, San Juan Province.

Discussion

Our results show that populations of the giant cactus *E. terscheckii* inhabiting drylands of northwestern Argentina are genetically

diverse and are highly structured at local scales. We found positive correlations between the distance at which maximum autocorrelation and kinship values were reached and vegetation area and patch

Table 4. Pairwise kinship coefficients, spatial autocorrelation parameters, and within-population inbreeding coefficient of *Echinopsis terscheckii* at 4 locations in northwestern Argentina

Site	r_{\max}	Dr_{\max}	k_{\max}	Dk_{\max}	b	Sp	F
LH	0.205	100	0.168	125	-0.02	0.015	-0.202 ^a
LC	0.212	50	0.158	25	-0.04	0.048	0.181 ^b
PA	0.142	25	0.150	25	-0.08	0.094	0.103 ^b
VF	0.269	25	0.398	25	-0.06	0.094	0.418 ^c

r_{\max} , is the maximum value of correlation; Dr_{\max} , is the distance in meters at which the correlation reaches the maximum value; k_{\max} , is the maximum value of the kinship coefficient; Dk_{\max} , is the distance in meters at which the maximum value of kinship coefficient occurs; b slope of the linear regression of kinship coefficients on the logarithm of geographic; Sp , is the estimation of SGS according to [Vekemans and Hardy \(2004\)](#); F , is the mutiocus fixation index by [Wright \(1931\)](#). Superscript letters indicate significant differences $P < 0.05$ paired by locus t -test. LH: Las Higuerrillas, Salta Province; LC: Qda. La Chilca, Catamarca Province; PA: Padercitas, La Rioja Province; VF: Valle Fértil, San Juan Province.

Table 5. Population genetic structure and family groups of *Echinopsis terscheckii* at 4 localities in northwestern Argentina

Site	K	Number of clusters	Number of families	Fullsibs	Halfsibs
LH	1	1	8	25 (12%)	99 (47%)
LC	2	2	16	21 (5%)	70 (16%)
PA	3	3	20	15 (4%)	67 (17%)
VF	7	2	20	14 (3%)	48 (11%)

The number of clusters (K) identified by Geneland and the other structuring parameters obtained by Colony are shown. LH: Las Higuerrillas, Salta Province; LC: Qda. La Chilca, Catamarca Province; PA: Padercitas, La Rioja Province; VF: Valle Fértil, San Juan Province.

size. Also higher NDVI values, that is, vegetation cover, were associated with lower number of patches and greater distances among them. Hence, in benign climates *E. terscheckii* populations consisted of sparse individuals growing in areas with greater vegetation cover yet clusters of genetically related individuals occurred at larger patch sizes. Under driest climatic settings neighborhoods of closely related genotypes with high relatedness and inbreeding values consisted of individuals that clustered at small distances.

Study sites occurring along a precipitation, and thus a productivity and therefore also a natural selection gradient, yielded concordant patterns of vegetation cover within each north and south sectors. Sites LC and VF under dry conditions had relatively lower greenness, that is, open vegetation, and consisted of a greater number of small patches which in turn were closer to each other. In contrast, the most humid location, LH, consisted of larger patches and had greater plant coverage. This is consistent with the expectation of vegetation patterns in strongly water-limited ecosystems with discontinuous plant cover not exceeding 60% ([Aguar and Sala 1999](#)). Thus, proxy measurements including NDVI and digitized vegetated patches proved to be useful for describing vegetation cover in drylands. These variable patterns of vegetation cover, in turn, were associated with variable spatial distribution of genotypes. Fine-grained spatial structure resulted in genetically structured and diverse populations.

