



Conservation implications of the mating system of the Pampa Hermosa landrace of peach palm analyzed with microsatellite markers

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Abstract

Peach palm (*Bactris gasipaes*) is cultivated by many indigenous and traditional communities from Amazonia to Central America for its edible fruits, and is currently important for its heart-of-palm. The objective of this study was to investigate the mating system of peach palm, as this is important for conservation and breeding. Eight microsatellite loci were used to genotype 24 open-pollinated progenies from three populations of the Pampa Hermosa landrace maintained in a progeny trial for genetic improvement. Both the multi-locus outcrossing rates (0.95 to 0.99) and the progeny level multi-locus outcrossing rates (0.9 to 1.0) were high, indicating that peach palm is predominantly allogamous. The outcrossing rates among relatives were significantly different from zero (0.101 to 0.202), providing evidence for considerable biparental inbreeding within populations, probably due to farmers planting seeds of a small number of open-pollinated progenies in the same plot. The correlations of paternity estimates were low (0.051 to 0.112), suggesting a large number of pollen sources (9 to 20) participating in pollination of individual fruit bunches. Effective population size estimates suggest that current germplasm collections are insufficient for long-term *ex situ* conservation. As with most underutilized crops, on farm conservation is the most important component of an integrated conservation strategy.

Keywords: *Bactris gasipaes*, bi-parental inbreeding, genetic improvement, genetic conservation, outcrossing.

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Introduction

The mating system and pollen and seed dispersal mechanisms influence levels of genetic diversity within and among plant populations (Hamrick *et al.*, 1993), contributing to their genetic structure. Hence, studies of the mating system and gene flow are fundamental to support both genetic improvement (Allard, 1960) and conservation (Frankham *et al.*, 2004) programs in plants. Estimates of outcrossing rates and the number of pollen donors within and among progenies and populations can elucidate the genetic structure of these populations, aid in the choice of the most suitable method for estimating genetic parameters,

and contribute to the study of the inheritance of quantitative traits, selection of superior genotypes, and determination of sample sizes for germplasm collections. Conservation strategies are likely to become more complex and difficult to manage as climate change accelerates during this century (Jarvis *et al.*, 2008; Maxted *et al.*, 2008), and information on the mating system is essential to contribute to this conservation effort.

The peach palm (*Bactris gasipaes* Kunth) is the only domesticated Neotropical palm (Clement, 1988; Mora Urpí *et al.*, 1997). It was domesticated for its fruit, which are drupes with an edible starchy-oily pulp surrounding the single seed. The fruits are cooked in salted water to eliminate anti-nutritional factors and consumed as snacks throughout its range (Clement, 2008). It was once a staple food for Native American populations from western Amazonia to southern Central America (Mora Urpí *et al.*, 1997).

Peach palm is increasingly important as a cultivated source of high quality hearts-of-palm, and the respective agribusiness is undergoing strong expansion, mainly in south-eastern Brazil, Costa Rica and Ecuador (Clement, 2008; Graefe *et al.*, 2013). Commercial propagation of peach palm uses open-pollinated seeds (Mora Urpí *et al.*, 1997). The majority of seeds available in the market today originate from the Pampa Hermosa landrace, Yurimaguas, Peru (Alves-Pereira *et al.*, 2012), as the spineless plants are more easily handled and more productive than those of other landraces (Mora Urpí *et al.*, 1999). The expansion of the hearts-of-palm plantations already constitutes a viable alternative to the unsustainable exploitation of hearts-of-palm from wild palms, such as the endangered *Euterpe edulis* in the Atlantic forest of southern and southeastern Brazil.

Peach palm phenology is strongly seasonal, with scattered flowering at the end of the Amazonian dry season, a high degree of synchrony at the beginning of the rainy season, and scattered flowering as rains become more abundant (Mora Urpí *et al.*, 1997). The whole flowering period lasts two to three months. The monoecious inflorescences appear in the axils of senescent leaves, each with thousands of unisexual staminate and hundreds of pistillate flowers (Mora Urpí and Solís, 1980). Owing to protogyny, the difference between female and male anthesis in the same inflorescence (24 hours), the peach palm is considered to be predominantly outcrossed. There are three pollination mechanisms (Mora Urpí and Solís, 1980): the first and most important is mediated by small curculionid weevils of the genera *Andranthobius* and *Phyllotrox*, which have limited flight ranges (100 e 500 m); the second is wind; and the third, gravity.

Although outcrossing predominates in the species, self-pollination may occur. A system of genetic self-incompatibility was suggested (Mora Urpí and Solís, 1980), although Clement and Arkcoll (1984) observed that self-compatibility varied from 0% to 88% among individual trees in the Putumayo landrace with controlled pollination. Because peach palm is caespitose (multi-stemmed), there is a possibility of natural self-pollination among stems in the same clump. In addition, Clement (1988) observed that the traditional agricultural practice is to plant various seedlings from open-pollinated progenies in the same plot, which favors mating among relatives. Cole *et al.* (2007) detected considerable inbreeding ($F = 0.105-0.210$) in peach palm populations belonging to peasant and indigenous communities in northeastern Peru, confirming that traditional practice favors a certain level of biparental inbreeding, even though there is significant gene flow mediated by farmer seed sourcing (Adin *et al.*, 2004). Alves-Pereira *et al.* (2012) found similar levels (0.156-0.210) within the Pampa Hermosa landrace with a larger sample. Generations of generally unconscious selection for yield will maintain productivity even as heterozygosity decreases due to inbreeding (Hancock, 2004).

