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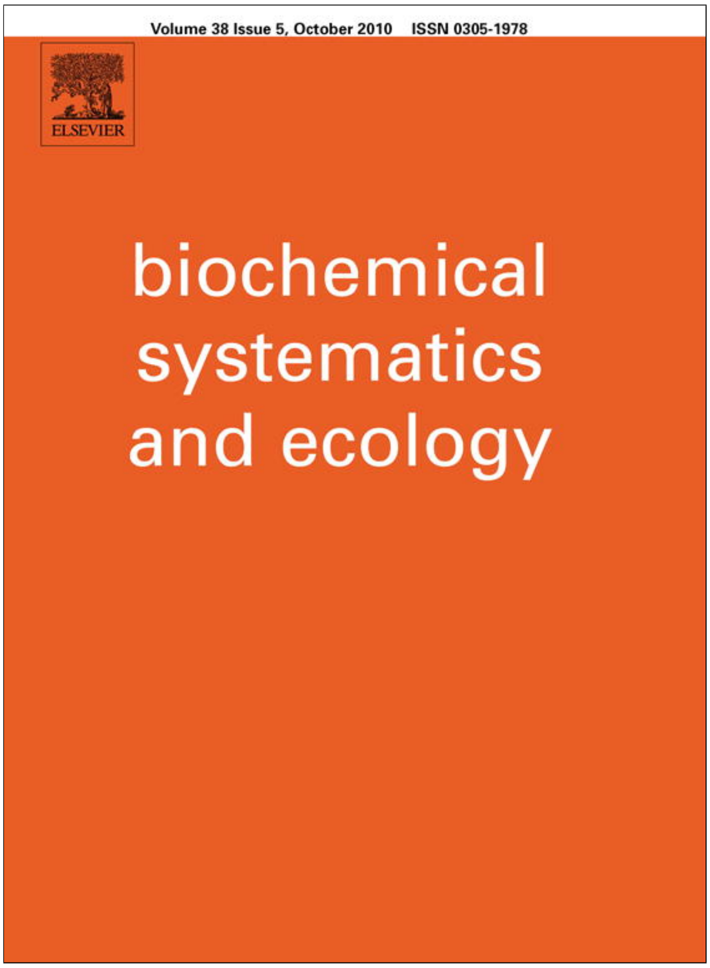

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Fine-scale spatial genetic structure in two Mexican cycad species *Dioon caputoi* and *Dioon merolae* (Zamiaceae, Cycadales): Implications for conservation

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ABSTRACT

An understanding of how genetic variation is distributed locally is not only vital to the diagnosis of the conservational status of a species, but also to prioritize actions of sustainable management. We compared the fine-scale spatial genetic structure (SGS) of two Mexican cycads, *Dioon caputoi* and *Dioon merolae*, which differ in aspects such as distribution range, density and population size, and habitat requirements, with the premise that this interspecific comparison will help to contrast the effects of these aspects on local genetic organization and thereby infer the possible causes of differences in distribution. We estimated the autocorrelation index r_{ij}, for the delineation of genetic neighborhoods. We used 14 alloenzymatic loci in *D. caputoi* and ten in *D. merolae*, and found that populations of *D. caputoi* have higher autocorrelation coefficients ($r_{ij} = 0.113$ and 0.082) than that of *D. merolae* ($r_{ij} = 0.034$). Contrary to expectations, we found that the inferred dispersal distance is not lower in *D. caputoi*, of narrow distribution compared to *D. merolae*. Contrasting population densities and habitat types may explain these differences, even when both species have the same type of seed and pollen dispersal vectors. We conclude that wide distribution and greater population density of *D. merolae* (compared to *D. caputoi*) is probably not due to greater dispersal ability, but to a higher capacity for colonization.

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1. Introduction

The conservation of plant species with restricted geographic distributions and/or low population sizes requires the application of theoretical and practical tools, both genetic and demographic (Lande, 1988). Population processes may partially be approached from different angles or scales: e.g., macroecology, community ecology and population ecology (Escudero et al., 2003). However, what happens within a population may partly be the result of geographic patterns of a greater scope and scale, so that a local pattern may be influenced by elements that arise at a larger scale (Levin, 1992), e.g., the effect of environmental conditions on the dispersal and/or germination of seeds (Bond and Wilgen, 1996; Nathan and Muller-Landau, 2000). Vice versa, local processes can have implications at regional scales; e.g. limitations on the dispersal distances of

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individuals or propagules may result in genetic isolation by distance between one population and another (Epperson and Li, 1997); local management of populations may have an impact throughout the genetic diversity levels of a species (Tinoco et al., 2005; Parra et al., 2008).

