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High levels of genetic variability and inbreeding in two Neotropical dioecious palms with contrasting life histories

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We characterized the population genetics of two Neotropical dioecious palm species of *Chamaedorea* with contrasting life strategies from the region that is both the northernmost extent and most species rich of the genus. *Chamaedorea tepejilote* is a common, wind-pollinated arboreal understory palm. Although most adult plants reproduce each year, only a few individuals produce the majority of flowers and seeds. *Chamaedorea elatior*, conversely, is an uncommon climbing subcanopy palm with entomophilous flowers (insect-pollinated characteristics). Most of the mature palms do not reproduce in consecutive years and fruiting is episodic. Isozymes with a total of 107 alleles for 27 loci of 17 enzymes from six populations were assessed. For both species, co-occurrence of high levels of genetic variation and homozygosity was observed (*C. tepejilote*: *H*e: 0.385–0.442, *f*: 0.431–0.486; *C. elatior*: *H*e: 0.278–0.342, *f*: 0.466–0.535). Genetic differentiation of *C. elatior* was much lower ($\theta = 0.0315$) than that for *C. tepejilote* ($\theta = 0.152$). The contrast in differentiation may be influenced by differences in the spatial scale of the genetic neighborhoods of the two species. The simultaneous maintenance of inbreeding and of a large number of alleles within the populations is attributable to the low and variable number of mating pairs. Demographic studies indicate that this pattern could be explained by low reproductive frequency among individuals and over years in *C. elatior* and by reproductive dominance in *C. tepejilote*.
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Introduction

The genetic reservoirs that are present in remnant tropical rainforests have been strongly influenced by the constantly changing status of their populations over relatively recent time periods as well as by the long-term evolutionary history of the species. Genetic variation patterns have resulted from diverse processes at different spatial and temporal scales. In Neotropical rainforests, genetically distinct regional populations of some plant species have been found among areas that differ in biogeographic history, suggesting long-term regional isolation (Aide and Rivera, 1998; Hamilton, 1999; Dick *et al*., 2003; Novick *et al*., 2003). Geographically peripheral populations have sometimes shown distinctive genetic patterns (Lavin *et al*., 1991; Chase *et al*., 1995; Gillies *et al*., 1999; Cavers *et al*., 2003). The evolutionary connectedness between populations depends on their location within the species range. For example, edge effects, such as low genetic variation or geographic distinctiveness, have been reported for most northerly populations of species that migrated into North America from South America (Chase *et al*., 1995; Chamberlain, 1998; Cavers *et al*., 2003). Conversely, species in groups that diversified in North Mesoamerica could be predicted to show higher levels of genetic variation than conspecific populations resulting from range expansion toward the South. At a local scale, the low genetic differentiation observed among conspecific populations of many Neotropical plants (Loveless, 1992; Hamrick, 1994; Murawski, 1995; Nason *et al*., 1996a; White *et al*., 1999; Lemes *et al*., 2003) indicates that either the populations have been derived from one another in the fairly recent past or that there are high levels of gene flow. Thus, both regional genetic differentiation and high genetic similarity at local scales depend on biogeographic history and gene flow (Caron *et al*., 2000; Novick *et al*., 2003; Dick *et al*., 2003).

For a single species, population subdivision could increase when evolutionary and ecological independence among populations is induced by landscape heterogeneity. In addition, low density or rare plants with discontinuous populations with a wide regional distribution are expected to show lower levels of genetic differentiation than high-density populations of common plants—given that the comparisons are made at similar spatial scales (Ellstrand, 1992; Cole, 2003; Degen *et al*., 2004). For example, Hall *et al* (1994a) reported substantial differentiation at a small geographic scale for the abundant tree *Pentaclethra macroloba* in comparison to *Cordia alliodora* (Chase *et al*., 1995) in areas where the overall abundance of the latter species was substantially lower than that of the former. In general, because biogeographic history and habitat heterogeneity are

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main determinants of genetic structure among populations, the genetic assessment of congeneric species in areas of overlap could clarify the influence of geographical barriers, population density or neighborhood size on population genetic differentiation.

The genetic diversity of a population is generally reorganized through mating and recombination following colonization, and then structured through localized genetic drift and possibly selection (Wright, 1978; Lefèvre *et al*., 2004). After a species' initial establishment, most recruitment is from local sources (Olivieri and Gouyon, 1997) resulting in an enduring genetic patch structure (Wright, 1943). One major effect of such structure is the establishment of genetic isolation by distance, which depends on the effective population size (Malécot, 1948; Sokal and Wartenberg, 1983; Epperson *et al*., 1999). In obligate outcrossing species, homozygote excess within populations ensues from mating between neighboring relatives (Epperson and Álvarez-Buylla, 1997), temporal reproductive isolation (Robertson, 1964; Hendry and Day, 2005), episodic reproductive events (Epperson and Chung, 2001) and nonrandom dispersal (Garant *et al*., 2005; Postma and Noordwijk, 2005). Thus, all those forms of nonrandom mating, and their implications for recombination and gene movement, have a major effect on genetic variation patterns of outcrossing plants. Even for populations having high numbers of reproductive adults, effective population sizes may be small due to asynchronous reproduction (for example, Hall *et al*., 1994a; Chase *et al*., 1996; Degen *et al*., 2004).

This article reports a population genetic study of two demographically well-characterized species of *Chamaedorea*, the most speciose genus of neotropical palms (Henderson *et al*., 1995). The study species, *Chamaedorea elatior* and *Chamaedorea tepejilote*, are both long-lived, dioecious obligate-outcrossers, but they differ in growth form, spatial, and geographical distributions, genetic neighborhood size and individual reproductive periodicity over years. Few comparisons of this type have been made in non-woody tropical rainforest species (Sarukhán *et al*., 1984). The research reported is part of an ongoing project on the evolutionary biology and conservation of *Chamaedorea* palms in Mexican tropical rainforest. Based on fossil pollen, it was concluded that the genus has been in the region since at least the Pliocene (Graham and Dilcher, 1998). A highly valued and overexploited non-timber resource at a world-wide scale since the 19th century (Hodel, 1992; Oyama, 1992; Vovides and Garcia-Bielma, 1994), local *Chamaedorea* populations have been going extinct from both habitat destruction and a long anthropogenic history of extraction of seeds, leaves and entire individuals from otherwise undisturbed forests (Oyama, 1992, 1993). Because effects of selective harvesting on population viability for a given species depend on conditions before management practices, an assessment of their genetic effects requires information about organization of genetic variation of pristine populations (Hall *et al*., 1994b; Nason *et al*., 1996b). In addition, the habitat heterogeneity associated with mountains has been suggested to be a major factor in regional differentiation in Mesoamerican rain forests (Dick *et al*., 2003). Accordingly, we sampled the most northerly undisturbed mountainous area (Figure 1) within the larger of the two centers of distribution of the genus (Hodel, 1992).