Microsatellite markers are considered ideal for studying mating systems as they exhibit simple Mendelian inheritance, are multi-allelic and have co-dominant expression (Powell *et al.*, 1996). Studies on mating systems using microsatellites have been conducted for numerous Neotropical tree species (Ward *et al.*, 2005; Lemes *et al.*, 2007; Carneiro *et al.*, 2007), and pan-tropical (Akuba *et al.*, 2009) and Neotropical palms (Eguiarte *et al.*, 1992; Gaiotto *et al.*, 2003; Conte *et al.*, 2008; Abreu *et al.*, 2011; Nazareno and Reis, 2012). Mating system information is especially important for *ex situ* conservation. In coconut (*Cocos nucifera*), the tall varieties are strongly outbreeding while the dwarf varieties are strongly inbreeding, which led to a recommendation of different sample sizes for population conservation (Konan *et al.*, 2008): 90 plants from at least 40 seed parents for tall populations and 45 plants from at least 10 seed parents for dwarf populations. No such study has been done in peach palm to date and this one will provide important new information to guide future improvement and conservation.

The objective of this study was to investigate the mating system of peach palm using microsatellite markers in order to provide information to interpret the genetic structure of progenies and populations of the Pampa Hermosa landrace, and to assist planning in heart-of-palm improvement and *ex situ* conservation programs. The following questions were addressed: *i*) What are the outcrossing, mating among relatives, and paternity correlation rates in the populations? *ii*) What are the effective number of pollen donors, average co-ancestry coefficients and the variance effective sizes within progenies and populations? *iii*) What are the conservation implications of this information, both *in situ* and *ex situ*?

Materials and Methods

Plant material

A progeny trial with 257 open-pollinated progenies collected at peak fruiting season from populations in traditional communities along three rivers (Cuiparillo, Shanusi, Paranapura) in the region of Yurimaguas, Peru, was established in 1991 at the National Research Institute for Amazonia's Tropical Fruit Experimental Station, 38 km north of Manaus, Amazonas, Brazil. The original collection concentrated on plants with spineless stems and leaf petioles identified in home gardens and swidden plots in traditional and indigenous communities, occasionally with plants collected in the same community within pollination distance of each other. The sampling for our analysis concentrated on a sub-set of the trial with 24 progenies selected for length of the heart-of-palm, including nine progenies from the Cuiparillo River ($n = 121$ plants), seven from the Shanusi River ($n = 133$) and eight from the Paranapura River ($n = 105$) (see Table 1 for individual progeny numbers).

Microsatellite analysis

DNA was extracted from 100 mg of apical meristem of a lateral sucker using DNAzol Kit (Life Technologies) and DNA quantity was estimated by comparison with phage λ (100 ng/ μ L, Fermentas) known standard concentrations in 0.9% ethidium bromide-stained agarose gels. Microsatellite loci amplifications were performed by PCR using eight pairs of primers developed for peach palm by Rodrigues *et al.* (2004). The PCR mixture (10 μ L) contained 1X PCR buffer (10 mM TrisHCl, 50 mM KCl, pH 8.4), dNTP (250 μ M), MgCl₂ (2.5 mM), primer forward tail M13 (0.25 μ M), forward primer M13 label with FAM or HEX or TAMRA fluorochromes (0.25 μ M), reverse primer (0.5 μ M), *Taq* DNA polymerase (Lambda Gene, LGC) (1.5 U), and 15 ng of genomic DNA. The amplifications were performed in an MJ Research PTC-100 thermal cycler according to the conditions described in Rodrigues *et al.* (2004). Fluorescence-labeled PCR products were analyzed in an automated sequencer (Mega BACE 1000). Alleles were sized using ET-ROX-400 size standard (GE Healthcare, England). Data collection and analysis were performed using Genetic Profiler and Fragment Profiler software (GE Healthcare, England).

Data analysis

Micro-checker (Van Oosterhout *et al.*, 2004) was used to identify possible null alleles and other genotyping errors. There was a suggestion of the presence of null alleles at some loci in some progenies in one population, which was corrected by elimination of suspected individuals. Possible linkage disequilibrium was evaluated between all loci pairs in each population using a G-test. Statistical significance of LD was calculated based on 1,000 permutations using the Bonferroni correction for $p = 0.05$. These analyses were run using FSTAT 2.9.3.2 (Goudet, 2002).