The populations of *E. terscheckii* we studied, showed high levels of genetic diversity, similar to patterns found using isozymes in other columnar cacti (reviewed by [Figueredo et al. 2010](#)). These include *C. gigantea*, *Cereus repandus*, *Lophocereus schottii*, *Pachycereus pringlei*, *Pilosocereus lanuginosus*, *Praecereus euchlorus*, *Stenocereus griseus*, and *Stenocereus thurberi* ([Hamrick et al. 2002](#); [Nason et al. 2002](#); [Nassar et al. 2003](#); [Morales et al. 2005](#)). High genetic diversity is usually associated with outcrossed reproductive systems of columnar cacti ([Nassar et al. 2003](#); [Figueredo et al. 2010](#)). The self-incompatible breeding system of *E. terscheckii* ([Ortega-Baez et al. 2010](#)), in combination with long-distance seed dispersal by birds probably result in genetically diverse populations.

Individual genotypes of *E. terscheckii* were neither randomly nor uniformly distributed at small spatial distances. Clusters of similar genotypes were identified in all studied populations; still they occurred at different spatial scales and showed kinship values that depended on site conditions, including climate and vegetation cover. This was especially true at Valle Fértil, which had the driest conditions, and displayed the strongest SGS with the highest kinship coefficient and correlation values of k_{\max} 0.398 and r_{\max} 0.269, respectively.

Only one previous study in Cactaceae analyzed the spatial genetic aggregation of genotypes at fine-scale spatial distances. This study of the columnar cactus *P. tillianus*, that grows in the Venezuelan Andes under annual rainfall of 450–550 mm found clusters of related genotypes at spatial scales <30 m ([Figueredo et al. 2010](#)). The significant SGS was explained by local establishment of closely related seeds produced by partial self-compatibility and biparental inbreeding ([Figueredo et al. 2010](#)). Likewise, genetic structure recorded in *E. terscheckii* occurred at distances of 25–50 m under more arid conditions (less than 400 mm/year), whereas in the most humid location genetically similar genotypes clustered at larger distances, that is, 100–125 m. Although self-incompatible, *E. terscheckii* populations, under arid and desert climates with a patchy spatial structure, may enhance local activity of pollinators favoring mating events between spatially closer and genetically related individuals (biparental inbreeding). Moreover, xerophilous deciduous forests under higher precipitation regimes consisting of sparse *E. terscheckii* individuals resulted in larger and more distant neighborhoods each producing a greater proportion of full- and half-sibs. Negative within-population inbreeding, and thus heterozygous excess, measured at the wettest LH site may be due to outbreeding, that is, mating of distantly related or unrelated individuals belonging to genetically distinct clusters and/or families.

All studied populations showed significant SGS, and the strength of the aggregated pattern was associated with vegetation cover and climatic conditions of studied locations. In particular, higher

precipitation was significantly related to larger vegetated patches and greater distances at which maximum kinship and autocorrelation values were attained. Higher average NDVI values, as a measure of vegetation greenness, were negatively correlated with the number and distance between vegetated patches. In addition, populations from relatively wetter settings consisted of fewer clusters and also a small number of families of related genotypes. The opposite results were obtained for water-stressed populations. These results suggest that a gradient in resource availability determines vegetation structure which in turn seems to be associated with the degree of genetic and family structure. Such structuring appears to be coarse-scale at the more humid extreme and fine-scale under arid conditions. Thus, the evidence presented here supports the stress gradient hypothesis which predicts that species' interactions grade from being more mutualistic (e.g., facilitation) under stressful abiotic conditions to more antagonistic (e.g., competition) or neutral under benign conditions. We extend these predictions in our stress gradient genetic hypothesis (SGGH). As interactions such as facilitation prevail in stressful environments, that is, under water shortage, genetic structure will reflect vegetation patterns. This relates to the fact that nurse plants buffer extreme conditions for plant establishment in drylands. Therefore, in open habitats there will be small vegetation patches which produce a fine-scale genetic structure. Under benign climates such as xeric forests under higher precipitation regimes and vegetation cover, competition for resources as light provides little opportunities for establishment under close canopies of relatively shade-intolerant slow-growing species as *E. terscheckii* whose seeds are photoblastic (Ortega-Baes and Rojas-Ar chiga 2007) and thus are light-dependent for germination. As a consequence, *E. terscheckii* growing in xerophitic forests will tend to have a sparse distribution and a coarse-scale population structure consisting of bigger patches of genotypes clustered at larger spatial scales which can be associated to the potential long-distance foraging behavior of seeds by birds.