In this study, patterns of population genetic variation of the two study species were used to address the following questions (1) What are the levels of genetic variation of *C. elatior* and of *C. tepejilote* in a mountainous region within the area where the genus diversified? (2) What do allelic patterns reveal about the maintenance of population genetic differentiation at a local scale? (3) Do their contrasting reproductive dynamics support similar deviation levels from Hardy–Weinberg equilibrium? (4) Since dioecy within the study populations is promoting outcrossing, but there are other factors of life history that favor nonrandom mating, do populations harbor simultaneously high levels of genetic variation and inbreeding?

Materials and methods

Study system

Chamaedorea is the largest, terminal and most speciose genus of a chain of related genera of palms (Uhl and Dransfield, 1987). In this genus, vegetative characters (size, color, leaves or growth form) are highly variable among species, within populations and within individuals. Species and subgenera have been mostly diagnosed using discrete inflorescence and flower attributes (Hodel, 1992). *Chamaedorea pinnatifrons* and *C. tepejilote* are two of the more widely distributed species showing a high degree of morphological variation over an ecologically differentiated gradient with uncertain taxonomic status. Quero (1994) recognized two different species (*Chamaedorea concolor* and *C. pinnatifrons*) within the morphological variation that Hodel (1992) categorized as *C. pinnatifrons*. Hodel (1992) distinguished two species (*Chamaedorea alternans* and *C. tepejilote*) within the morphological variation that Henderson *et al*. (1995) considered to be one species, *C. tepejilote*. Recently, Bacon and Bailey (2006) showed amplified fragment length polymorphisms differentiation between these two morphological groups. They also suggested that *C. alternans* and *C. tepejilote* are not sister species (Thomas *et al*., 2006).

In this study, we selected two species showing contrasting traits of pollination, growth form, plant density and individual reproductive periodicity. They are *C. tepejilote* and *C. elatior*. Since the status of *C. tepejilote* and *C. alternans* is unresolved, we will use the name *C. tepejilote* for the latter (on the basis of nomenclatural priority).

C. tepejilote is a dioecious, arboreal palm distributed from Mexico to Colombia and reaches up to 5 m in height. Its small and greenish-yellow flowers are wind-pollinated (Otero-Arnaiz and Oyama, 2001). Several species of birds and bats eat their fruits (Trejo-Pérez, 1989) and may disperse the seeds, but fruits are mainly dispersed by gravity. Most seeds are eaten by seed predators (Oyama, 1991, 1997). Most mature individuals produce reproductive structures during consecutive years in permanent study sites established previously in the lowland rainforest Los Tuxtlas of Mexico, where *C. tepejilote* is one of the most abundant understory plants (Oyama, 1990). In contrast, *C. elatior* is a dioecious, climbing species distributed from Mexico to Honduras (Aguilar, 1986; Henderson *et al*., 1995), and reaches over 20 m in length. Insect pollination is indicated by the

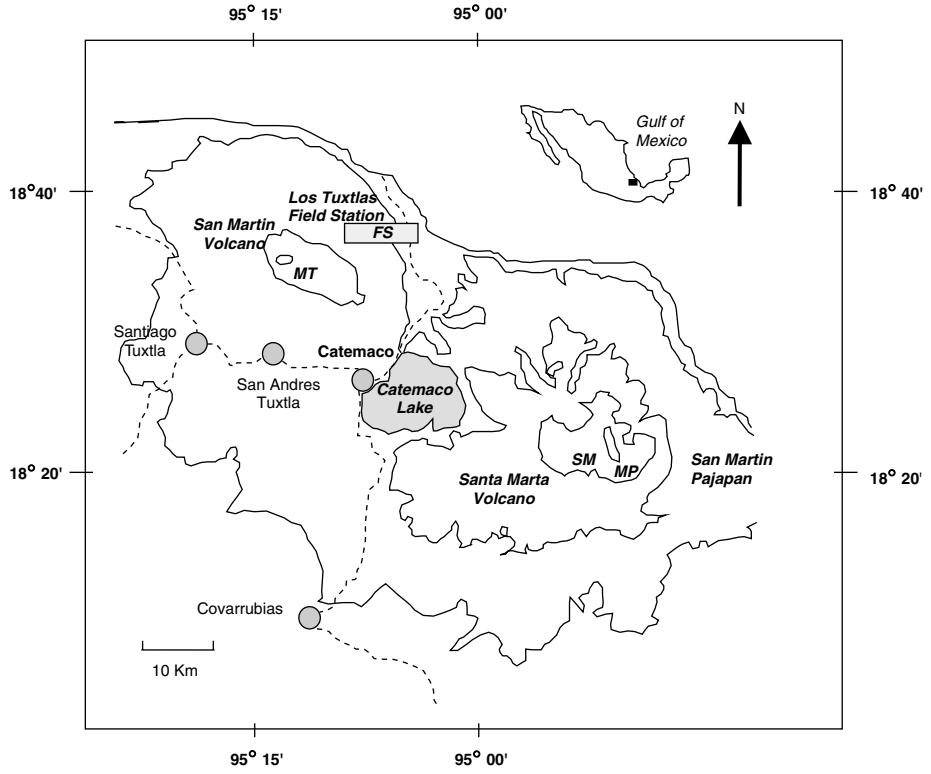

Figure 1 Map of Los Tuxtlas Region in Veracruz, Mexico showing the sampled populations of *C. elatior* and *C. tepejilote*. (1) Los Tuxtlas Field Station (FS), (2) Santa Marta (SM), (3) San Martin Pajapan (MP) and (4) San Martin Tuxtla (MT).

sticky pollen and the scented small greenish-yellow male flowers of this species (Henderson, 1986; Oyama, 1997). Pooled 4-year demographic data of one *C. elatior* population in Los Tuxtlas showed high mortality at adult stages (approximately 20%). Most reproductive individuals produced flowers or fruits only once during the study period (Luna, 1999).