The analysis of local distribution of genetic diversity, also known as fine-scale spatial genetic structure (SGS), is an approach to population genetics that is applied to analyze patterns of local gene flow and that indirectly helps infer how the forms of dispersal of organisms interact with the physical environment (Smouse and Peakall, 1999; Epperson, 2003). In plants, the SGS is expected to be consistent with the particular pollination and dispersal syndromes they present, since these are the main determinants of local gene flow (Epperson, 2003; Jones et al., 2006; Pardini and Hamrick, 2008). However, SGS is also a reflection of ecological and/or evolutionary factors operating in concert, leading to an interaction between the mechanisms of dispersal and the post-dispersal processes (Vekemans and Hardy, 2004; Zhou and Chen, 2010).

In this context, comparative studies are crucial for an understanding of the processes involved in the formation of SGS; e.g. regeneration processes or histories of colonization (Jones et al., 2006; Soto et al., 2007), differential role of pollen and seed flow (Ndiade-Bourobou et al., 2010), dynamics between different topographic zones within a population (Oshawa et al., 2007), life cycle stages of a population (Chung et al., 2003; Fuchs and Hamrick, 2010; Yao et al., 2010; Zhou and Chen, 2010).

In this study, we analyzed the SGS of two species of cycads in the genus *Dioon* Lindl.: *Dioon caputoi* De Luca, Sabato & Vázq. Torres and *Dioon merolae* De Luca, Sabato and Vázq. Torres. Both of these species exhibit similar pollination and dispersal systems but differ in their geographical area, population sizes, densities, and habitat types. From these characteristics, the population density and habitat requirements of each species are of particular interest for this study, since the population density largely determines the local genetic structure, as a decrease in the number of plants in the population increases the magnitude of the SGS (Doligez and Joly, 1997; Vekemans and Hardy, 2004).

The objective of this work is therefore to define the spatial patterns of local genetic diversity, contrasting two species which, by the nature of their distribution, abundance and habitat types may have different influences on the formation of such patterns. In this context, we hypothesize that: *D. caputoi*, with relatively lower population densities and more specific habitat requirements (in contrast to *D. merolae*), will be the species that presents more pronounced genetic structures at closer distance intervals.

2. Material and methods

2.1. Study sites

The study was conducted in two populations of *D. caputoi*: La Grana and El Guayabo, municipality of Santa María Caltepec (southern limits of Puebla State), bordering the northwest of Oaxaca State, Mexico (Cabrera-Toledo et al., 2008). Both populations have a complex topography characterized by scarce flat areas and a dominance of steep slopes. In the first population, the average slope is 43°27’, while in the second it is 63°36’. Average annual rainfall is 319 mm and the average annual temperature is 18 °C (García, 2004).

The population of *D. merolae* known as Loma Colorada is in the municipality of San Jerónimo Taviche, located in the Central Valleys region of Oaxaca (Flores-Vázquez, 2008). The local climate is warm subhumid with summer rainfall: (A) Cb (W_o) (W) (García, 2004). The average annual precipitation is 665.5 mm and average annual temperature is 18 °C (García, 2004). The incline in this area is about 20°.

2.2. Study species

The genus *Dioon* belongs to a group of gymnosperm plants, very long-lived, slow growing and with long life cycles; it is the most ancient group of seed plants (Norstog and Nicholls, 1997). *D. caputoi* has erect or creeping stems up to 2.5 m long and 20–25 cm in diameter, while *D. merolae* has erect stems that reach 5 m in height and up to 70 cm in diameter. The conservation status of these species is, according to the IUCN “Endangered” for *D. caputoi* and “Vulnerable” for *D. merolae* (IUCN, 2008).

D. caputoi has four populations of between 60 and 100 individuals, whereas *D. merolae* has at least ten populations of between 100 and 1000 plants. The populations of *D. caputoi* included in this study have a population density of approximately 0.002 Ind./m^2, whereas for *D. merolae*, the density is 0.01 Ind./m^2. Finally, *D. caputoi* is distributed exclusively in xeric scrub, while *D. merolae* inhabits tropical deciduous, pine and pine-oak forests.