Photoblastic seeds are often associated with persistent seed banks as shown in *Ferocactus wislizeni* a common cactus of the Sonoran Desert, whose seeds survived at least 18 months in and on the soil (Bowers 2000). A short-term persistent seed bank was documented in *Echinopsis atacamensis*, a closely related species to *E. terscheckii*, with small seeds and enforced dormancy that is commonly found at high-elevation areas of the Puna in Northwestern Argentina (de Viana 1996). Thus, the elevated SGS measured in *E. terscheckii* may also result from transient seed banks that may accumulate related genotypes in close proximity from mother plants. In addition, massive but infrequent recruitment events are associated with favorable rainfall and temperature periods in drylands. The absence of germination and seedling establishment of the cactus *Pachycereus schottii* was attributed to drought (Holland and Molina Freaner 2012), and indicated that nurse plants could only facilitate seedling recruitment under sufficient precipitation conditions. Apparently, infrequent events such as "El Ni o" may increase the number of new recruits in some populations (God nez-Alvarez et al. 2003). For instance, heavy occasional rainfall was recorded at Valle F rtil population (German Poblete, Universidad Nacional de San Juan, Argentina, personal communication) and has been documented elsewhere (Dore 2005) which may result in abundant seedling establishment that may explain the high inbreeding measured at this driest location.

Conclusions

Drylands and deserts can be considered fragile ecosystems where recruitment is greatly influenced by the regional climate and local

conditions, such as the surrounding vegetation. Plant cover either may facilitate seedling establishment and survival in arid open shrublands and deserts while inducing competition for light in xerophilous forests under higher precipitation regimes. Hence physical gradients impact on interactions among species, which in turn influence the SGS of the giant columnar cactus that ranges from fine-grained at the arid end to coarse-grained at the mesic end of the environmental spectrum. The strong family structure found under drought is probably reinforced by occasional massive seedling recruitment due to increased heavy precipitation regimes as expected under climate change.

Drylands are currently threatened by human activities such as habitat loss and population decline of native plants and animals. In particular, the demise of lowland populations of *E. terscheckii* that occupy transitional areas is due to the advancement of the agricultural frontier by soybean plantations. It was estimated that 66.4% of *E. terscheckii*'s range has become fragmented in the past 30 years (Ortega-Baes and Lowry 2013). On the other hand, for this species, collection of live specimens for ornamental purposes as well as wood extraction for crafts and construction jeopardize natural populations. Passive restoration and sustainable use practices of *E. terscheckii* populations affected by anthropogenic disturbances are difficult particularly due to the fact that it is slow growing and has limited natural recruitment. In spite of these limitations, the information provided here can be useful in germplasm collection for conservation actions. In order to preserve genetic diversity of *E. terscheckii*, seeds should be collected using fine-grained schemes (<50 m) in open areas as deserts and arid shrublands, whereas seed collection zones in xerophilous forests under higher precipitation regimes should follow coarse-scale (>100 m) strategies.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Availability

Data consisting of sampling locations, vegetation cover, and isozyme data was deposited in Dryad.

References

Aguiar MR, Sala OE. 1999. Patch structure, dynamics and implications for the functioning of arid ecosystems. *Trends Ecol Evol.* 14:273–277.