Study populations and field methods

In the mountainous area of Los Tuxtlas Region (Mexico), we explored the two main massifs, Santa Marta Mountain and the San Martin Volcano (Figure 1). Annual rainfall is approximately 4700 mm and annual mean temperature is about 23°C. Full descriptions of the region are given by González *et al*. (1997). This region has extensive areas of pristine rainforest and the highest number of *Chamaedorea* species on the Atlantic coast of Mexico (Zarco-Espinosa, 1999). For each species, three undisturbed tropical rainforest areas in Los Tuxtlas were selected. For each species, individuals taller than 50 cm (juvenile and adults) located at least 5 m away from each other were sampled from rainforest patches of several hundred hectares, where a large population of either or both of the two studied species occurred. In total, 346 individuals of *C. elatior* and 347 of *C. tepejilote* were sampled. The *C. elatior* samples consisted of 197 individuals from the Los Tuxtlas Field Station 'eFS' (95°06′44″W, 18°35′22″N, 150–250 m a.s.l.), 78 from Santa Marta 'eSM' (94°58′44″W, 18°25′31″N, 1224 m a.s.l.) and 71 from San Martin Pajapan 'eMP' (94°44′28″W, 18°18′43″N, 700 m a.s.l.). For *C. tepejilote*, we collected exclusively individuals that Hodel (1992) defined as *C. alternans*, but the most recent taxonomic status sensu Henderson *et al*. (1995) is used here because this includes the morphological variation previously considered as *C. alternans*. Thus, 219 palms from Los Tuxtlas Field Station 'tFS' (95°06′16″W, 18°35′45″N, 150 m a.s.l.), 63 from Santa Marta 'tSM' (94°59′45″W, 18°25′25″N, 1224 m a.s.l.) and 65 from San Martin Tuxtla 'tMT' (95°10′49″W, 18°33′08″N, 1200 m a.s.l.) were sampled. From each individual, three pinnae from the youngest leaf were washed, placed in polyethylene bags, frozen in liquid nitrogen and stored at −70°C until processed for enzyme analysis.

At the Field Station, numbers of palms surpassed those sampled in the other localities because every palm of either *C. tepejilote* or *C. elatior* had been recorded and mapped within permanent study sites during a 4-year period (Oyama, 1990; Luna, 1999). Therefore, to standardize both the strategy and size of sampling, resampling ($N = 63$) was carried out within the long-term study sites. Thus, the first series of statistical analyses is termed 'unequal sizes' and the second 'equal sizes'.

Protein electrophoresis and statistical methods

A 1 cm² leaf tissue sample per individual was finely sliced and ground in an extraction buffer consisting of three parts YO buffer (Yeh and O'Malley, 1980) and one part Veg II buffer (Cheliak and Pitel, 1984). Enzyme analyses were carried out following the standard procedures for starch gel electrophoresis (Soltis and Soltis, 1989; Kephart, 1990). Seventeen enzymes were dyed in three buffer systems. (1) C system (Stuber *et al*., 1988) resolved: DIA (E.C. 1.6.99), G6PD (E.C. 1.1.1.49),

GDH (E. C. 1.4.1.3), EST (E.C. 3.1.1.), LAP (E.C. 3.4.11.1), GOT (E.C. 2.6.1.1), CPX (E.C. 1.11.1.7), APX (E.C. 1.11.1.7) and RUB (E.C. 4.1.1.39). (2) D system (Stuber *et al*., 1988) resolved MDH (E.C. 1.1.37), PGM (E.C. 5.4.2.2), ME (E.C. 1.1.1.40) and PGI (E.C. 5.3.1.9). (3) Morpholin-citrate (Wendel and Weeden, 1989) resolved ACPH (E.C.3.2.3.2), IDH (E.C. 1.1.1.41), 6PGD (E.C. 1.1.1.44) and SDH (E.C. 1.1.1.25). Staining protocols were modified to obtain optimal resolution of genotypes (Luna, 1999). Assignment of genotypes was consistent with the known enzyme structure (Soltis and Soltis, 1989; Wendel and Weeden, 1989). Putative loci and alleles were designated sequentially. Loci and alleles that were most anodally migrating were denoted in sequence by '1', '2', and so on.

The genetic variation of *C. elatior* and *C. tepejilote* was characterized by measuring the number of alleles (A), total alleles (A_T), exclusive alleles (A_X), effective alleles per locus ($Ae = 1/\Sigma pi^2$, where pi is the frequency of allele i (Nei, 1987)) and the percent of polymorphic loci (P). Comparisons between A and Ae among populations and between species were performed using the Wilcoxon test (Sokal and Rohlf, 1979). Genetic diversity was assessed by calculating the observed heterozygosity (Ho), the expected heterozygosity (He) under Hardy–Weinberg equilibrium 'HW' (Levene, 1949), and the Nei (1987) measures: within populations (H_S), among populations (D_{ST}), the total diversity (H_T) and the genetic differentiation coefficient (G_{ST}). Differences in levels of genetic diversity between species were tested for significance using Mann–Whitney U-tests (Sokal and Rohlf, 1979). An inbreeding coefficient per population was estimated by $f = (He - Ho)/He$, and significant deviations from HW were determined by $X^2 = f^2N(k-1)$, d.f. $= [k(k-1)]/2$, where N is the sample size and k the number of alleles (Li and Horvitz, 1953). Nei's (1972, 1978) genetic differentiation between pairs of populations was summarized in an unweighted pair group method with arithmetic mean (UPGMA) phenogram (Sneath and Sokal, 1973). Fixation indices per locus and population differentiation were tested for significance by following Markov Chain Monte–Carlo methods (Raymond and Rousset, 1995) using the tools for population genetic analyses (TFPGA) program, version 1.3 (Miller, 1997). Genetic structure was measured by f, F and θ statistics (Weir and Cockerham, 1984), which estimate F_{IS}, F_{IT} and F_{ST} (Wright, 1951), respectively. Thus, excess of homozygosity is indicated by statistically significant positive values of f and F and deficits by negative values. The θ values may indicate from equal ($\theta = 0$) up to entirely different ($\theta = 1$) allele frequencies among populations. Li and Horvitz's (1953) X^2 statistic was used to test if f and F values per locus were significantly different from zero or not, and significance of F_{ST} per locus was determined by $X^2 = 2N\ F_{ST}\ (k-1)$, d.f. $= (k-1)(s-1)$, s is the number of subpopulations (Workman and Niswander, 1970). Confidence intervals at 95% for the mean f, F and θ statistics were calculated by a bootstrap procedure and the variances of each statistic was estimated by jackknifing over loci, using the TFPGA (Miller, 1997) computer program. Gene flow was indirectly estimated from $Nm = ((1/F_{ST}) - 1)/4$ (Wright, 1951) and also from G_{ST}. Populations were geo-referenced and pairwise geographical distances were calculated in ArcView and ILWIS programs.