2.3. Sample collection

We obtained samples from 23 individuals in La Grana and 44 in El Guayabo (juveniles and adults; seedlings were very rare), which represents over 50% of the population in both locations. The spatial location of each plant was recorded in a Cartesian plane (lines of $\sim$150 m and 200 m in length). In the Loma Colorada population of *D. merolae*, the same procedure was performed, but in a grid of 1 ha, where 116 individuals were sampled. In all the sampled populations, the plants have a non-uniform distribution, characterized by aggregations of less than four plants in approximately 20 m^2 in the case of populations of *D. caputoi*, and less than 20 plants in 20 m^2 in those of *D. merolae*. Tissue samples were transported on ice to the laboratory and stored at -70 °C, to prevent the denaturation of enzymes prior to their extraction.

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2.4. Electrophoresis

Enzymes were extracted following the protocol of González-Astorga et al. (2003). Electrophoresis was carried out in two buffer systems: system R, which was run at 4 °C for 8.5 h (constant current of 35 mA and voltage of 200 V); and system PK, which was run at 4 °C for 7 h (constant current of 50 mA and voltage of 200 V). For each sample, a note was made of the genotypic variation in those enzymes that showed a legible staining. In *D. caputoi*, eight enzymes were analyzed with the system R: Anodic Peroxidase (APX), Malate-dehydrogenase (MDH), Menadione-reductase (MNR), Isocitrate-dehydrogenase (IDH), Alcohol-dehydrogenase (ADH), Phosphoglucose-isomerase (PGI), Leucine-aminopeptidase (LAP) and Esterase (EST), and three enzymes with the system PK: Diaphorase (DIA), Glutamate-dehydrogenase (GDH) and Aconitate-hydratase (ACO).

In the case of *D. merolae*, eight enzymes showed legible staining, four with the system R: Malate-dehydrogenase (MDH), Anodic Peroxidase (APX), 6 - Phosphogluconate-dehydrogenase (6PGD) and Isocitrate-dehydrogenase (IDH); and four with the system PK: Glutamate-oxaloacetate-transaminase (GOT), Phosphoglucomutase (PGM), Malic Enzyme (ME), and Aconitate-hydratase (ACO).

2.5. Statistical analysis

The presence of alleles in all the enzymatic systems was obtained from the banding patterns observed in the gels and the genotypes were recorded under this scheme. To determine whether the study populations exhibited SGS, we used the program *GenAlex 6* (Peakall and Smouse, 2006). We did a spatial autocorrelation analysis, based on the use of Euclidean genetic distances (d_{ij}) (Smouse and Peakall, 1999). The autocorrelation coefficient r_{ij}, varies in the range of −1 to 1 and represents a measure of correlation between genetic similarity and geographical separation, located within a specific distance interval between pairs of individuals (Smouse and Peakall, 1999). Values statistically different from cero reflect a non random distribution, i.e. individuals at certain distance are less (negative values) or more related (positive values) than expected by chance (Smouse and Peakall, 1999). The significance of r_{ij} was obtained by the random permutations method, carried out by *GenAlex* (Peakall and Smouse, 2006), which consists of a random exchange of geographic locations of individual genotypes.

For *D. caputoi*, SGS analyses were performed in 14 polymorphic loci. With only 23 individuals in La Grana and 44 in El Guayabo, the allocation of fixed distance classes (e.g. every 5 m) produced a very low number of pairs of individuals per class. We therefore decided to choose the option in *GenAlex* that generates a proportional number of pairs in each class and adjusts the distances accordingly, so that with fewer classes of distance the program groups together more pairs per class. Under this scheme, we chose four distance classes for La Grana, and eight for El Guayabo, with varying distance intervals for each population (Fig. 1a and b).