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8.114

- Bertness MD, Callaway R. 1994. Positive interactions in communities. *Trends Ecol Evol.* 9:191–193.
- Bowers JE. 2000. Does *Ferocactus wislizeni* (Cactaceae) have a between-year seed bank? *J Arid Environ.* 45:197–205.
- 9.5 Brown AD, Pacheco S. 2006. Propuesta de actualización del mapa ecoregional de la Argentina. In: Brown AD, Martínez Ortiz U, Acerbi M, Corcuera J, editors. *Situación Ambiental Argentina 2005*. Buenos Aires (Argentina): Fundación Vida Silvestre. p. 59–60.
- Cabrera AL. 1971. Fitogeografía de la República Argentina. *Boletín de la Sociedad Argentina de Botánica.* XIV:1–2.
- 9.10 Cabrera AL. 1976. Regiones fitogeográficas argentinas. In: Kugler WF, editor. *Enciclopedia argentina de agricultura y jardinería*. Buenos Aires (Argentina): Ed. Acme. p. 1–85.
- Clark-Tapia R, Alfonso-Corrado C, Eguiarte LE, Molina-Freaner F. 2005. Clonal diversity and distribution in *Stenocereus eruca* (Cactaceae), a narrow endemic cactus of the Sonoran Desert. *Am J Bot.* 92:272–278.
- 9.15 Callaway RM, Walker LR. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology.* 78:1958–1965.
- Conkle MT, Hodgskiss PD, Nunnally LB, Hunter SC. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. In: Pacific Southwest Forest and Range Experiment Station, editor. *General Technical Report PSW-64*. Berkeley (CA).
- AQ3 9.20 de Viana ML. 1996. Distribución espacial de *Trichocereus pasacana* (Cactaceae) en relación al espacio disponible y al banco de semillas. *Rev Biol Trop.* 44/45:95–103.
- Diers L. 2008. Over chromosomen bij cactussen-ter herinnering aan Albert Buining (1). *Succulenta.* 87:83–92.
- 9.25 Dore MH. 2005. Climate change and changes in global precipitation patterns: what do we know? *Environ Int.* 31:1167–1181.
- Ellstrand NC, Roose ML. 1987. Patterns of genotypic diversity in clonal plant species. *Am J Bot.* 74:123–131.
- 9.30 Figueroa CJ, Nassar JM, García-Rivas AE, González-Carcacia JA. 2010. Population genetic diversity and structure of *Pilosocereus tillianus* (Cactaceae, Cereaceae), a columnar cactus endemic to the Venezuelan Andes. *J Arid Environ.* 74:1392–1398.
- Franco AC, Nobel PS. 1989. Effect of nurse plants on the microhabitat and growth of cacti. *J Ecol.* 77:870–886.
- 9.35 Godínez-Alvarez H, Valverde T, Ortega-Baes P. 2003. Demographic trends in the Cactaceae. *Bot Rev.* 69:173–203.
- Guillot G, Estoup A, Mortier F, Cosson JF. 2005a. A spatial statistical model for landscape genetics. *Genetics.* 170:1261–1280.
- Guillot G, Mortier F, Estoup A. 2005b. GENELAND: a computer package for landscape genetics. *Mol Ecol Notes.* 5:708–711.
- 9.40 Hamrick JL, Nason JD, Fleming TH, Nassar JM. 2002. Genetic diversity in columnar cactus. In: Fleming TH, Valiente-Banuet A, editors. *Columnar cacti and their mutualists: evolution, ecology and conservation*. The University of Arizona Press. p. 122–133.
- AQ4 Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. *Mol Ecol Notes.* 2:618–620.
- 9.45 Holland JN, Molina Freaner F. 2012. Hierarchical effects of rainfall, nurse plants, granivory and seed banks on cactus recruitment. *J Veg Sci.* 24:1053–1061.
- Hunt D. 2006. *The new cactus lexicon*. Milborne Port, Somerset (UK): Remous Ltd.
- 9.50 Jacquemyn H, Brys R, Vandepitte K, Honnay O, Roldán-Ruiz I, Wiegand T. 2007. A spatially explicit analysis of seedling recruitment in the terrestrial orchid *Orchis purpurea*. *New Phytol.* 176:448–459.
- Jones OR, Wang J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol Ecol Resour.* 10:551–555.
- 9.55 Kiesling R. 1978. El género *Trichocereus* (Cactaceae): I: Las especies de la República Argentina. *Darwiniana.* 21:263–330.
- King JN, Dancik BP. 1983. Inheritance and linkage of isozymes in white spruce (*Picea glauca*). *Can J Genet Cytol.* 5:430–436.
- Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu Rev Ecol Syst.* 27:237–277.
- 9.60 Loiselle BA, Sork VL, Nason JD, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *Am J Bot.* 82:1420–1425.
- Mandujano MC, Flores-Martínez A, Golubov J, Ezcurra E. 2002. Spatial distribution of three globose cacti in relation to different nurse-plant canopies and bare areas. *Southwestern Nat.* 47:162–168.
- 9.65 Mitton JB, Linhart YB, Sturgeon KB, Hamrick JL. 1979. Allozyme polymorphisms detected in mature needle of ponderosa pine. *J Hered.* 70:86–89.
- Moraes EM, Abreu AG, Andrade SC, Sene FM, Solferini VN. 2005. Population genetic structure of two columnar cacti with a patchy distribution in eastern Brazil. *Genetica.* 125:311–323.
- 9.70 Munguía-Rosas MA, Sosa VJ. 2008. Nurse plants vs. nurse objects: effects of woody plants and rocky cavities on the recruitment of the *Pilosocereus leucocephalus* columnar cactus. *Ann Bot.* 101:175–185.
- Murphy RW, Sites JW, Buth DG, Hufler CH. 1996. Proteins: isozyme electrophoresis. In: Hillis DM, Moritz C, Mable BK, editors. *Molecular systematics*. Sunderland (MA): Sinauer Associates. p. 51–120.
- 9.75 Nason JD, Hamrick JL, Fleming TH. 2002. Historical vicariance and postglacial colonization effects on the evolution of genetic structure in *Lophocereus*, a Sonoran Desert columnar cactus. *Evolution.* 56:2214–2226.
- Nassar JM, Hamrick JL, Fleming TH. 2003. Population genetic structure of Venezuelan *Chiropterophilous* columnar cacti (Cactaceae). *Am J Bot.* 90:1628–1637.
- 9.80 Niering WA, Whittaker RH, Lowe CH. 1963. The saguaro: a population in relation to environment. *Science.* 142:15–23.
- Ortega-Baes P, Lowry M. 2013. *Echinopsis terscheckii*. The IUCN Red List of Threatened Species 2013: e.T152300A620937. <http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T152300A620937.en>.
- 9.85 Ortega-Baes P, Rojas-Arérchiga M. 2007. Seed germination of *Trichocereus terscheckii* (Cactaceae): light, temperature and gibberellic acid effects. *J Arid Environ.* 69:169–176.
- Ortega-Baes P, Saravia M, Suhring S, Godínez H, Zamar M. 2010. Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biol.* 13:33–40.
- 9.90 Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes.* 6:288–295.
- Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics.* 28:2537–2539.
- 9.95 Peñuelas J, Filella I. 1998. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends Plant Sci.* 3:151–155.
- Peters EM, Martorell C, Ezcurra E. 2008. Nurse rocks are more important than nurse plants in determining the distribution and establishment of globose cacti (*Mammillaria*) in the Tehuacán Valley, México. *J Arid Environ.* 72:593–601.
- 9.100 Premoli AC, Kitzberger T. 2005. Regeneration mode affects spatial genetic structure of *Nothofagus dombeyi* forests in northwestern Patagonia. *Mol Ecol.* 14:2319–2329.
- Ranker TA, Haufler CH, Soltis PS, Soltis DE. 1989. Genetic evidence for allopolyploidy in the neotropical fern *Hemionitis* (Adiantaceae) and the reconstruction of an ancestral genome. *Syst Bot.* 14:439–447.
- 9.105 Rousset F. 2000. Genetic differentiation between individuals. *J Evol Biol.* 13:58–62.
- Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity.* 82:561–573.
- 9.110 StatSoft, Inc. (2004) *STATISTICA (computer program manual) version 7.0*. Tulsa.
- Valiente-Banuet A, Bolongaro-Crevenna A, Briones O, Ezcurra E, Rosas M, Nuñez H, Barnard G, Vazquez E. 1991. Spatial relationships between cacti and nurse shrubs in a semi-arid environment in central México. *J Veg Sci.* 2:15–20.
- 9.114