Results

Genetic variation

The 17 enzymes stained for gave a total of 28 banding zones (loci), and all but one of these were observed in both *C. elatior* and *C. tepejilote*. In part because samples for both species were done simultaneously and hence under the exact same gel and staining conditions, and because of consistency in inheritance patterns (Wendel and Weeden, 1989), we presume that the banding zones are the same allozyme loci. The only locus that was not shared, EST-4, was excluded from all statistical analyses. For the 27 remaining loci, 107 alleles from a total of 693 individuals in the samples of unequal sizes, and 102 alleles from 378 palms in the equalized samples were recorded. For unequal sample sizes, *C. tepejilote* showed 101 total alleles which included 13 exclusive alleles, whereas $A_T = 94$ and $A_X = 6$ from *C. elatior* were recorded, respectively. For equalized samples, A_T and A_X were equal to 94 and 13 for *C. tepejilote*; and 89 and 8 for *C. elatior*. A_T and A_X decreased from unequal to equal sampling size, as would be expected since more than one-half of the Field Station's exclusive alleles (A_X) were omitted. The distribution of exclusive, total and shared alleles is illustrated in Venn diagrams in Figure 2. Most loci had three or four alleles. After sample size standardization, the number of loci having three alleles increased but loci with four or five alleles decreased (Figure 3). Ae was substantially lower than A for each population and species ($P < 0.0001$). Thus, alleles with low frequencies were numerous and contributed substantially to allelic diversity (A_T). In the case of *C. tepejilote*, approximately 49–52.5% of the alleles had frequencies less than 0.21 and only 4–5% of the alleles had frequencies greater than 0.81 (Figure 4). Forty-three or 44 alleles (44 or 46%, respectively) had frequencies between 0.21 and 0.8. For *C. elatior*, the majority of alleles (57 or 62%) occurred at low frequencies, with 26–27% being higher than 0.61 and few alleles (11 or 15) with intermediate frequencies (Figure 4). The A values did not differ significantly between species, but those for Ae did ($Z = 4.52$, $P < 0.0001$). Mean Ae value was about 1.5 for *C. elatior* and 2.0 for *C. tepejilote*. The percent of polymorphic loci was very high for every population (Table 1). Only one locus, RUB-1, was monomorphic in all populations. In *C. tepejilote*, polymorphism ranged from 92.6 to 96.3%; and in *C. elatior* from 81.5 to 88.9. The highest population-specific level of monomorphism was observed in the eMP population: six out of 27 loci were monomorphic. High gene diversity in total population (H_T) and within populations (H_S) was observed for both *C. elatior* ($H_T = 0.340$ and $H_S = 0.329$) and *C. tepejilote* ($H_T = 0.483$ and $H_S = 0.426$), although both H_T and H_S were significantly greater in *C. tepejilote* (Mann–Whitney U-test, $P < 0.01$).

Genetic structure

Allele frequency heterogeneity per locus among populations was significant at $P < 0.05$ for most loci: 21 of the 26 loci from the three populations of *C. elatior*, and 23 of the 27 for those of *C. tepejilote*. Exact tests for population differentiation over all populations and loci were significant for each species at $P < 0.0001$. The analyses of all possible pairwise population comparisons (1 vs 2,