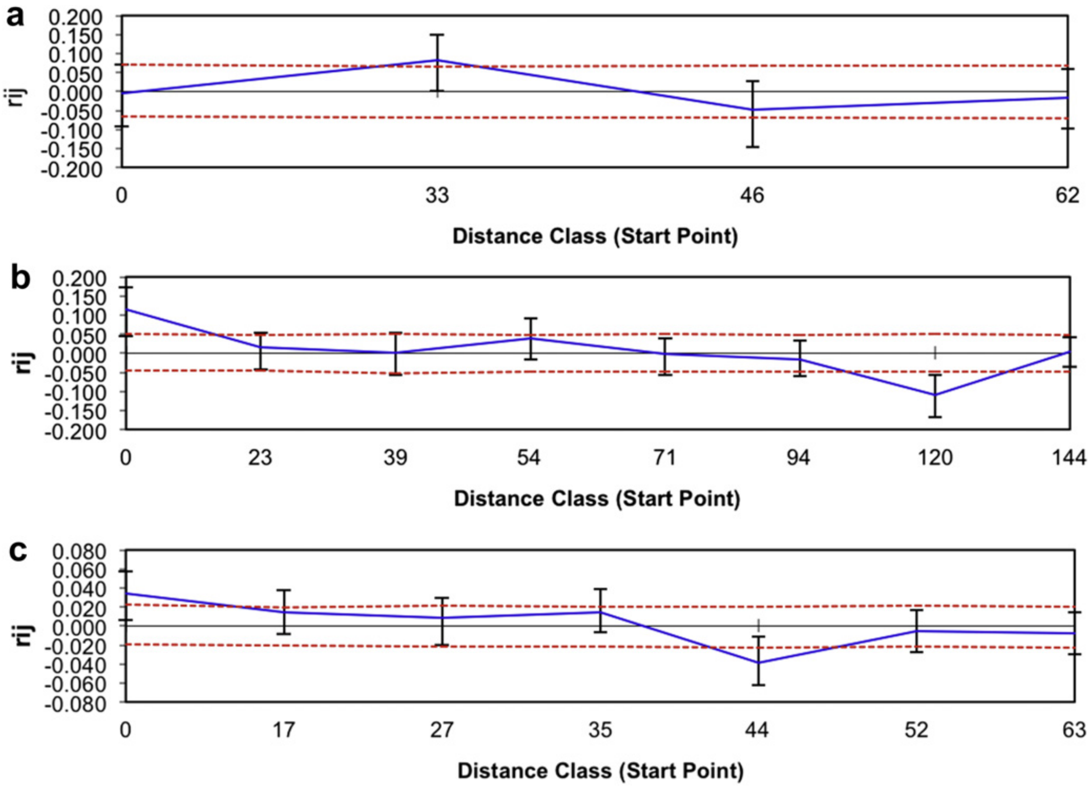

Fig. 1. Multilocus correlograms of r_{ij} (solid lines) and confidence intervals (broken lines) at 95% (100 bootstraps, 999 permutations). a) *D. caputoi*, La Grana; b) *D. caputoi*, El Guayabo; c) *D. merolae*, Loma Colorada.

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In the case of the population of *D. merolae*, ten polymorphic loci and eight distance classes were chosen (Fig. 1c). While the number of samples was higher in this species (116 individuals), we chose to perform the same procedure to form the distance classes, so that the results between species would be methodologically comparable.

3. Results

Both species showed a significant relationship between the genetic similarity of individuals and the geographical distances that separate them, i.e., we found significant SGS. In *D. caputoi*, this relationship in general was relatively higher (Fig. 1a and 1b) than in *D. merolae* (Fig. 1c). However, for the El Guayabo population of *D. caputoi*, this was only significant in the distance class 0–23 m ($r_{ij} = 0.113$, $p = 0.001$), indicating that only those individuals located at a distance of less than 23 m apart have more genetic similarity than that expected at random. For the La Grana population, only the class 33–46 m was significant ($r_{ij} = 0.082$, $p = 0.006$) with the index being lower than that of El Guayabo. This indicates that the genetic similarity between individuals is of lesser intensity than in El Guayabo, occurring at distances of less than 46 m and above 33 m.

In the studied population of *D. merolae* (i.e. Loma Colorada), we found that individuals separated by a distance of up to 17 m (Fig. 1c), are genetically more similar than would be expected by chance, but at a lower intensity ($r_{ij} = 0.034$, $p = 0.003$), than was observed in *D. caputoi* ($r_{ij} = 0.113$ and $r_{ij} = 0.082$).