- Valiente-Banuet A, Ezcurra E. 1991. Shade as a cause of the association between the cactus *Neobuxbaumia tetetzo* and the nurse plant *Mimosa luisana* in the Tehuacan Valley, Mexico. *J Ecol.* 79:961–971.
- Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol.* 13:921–935.
- 10.5 Xu C, Holmgren M, Van Nes EH, Maestre FT, Soliveres S, Berdugo M, Kéfi S, Marquet PA, Abades S, Scheffer M. 2015. Can we infer plant facilitation from remote sensing? A test across global drylands. *Ecol Appl.* 25:1456–1462.
- 10.10
- 10.15
- 10.20
- 10.25
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- 10.35
- 10.40
- 10.45
- 10.50
- 10.55
- Wright S. 1931. Evolution in Mendelian populations. *Genetics.* 16:97–159.
- Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. 1999. POPGENE 1.32, the user-friendly shareware for population genetic analysis. Edmonton: Molecular Biology and Biotechnology Center, University of Alberta. Available from: <http://www.ualberta.ca/~fyeh/download.htm> 10.60
- Zhen Z, Quackenbush LJ, Zhang L. 2016. Trends in automatic individual tree crown detection and delineation—evolution of LiDAR data. *Rem Sens.* 8:333. 10.65
- 10.70
- 10.75
- 10.80
- 10.85
- 10.90
- 10.95
- 10.100
- 10.105
- 10.110
- 10.114