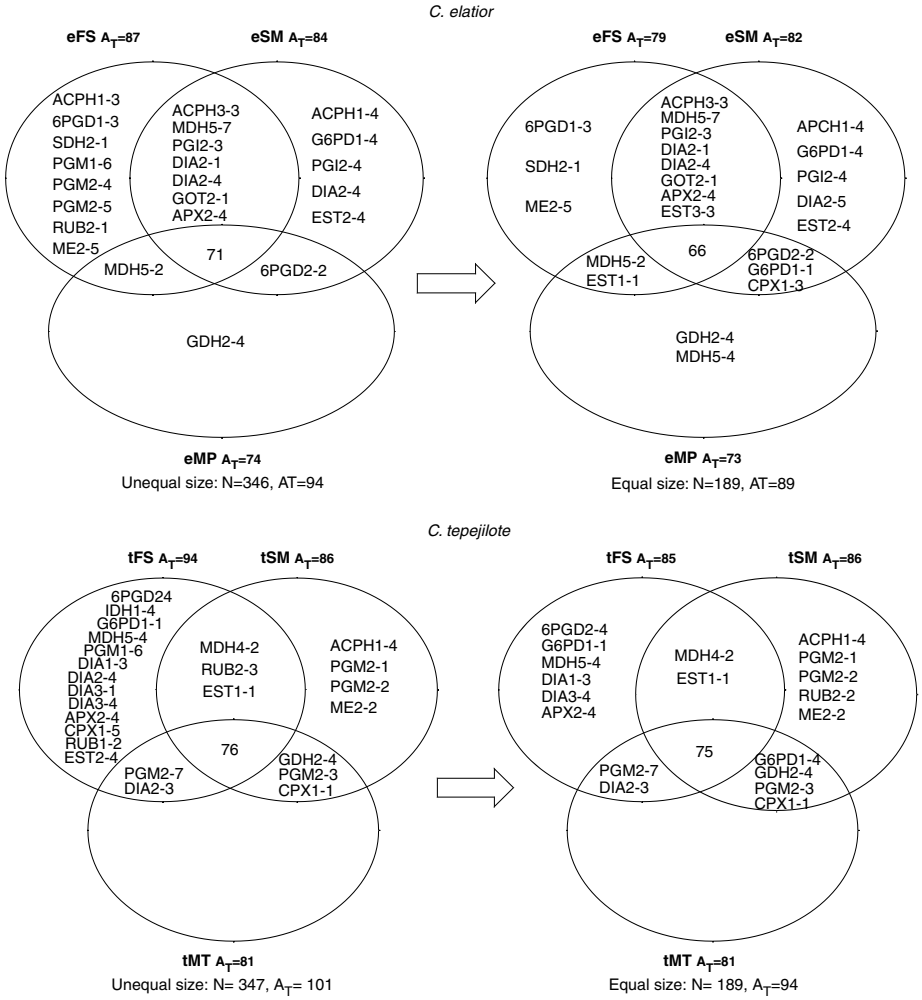

Figure 2 Allelic distribution in populations of *C. elatior* and *C. tepejilote* at Los Tuxtlas, Mexico. A_T = total alleles.

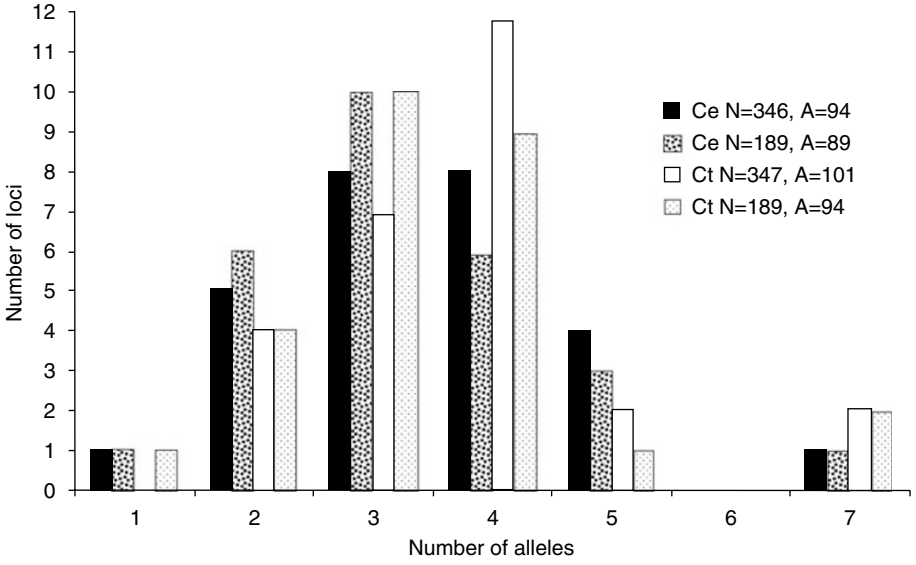

Figure 3 Number of alleles per locus for the populations of *C. elatior* and of *C. tepejilote* studied at Los Tuxtlas, Mexico.

1 vs 3 and 2 vs 3) also showed high levels of significance ($P<0.0001$). For *C. elatior*, 10 non-significant 'ns' loci and 16 significant 's' loci between eFS and eSM populations were found; 10 ns and 16 s, between eFS and eMP; and 21 ns and 5 s for eSM and eMP. In the case of *C. tepejilote*, the non-significant and the significant numbers of loci for

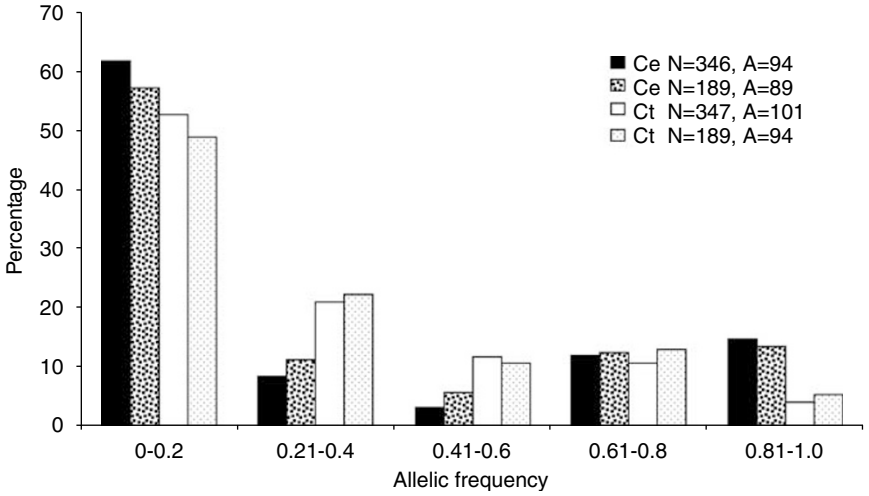

Figure 4 Distribution of allelic frequencies for *C. elatior* and *C. tepejilote* at Los Tuxtlas, Mexico.