4. Discussion

Populations of both species show a SGS similar to that reported by other authors using allozymes markers for plants with long generational cycles (Jones et al., 2006; Oshawa et al., 2007; Pardini and Hamrick, 2008 in Table 1). It is pertinent to mention that SGS studies are sensitive to sample sizes (Smouse and Peakall, 1999; Epperson, 2010). If sampling size and scale is biased for any reason, neighborhood sizes can change (Fenster et al., 2003; Epperson, 2003). The quantity of samples in this study (La Grana $n = 23$, El Guayabo $n = 44$) represents the 50% of each population in the case of *D. caputoi*, and 10% in the case of *D. merolae* (Loma Colorada $n = 116$). The latter, is comparable with samplings in other species with large population sizes (e.g. 10% in seedlings sampling: Fuchs and Hamrick, 2010). In this context, to counteract possible sampling bias we focused in gain statistical power increasing numbers of assayed loci (Epperson, 2010): 14 loci for *D. caputoi* and 10 loci for *D. merolae*, which is comparable with what is reported in other studies using allozymes (e.g. 11 loci: Jones et al., 2006; 10–11 loci: Pardini and Hamrick, 2008; 13 loci: Fuchs and Hamrick, 2010). With the same objective we used a multiloci analysis (Smouse and Peakall, 1999). Our results are also consistent with those reported for studies in plant populations in which SGS was assessed with the Moran's *I* statistic (Luna et al., 2005; Premoli and Kitzberguer, 2005, Table 1). Using this statistic, Epperson (2003) noted that, in plants with dispersion-pollination systems similar to those in this study (i.e., gravity-insects), there is an

Table 1
Spatial genetic structure (SGS) reported for long life cycle plants (including this study).

Reference	Species	Dispersion/Polinization vectors	Molecular marker	SGS (r_{ij}, I, others)	Distance[a] (m)	Pd[b] (ind/ha)
Gapare and Aitken, 2005	*Picea sitchensis* (Pinaceae)	Wind/Wind	Sequence -tagged site	$p_{ij} = 0.2$	500	–
Luna et al., 2005	*Chamaedora elatior* (Arecaceae)	Gravity/Entomophilus	Allozymes	$I = 0.034$	<10	28
Luna et al., 2005	*Chamaedora Tepejilote* (Arecaceae)	Gravity/Wind	Allozymes	$I = 0.009$	<2.5	1097
Premoli and Kitzberguer, 2005	*Nothofagus dombeyi* (Fagaceae)	Gravity/Wind	Allozymes	$I = 0.1$; $I = 0.25$	<12, <16	96–5000
Jones et al., 2006	*Quercus rubra* (Fagaceae) (seedlings)	Gravity, squirrel, mice/Wind	Allozymes	$r_{ij} = 0.27$	<25	900
Jones et al., 2006	*Pinus strobus* (Pinaceae)	Wind/Wind		$r_{ij} = 0.05$	<10	90
Oshawa et al., 2007	*Quercus crispula* (Fagaceae)	Gravity, rodents and birds/wind	SSR	$r_{ij} = 0.04$ (adults); $r_{ij} = 0.04–0.1$ (seedling)	5	300
Soto et al., 2007	*Quercus suber* (Fagaceae)	Rodents/-	SSR	Nason's kinship = 0.025	75	85
Pardini and Hamrick, 2008	*Albizia julibrissin* (Fabaceae)	-/generalists: e.g. bees and hummingbirds	Allozymes	$r_{ij} = 0.06–0.11$	8–60	200
Fuchs and Hamrick, 2010	*Guaiacum sanctum* (Zygophyllaceae)	birds/bees, wasps	Allozymes	$r_{ij} = 0.02$ (seedlings and juveniles)	5/30 (seedling/juveniles)	–
This study	*D. caputoi*	Gravity and rodents/beetles	Allozymes	$r_{ij} = 0.113$; 0.082	<23, 33–46	23
Thid study	*D. merolae*	Gravity and rodents/beetles	Allozymes	$r_{ij} = 0.034$	<17	44

[a] Distance where SGS is significant $p < 0.05$.

[b] Population density.

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interval of $I = 0.15$ to 0.4, which is considered a high spatial structure. In this study, the SGS values for the populations of *D. caputoi* are slightly below this range ($r_{ij} = 0.113$ in El Guayabo and $r_{ij} = 0.082$ in La Grana), which is probably a reflection of populations composed primarily of adult plants (Cabrera-Toledo, 2009). A reduction of SGS in adult plant cohorts with limited dispersal is expected when the genetic relationships established between individuals of the early cohorts decrease as a result of the different mortality rates over the life cycle stages, known as "thinning processes" (Epperson and Alvarez-Buylla, 1997; Luna et al., 2005; Zhou and Chen, 2010).