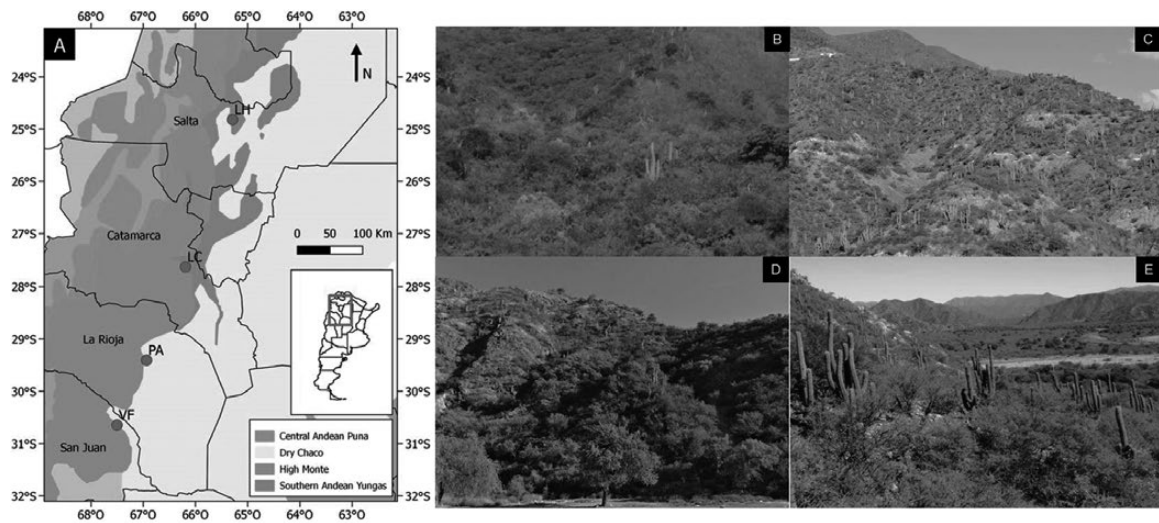


Figure 1.