Table 1 Genetic variation and differentiation between populations of *C. elatior* and of *C. tepejilote* in a lowland rainforest at Southern Mexico

	Chamaedorea elatior			Chamaedorea tepejilote		
	EFS	*eSM*	*eMP*	*tFS*	*tSM*	*tMT*
A	2.93 (0.21)	3.04 (0.19)	2.70 (0.20)	3.15 (0.20)	3.19 (0.21)	3.00 (0.20)
Ae	1.64 (0.14)	1.61 (0.13)	1.48 (0.13)	1.72 (0.13)	1.91 (0.14)	1.87 (0.15)
P	88.9	88.9	81.5	92.6	96.3	92.6
Ho	0.159 (0.024)	0.181 (0.026)	0.143 (0.027)	0.219 (0.028)	0.235 (0.027)	0.219 (0.029)
He	0.342 (0.036)	0.339 (0.033)	0.278 (0.036)	0.385 (0.031)	0.442 (0.032)	0.426 (0.035)
f	0.535***	0.466***	0.486***	0.431***	0.468***	0.486***
eFS	—	0.0235	0.0246			
eSM	23086	—	0.0066			
eMP	49849	28201	—			
tFS				—	0.1825	0.0371
tSM				22225	—	0.1881
tMT				9481	24418	—

In the upper section, the mean population values of alleles (*A*), effective alleles (*Ae*), percentage of polymorphic loci (*P*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Los Tuxtlas Field Station (tFS), Santa Marta (tSM), San Martin Tuxtla (tMT) and the inbreeding coefficient *f* are shown. In the lower section, Nei's (1978) genetic distance is indicated above the diagonal, while geographical distances (in meters) are below the diagonal.
Standard error is shown within parentheses.
***$P < 0.001$.

each pairwise comparison were: 7 ns and 20 s between tFS and tSM, 12 ns and 15 s between tFS and tMT, and 12 ns and 15 s between tSM and tMT. *C. tepejilote* showed higher values of Nei's (1978) genetic distance than did *C. elatior* (Table 1), the lowest value (0.007) of which occurred between eSM and eMP. This node was supported by 22 loci (81.5%). For *C. tepejilote*, the pair of tFS and tMT populations showed the lowest distance (0.037), with a consistency index of about 37% and 10 loci supporting this node. Nei's (1972) genetic similarity supports the idea that genetic heterogeneity between populations of *C. tepejilote* is larger than that between populations of *C. elatior* (Figure 5). For instance, the smallest genetic distance (0.0371) between the two geographically closest populations of *C. tepejilote* is higher than that between the farthest populations of *C. elatior* (0.0371 vs 0.0246), even when Euclidean distance (9481 vs 49 849 m) is almost fivefold longer for the latest populations (Table 1).

Based on a total of 156 fixation indexes calculated, excess homozygosity was indicated by significantly positive *f* estimates in 62 (79.5%) of 78 cases for the *C. elatior* populations and 58 (74.4%) of 78 for the *C. tepejilote* populations. Overall significant deficits of heterozygotes were found for every population. For both species, excess of homozygous individuals was further indicated by the positive F_{IT} and F_{IS} values of most loci (Table 2). For the *C. elatior* populations, $\theta = 0.0315 \pm 0.005$ and for the *C. tepejilote* populations, $\theta = 0.152 \pm 0.035$. In addition, the absolute difference among populations (D_{ST}) and the coefficient of relative differentiation (G_{ST}) were 0.011 and 0.033, respectively, for *C. elatior*; and 0.057 and 0.118 for *C. tepejilote*. Thus, most genetic variation (96.7 and 88.2%, respectively) was found within populations. The mean estimates of gene flow using G_{ST} and F_{ST} were from 1.4 to 1.9 for *C. tepejilote* and from 7.3 to 7.7 for *C. elatior*. Pairwise comparisons of D_{ST}, G_{ST} and *N*m between species were significant (Mann–Whitney *U*-test, $P < 0.05$).

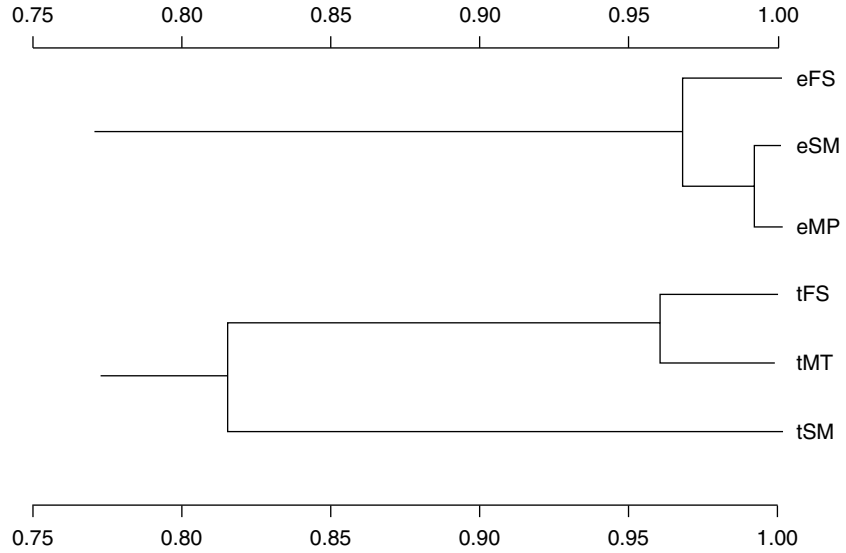

Figure 5 Nei's (1972) genetic similarity among populations of *C. elatior* and *C. tepejilote* at Los Tuxtlas, Mexico.

Table 2 Wright's F statistics for *C. elatior* and of *C. tepejilote* based on 27 loci from populations studied at Los Tuxtlas, Mexico