The SGS of *D. merolae* ($r_{ij} = 0.034$) is considerably lower than that of *D. caputoi*; is more similar to that reported for plants pollinated and/or dispersed by vectors of greater "reach" (i.e., wind and birds), where the SGS varies in a range of $I = 0.03$–0.06 (Epperson, 2003). Given the similarity of dispersion-pollination mechanisms, it could be expected that the two species studied (*D. caputoi* and *D. merolae*) would have relatively similar SGS, yet this was not the case. We suggest that two aspects of rarity which might have been interacting with each other since the current adult populations was established, could be relevant to our results: 1) population density, along with other processes intrinsic to the populations; and 2) the influence of habitat type, relevant in the dispersion and establishment of seeds in these species.

D. merolae, with a relatively more dense population (ca. 100 Ind./ha) shows a lower SGS, while *D. caputoi*, with lower density (ca. 20 Ind./ha) has higher SGS. In plant species with large population sizes and densities, the seeds of which are dispersed by gravity, it is more likely that survivors of different cohorts will be grouped within a relatively narrow space, thus diminishing genetic relations between plant groups (cf. Chung et al., 2003; Luna et al., 2005). We hypothesize that this situation occurred within the history of *D. merolae* population (Loma Colorada), which suggests that this population has held high density (relative to *D. caputoi*) since its establishment.

The dynamics of seed dispersal is the life cycle stage that determines the local genetic structure to a greater extent than that of pollen movement (Dow and Ashley, 1996; Dyer, 2007; Ortego et al., 2010). When the pollen is widely distributed and seed dispersal is highly localized, inbreeding is not expected but the spatial aggregation of seedlings can produce a significant SGS (Zhou and Chen, 2010). This is consistent with results previously reported in *D. caputoi* and *D. merolae*: no inbreeding was found, but there was a heterozygote excess (Cabrera-Toledo et al., 2008, 2010). This could be explained by a probable wider spatial dynamic of pollen, as has been reported in *Ficus cyrtophylla* (Zhou and Chen, 2010) which shows similar pollination cycles to cycads, where pollination events depend on synchrony between the receptivity of the female reproductive structures and the availability of pollen, and are not necessarily the closest plants, the phenology of which is very asynchronous (Terry et al., 2005, 2007). Thus, the movement of pollen could be promoting a high diversity at the global level while low seed dispersal promotes local structuring.

Seeds clustering in burrows, typical of cycad populations where rodents are considered secondary dispersers or even predators in some cases (Norstog and Nicholls, 1997) are also typical in *D. caputoi* and *D. merolae* populations. However we observed that the availability of seeds of different cones is higher in *D. merolae*, which increases the likelihood of grouping of unrelated seeds by the rodents. Moreover, the conditions of precipitation (665 mm) per year in Loma Colorada increase the likelihood that dispersers leave seeds in more moist sites, favorable for germination, compared with the precipitation that occurs in the localities of *D. caputoi* (310 mm), which are characterized by very dry sites with low recruitment (Cabrera-Toledo, 2009). These two ecological filters, i.e. seed limitation and establishment limitation, are critical in the successful establishment of a plant and the subsequent structuring of plant populations (Norden et al., 2009). These elemental differences in niche types of secondary dispersers can influence their dispersal efficiency (Schupp, 2007), and may be having a differential effect on both species; this is, even when in both species dispersal occurs by gravity and/or rodents, dispersal efficiency is probably greater in *D. merolae*. This offers an alternative explanation for the differences between the SGS in both species.

Finally, the results of this study help us to infer that the wide distribution of *D. merolae* (in contrast to that of *D. caputoi*) is probably not a consequence of greater dispersal, but rather of better colonization capacity. Evidently, we cannot generalize these local genetic structure values to other populations in the overall distribution of the species. As we mentioned in the Introduction, biotic and abiotic factors can influence local conditions. Fine genetic scale studies are frequently evaluated within one population regardless of the distribution range of the species, and statements derived from them are specific to the population of concern (e.g. Fuchs and Hamrick, 2010; Jacquemyn et al., 2006; Mizuki et al., 2010; Ortego et al., 2010; Yao et al., 2010). However, it is pertinent to mention that our previous research reports that genetic diversity distribution in *D. caputoi* as well as in *D. merolae*, is mostly within populations (83% and 81%, respectively) (Cabrera-Toledo et al., 2010); this means that just one population represents a great proportion of overall genetic diversity of the species.

We recommend that the recollection of seeds take place in maternal plants with low probability of genetic relation. For *D. caputoi* populations we mean, those separated by distances of not less than 20 m for the population El Guayabo, and 40 m for La Grana; for *D. merolae*, in Loma Colorada not less than 17 m. Following this procedure should diminish the chances of interbreeding and make reforestations with closely related plants.

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