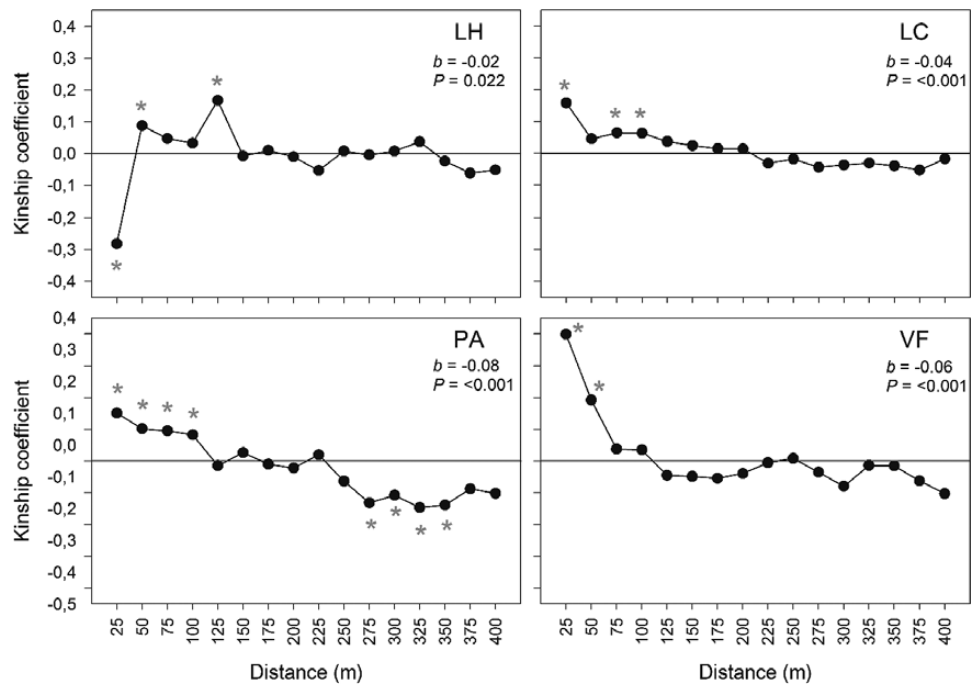


Figure 2.

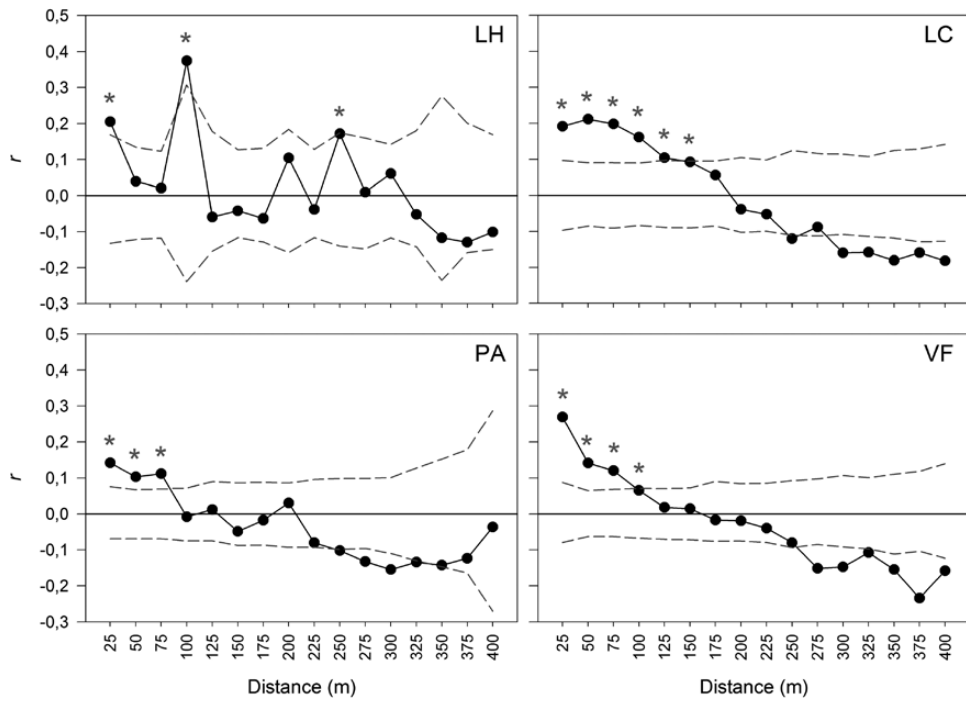


Figure 3.