Locus	Chamaedorea elatior			Chamaedorea tepejilote		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
ACPH-1	0.4369***	0.4293***	−0.0134	0.6054***	0.7600***	0.3918***
ACPH-3	0.2725***	0.2966***	0.0331***	0.5659***	0.5975***	0.0727***
6PGD-1	0.6771***	0.6879***	0.0335*	0.4430***	0.4397***	−0.0059
6PGD-2	0.1421	0.1582	0.0188*	0.2083*	0.4360***	0.2876***
IDH-1	0.7842***	0.7977***	0.0625***	0.6202***	0.6614***	0.1086***
SDH-2	0.6327***	0.6356***	0.0079	0.3703***	0.4296***	0.0942***
G6PDH-1	0.2136***	0.2181***	0.0057	0.1538*	0.1510	−0.0033***
GDH-2	0.4317***	0.4497***	0.0317***	0.5912***	0.6338***	0.1042***
LAP-2	0.2751***	0.2734***	−0.0024	0.4887***	0.5169***	0.0551***
MDH-4	0.4563***	0.4818***	0.0468***	0.5477***	0.5467***	−0.0022
MDH-5	0.4454***	0.4599***	0.0261***	0.3243***	0.3662***	0.062***
PGI-1	0.8991***	0.9097***	0.1057***	0.9273***	0.9296***	0.032***
PGI-2	0.5076***	0.5209***	0.0269***	0.2646***	0.2948***	0.041***
PGM-1	0.7822***	0.7884***	0.0288**	0.6591***	0.6649***	0.0171*
PGM-2	0.6819***	0.7033***	0.0672***	0.1807*	0.5401***	0.4387***
DIA-1	0.6828***	0.6946***	0.0372**	0.6702***	0.7403***	0.2123***
DIA-2	0.7713***	0.7728***	0.0069	0.5886***	0.7884***	0.4856***
DIA-3	0.6518*	0.6501***	−0.0048	0.6626***	0.6832***	0.061**
GOT-2	0.1973**	0.2122***	0.0186**	−0.0137	0.0068	0.0202*
APX-2	0.4092***	0.4244***	0.0258***	0.6500***	0.6560***	0.0171**
CPX-1	0.4312***	0.4550***	0.0419***	0.5805***	0.6777***	0.2316***
RUB-1						
RUB-2	0.6399***	0.6758***	0.0997***	0.4807***	0.4794***	−0.0024
ME-2	0.6986***	0.7087***	0.0335***	0.6384***	0.6613***	0.0632***
EST-1	0.3604***	0.3741***	0.0214	0.1534	0.1567	0.0039
EST-2	0.3616***	0.3722***	0.0166*	0.2224*	0.2663**	0.0564***
EST-3	0.4789***	0.4786***	−0.0006	0.3822***	0.6111***	0.3705***
Mean	0.5012***	0.5169***	0.0315***	0.4434***	0.5274***	0.1509***
SD	0.0425	0.0427	0.0051	0.0425	0.0403	0.0352
95% CI	0.4219–0.5838	0.4368–0.6001	0.0223–0.0419	0.3619–0.5226	0.4485–0.6018	0.0856–0.2178

Statistical test according to Li and Horvitz (1953) for F_{IS} and F_{IT}; and according to Workman and Niswander (1970) for F_{ST}.
***$P<0.001$; **$P<0.01$; *$P<0.05$.

Discussion

C. tepejilote and *C. elatior* showed very high levels of isozyme variation in comparison to values in other plants. Polymorphism and expected heterozygosity for *C. tepejilote* (*P*: 92.6–96.3, *He*: 0.385–0.442) and *C. elatior* (*P*: 81.5–88.9, *He*: 0.278–0.342) substantially exceed the corresponding average values (*P*: 65.5 and *He*: 0.180) observed for other long-lived perennial outcrossing species (Hamrick and Godt, 1997). Both high numbers of exclusive alleles and very large numbers of total alleles within populations were also found. Deficits of heterozygotes were also observed for most loci in all study populations. In contrast, less than one-tenth of the

genetic variation occurred among populations, which would normally be taken to indicate high levels of gene flow. It is probably that other life history factors, such as the influence of biogeographic history and current gene dispersal have affected the standing genetic patterns of *C. elatior* and *C. tepejilote*.

Both theoretical and empirical studies provide reasons to expect that dioecy, perenniality and wide distributions contribute to minimize population differentiation and slow the processes of genetic erosion in *C. elatior* and *C. tepejilote*. *C. tepejilote* showed higher values of genetic variation within and among populations than *C. elatior*. Our findings are in agreement with both the highest *P* and the lowest G_{ST} values reported for long-lived outcrossing plants, as well as lower G_{ST} values documented for tall plants with low population density relative to plants with the opposite traits (Hamrick and Godt, 1997; Takeuchi *et al*., 2004). Similar to *C. elatior*, Neotropical populations of *Swietenia humilis* (White *et al*., 1999), *Carapa guianensis* (Dayanandan *et al*., 1999) and *Symphonia globulifera* (Aldrich *et al*., 1998) have also been shown to have low genetic differentiation and high allelic diversity. In addition to *C. tepejilote*, moderate genetic differentiation and high genetic variation within populations were reported for *P. macroloba* (Hall *et al*., 1994a), *Swietenia macrophylla* (Gillies *et al*., 1999; Lemes *et al*., 2003) and *Caryocar brasiliense* (Collevatti *et al*., 2001a). The larger number of exclusive alleles within *C. tepejilote* populations is consistent with the G_{ST} measures in indicating more-restricted gene flow in comparison to that of *C. elatior*.

It is important to compare these results to earlier findings on a very different spatial scale between contiguous adults within localized populations. It was found that while genetic autocorrelations were low in both species, values for *C. elatior* were somewhat greater than *C. tepejilote* (Luna *et al*., 2005). Consequently, at the localized scale (on the order of 100's of meters to a few kilometers), there is greater genetic differentiation for *C. elatior* than *C. tepejilote*, opposite the present results. The values corresponded to those expected for Wright's neighborhood sizes of 100 reproductive individuals for *C. elatior* and 300 reproductive individuals for *C. tepejilote*. If density were ignored, this finding would imply that dispersal distance is more limited in *C. elatior* than *C. tepejilote*. However, Wright's neighborhood is always relative to density, which differed greatly between the two species, being much higher in *C. tepejilote*. The areas occupied by neighborhoods are estimated at 1.0 Ha for *C. tepejilote* vs 13.2 Ha for *C. elatior*. Thus in the sense used by Wright (1943), the area over which there is effective gene flow is much larger in *C. elatior*. If one projects these results to the much greater spatial distances between the study populations examined here, then they are consistent with observed lesser differentiation for *C. elatior*. In other words, the almost threefold higher F_{ST} value among the populations of *C. tepejilote* can be explained by differences in the spatial scale over which the two species' neighborhood sizes are harbored. Finally, we note that criteria for obtaining unbiased values of population genetic differentiation for among-species comparisons remained unclear (Cole, 2003), although one relative measure of population genetic differentiation has been recently developed (Hedrick, 2005).

The spatial scale of sampling is typically related to the estimated magnitude of population structuring. Low genetic differentiation has been found for many tropical tree species sampled within a single natural population or among multiple populations separated by less than 100 km (O'Malley and Bawa, 1987; Hamrick and Loveless, 1989; Murawski and Bawa, 1994; Boshier *et al*., 1995; Hall *et al*., 1994b, 1996; Nason *et al*., 1996b), but high population differentiation has also been detected at similar geographical scales (Hall *et al*., 1994a). At a regional level, *C. alliodora* showed significant differences between Atlantic and Pacific populations, although the overall amount of genetic structure was low (Chase *et al*., 1995). For *C. elatior* and *C. tepejilote*, the significant genetic heterogeneity among populations supports the idea that local demes may deviate significantly from the population mean allele frequency even if genetic differentiation equals 0.5 (Wright, 1978).

In marked contrast to the general expectation and usual observation that the lowest levels of inbreeding in plants occur in obligate outcrossing species, large excesses of homozygotes were observed for both species, and these occurred consistently among loci and populations. Moreover, the levels of inbreeding are far higher than those that would be expected from simple forms of mating by spatial proximity (Epperson, 1990). Detailed demographic data from the permanent sites at the Field Station have demonstrated that both species blossom once a year (Oyama, 1990; Luna, 1999). In the case of *C. tepejilote*, although almost all adult plants reproduced every year, only a few individuals produced most of seeds and did so consistently during four successive years (Oyama, 1990). Moreover, strong winds that transport the copious pollen that is released during the storm season could enhance mating-pair heterogeneity. In any case, the strong reproductive dominance displayed by small numbers of individuals may greatly increase the genetic similarity among contemporary offspring. A contrasting pattern was apparent in *C. elatior*. Almost all fruits that developed were on palms that reproduced only one specific year. Most of reproductive palms (68%) flowered only once during the 4-year study period. The majority of seeds were produced by about one-tenth of the female plants. Given the small number of maternal plants, the seeds produced each year are highly related and, differ from other years. This mating pattern would contribute to mating-pair heterogeneity in the long term, which is otherwise unexpected for populations having small effective sizes.

The role of male reproduction is also important. For *C. tepejilote*, there is a unimodal temporal distribution of pollination activity (Otero-Arnaiz and Oyama, 2001) and some male individuals remained reproductively dominant (that is, produce larger numbers of male flowers) over the 4-year period (at least). These effects will combine to produce unequal individual contributions to the population genetic structure and even greater inbreeding. Such temporal restriction on gene flow within populations composed of a mixture of individuals that reproduce at different—and often heritable—times has been called 'isolation by time' (Hendry and Day, 2005).

Robertson (1964) examined the case where a population effectively splits into sublines and there is some mixing of the sublines. Within sublines, inbreeding and

genetic drift are increased, but genetic drift of the entire population is decreased over the long term. The demographic data indicate that this type of population structure may occur in both study species, for slightly different reasons. The sublines would maintain both high levels of polymorphism and explain the homozygote excess (Sonesson and Meuwissen, 2001).

Various environmental heterogeneity and genetic factors have been proposed to explain interindividual variability in growth, survivorship and reproduction (Kohyama, 1981; Solbrig, 1981; Primack and Antonovics, 1982; Sarukhán *et al*., 1984; Oyama, 1990; Peters, 1991; Hendry and Day, 2005). Studies of five tropical arboreal species (Bullock, 1982; Piñero and Sarukhán, 1982) found high reproductive output for a minority of individuals. It was suggested that other individuals would later emerge as frequent reproducers. In the case of the local abundant species *P. macroloba*, Hall *et al*. (1994a) hypothesized that asynchronous reproduction resulted in a small genetic neighborhood. Degen *et al*. (2004) also found low effective density (1.3–1.6 trees/Ha) within a population of *S. globulifera* having a high adult density. In that study, and in *Acacia melanoxylon* (Muona *et al*., 1991), non-random mating enhanced by a limited number of pollen donors was suggested. For *Pithecellobium elegans* (Chase *et al*., 1996), temporal variation of individual flowering was reported, in which the pollen pool was dominated by some particular individuals that produced massive flowers during one period, but then were minimal contributors to the pool in other years. Recently, high levels of polymorphism together with significant levels of inbreeding have been demonstrated in microsatellite studies of the tropical plants *S. globulifera* (Aldrich *et al*., 1998; Degen *et al*., 2004), *S. humilis* (White *et al*., 1999), *Melaleuca alternifolia* (Rossetto *et al*., 1999), *S. macrophylla* (Lemes *et al*., 2003; Novick *et al*., 2003) and within juvenile cohorts of *Elaeocarpus grandis* (Rossetto *et al*., 2004). In addition, heterozygote deficits in tropical plants have been found in populations of *Piper amalago* (Heywood and Fleming, 1986), *Austromyrtus* spp. (Shapcott and Playford, 1996), *Syzygium nervosum* (Shapcott, 1998), *Ancistrocladus korupensis* (Foster and Sork, 1997), *Capsicum annuum* (Hernández-Verdugo *et al*., 2001), *Escontria chiotilla* (Tinoco *et al*., 2005) and the dioecious species *Chamaedorea tuerckheimii*, *Carica papaya* and *Carica cauliflora* (Oyama *et al*., unpublished data). Inbreeding was also detected in populations of the trees *Pithecellobium pedicellare* (O'Malley and Bawa, 1987) and *Bertholletia excelsa* (O'Malley *et al*., 1988). Inbreeding has often been attributable to self-fertilization or kin mating. Within populations showing flowering synchrony, a Wahlund effect as generator of nonrandom mating has sometimes been discounted (Novick *et al*., 2003). However, as we have suggested here, synchronization at the population level does not exclude reproductive variability at the individual level. In other cases, an excess of homozygotes was not initially attributed to inbreeding (Collevatti *et al*., 2001a), but was later confirmed (Collevatti *et al*., 2001b). Accordingly, long-term studies that assess the effects of individual reproductive variation on population genetic structure can reveal critical aspects of the mating system and their effects on the structure of genetic variation. As comparable analyses become available, the importance of reproductive isolation by time and its influence on levels of identity by descent should be clarified.

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