Mating system analysis was based on the mixed mating (Ritland and Jain, 1981) and correlated mating models (Ritland, 1989, 2002) using the multilocus mating system program MLTR (Ritland, 2004). The EM (Expectation-Maximization) algorithm was used due to the small number of progenies analyzed per population, as suggested by Ritland (2004). We estimated the following mating system parameters: mean multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), fixation index of maternal parents (F), correlation of paternity (r_p), correlation of selfing (r_s), and the correlation of t among loci ($r_{t(t)}$). All parameters were estimated at the population level and t_m was also estimated at the individual progeny level. Standard errors (SE) were estimated by bootstrapping over progenies using 10,000 replicates.

Biparental inbreeding was determined by the difference between the multilocus and single-locus outcrossing rates ($t_m - t_s$). The correlation of paternity measures the proportion of outcrossed offspring that are full-sibs, the corre-

lation of selfing measures the individual variation in outcrossing rate (Ritland, 1989), the correlation t among loci corresponds to the fraction of inbreeding due to uniparental inbreeding (selfing), and $(1 - r_{t(t)})$ to the fraction of inbreeding due to the biparental inbreeding.

The effective number of pollen donors was estimated by $N_{ep} = 1/r_{p(m)}$ (Ritland, 1989). The coefficient of co-ancestry among plants within progenies (Θ) was estimated from the coefficient of correlation of relatedness among plants within progenies (r_{xy}), following Ritland (1989). In non-inbred diploids, the co-ancestry coefficient is half of r_{xy} , so $\Theta = r_{xy}/2$. The co-ancestry within families was then estimated as

$$\Theta = 0.125(1 + \hat{F}_a)[4\hat{s} + (\hat{t}_m^2 + \hat{t}_m\hat{s}\hat{t}_s)(1 + \hat{r}_{p(m)})]$$

where, s is the self-fertilization rate ($\hat{s} = 1 - \hat{t}_m$) and F_a is the inbreeding coefficient in the parental population (Ritland, 1989). The variance effective population size was estimated according to the expression: $N_{e(v)} = 0.5/\Theta$ (Cockerham, 1969). When multiplied by progeny number, this provides an estimate of $N_{e(v)}$ of the population when two presuppositions are met: *i*) the seed trees that produced the progenies are not related; *ii*) the seed trees did not cross with one another and received a different pollen gene pool (no overlapping pollen gene pool among fruiting tree) (A.M. Sebbenn, pers. obs.). Given the geographical extent of the original collection expedition, of which roughly 10% was used here, we believe that these presuppositions are met with very small probability of error.

Results

The three populations had low levels of linkage disequilibrium with $p < 0.05$, with a mean of 14% and a range of 7% to 25%. Since progenies are expected to show disequilibrium, because all of the offspring have at least one allele at each locus from the mother tree, it is impossible to identify other sources of disequilibrium in the populations. Tarazi *et al.* (2010) showed that this disequilibrium due to progeny-specific genetic expectations does not affect estimates of genetic diversity and structure, mating system or paternity analysis.

The estimates of the multilocus outcrossing rate (t_m) were high for all three populations (≤ 1), but significantly different from 1.0 for the Cuiparillo and Shanusi populations, suggesting a mixed mating system with predominance of outcrossing. The individual multilocus outcrossing rates per progeny were also high in all progenies (based on standard errors, only progeny 7 from the Shanusi population and progeny 2 of the Paranapura population presented values statistically different from 1.0.) of the three populations, with low variation among progenies. The low estimate of the correlation of outcrossing (r_t) agrees with the low variation observed in the outcrossing rates among progenies within populations, confirming the

Table 1 - Estimates of peach palm mating system parameters in progenies from populations cultivated along three rivers in the distribution of the Pampa Hermosa landrace, near Yurimaguas, Peru.

	Cuiparillo	Shanusi	Paranapura
Multilocus outcrossing rate (t_m) in progeny level			
Progeny 1	1.00 (0.00) - [12]	1.00 (0.00) - [30]	1.00 (0.00) - [10]
Progeny 2	0.95 (0.05) - [20]	1.00 (0.00) - [27]	0.92 (0.08) - [12]
Progeny 3	1.00 (0.00) - [14]	1.00 (0.00) - [24]	1.00 (0.00) - [12]
Progeny 4	1.00 (0.00) - [15]	1.00 (0.00) - [17]	1.00 (0.00) - [12]
Progeny 5	1.00 (0.00) - [12]	1.00 (0.00) - [15]	1.00 (0.00) - [25]
Progeny 6	0.92 (0.01) - [11]	1.00 (0.00) - [10]	1.00 (0.00) - [12]
Progeny 7	0.94 (0.05) - [16]	0.90 (0.07) - [10]	1.00 (0.00) - [10]
Progeny 8	1.00 (0.00) - [11]		1.00 (0.00) - [12]
Progeny 9	1.00 (0.00) - [10]		
Mating system parameters in population level			
Multilocus outcrossing rate: t_m	0.95 (0.93-0.97)	0.98 (0.97-0.99)	0.99 (0.98-1.00)
Single locus outcrossing rate: t_s	0.85 (0.82-0.88)	0.78 (0.74-0.82)	0.88 (0.85-0.91)
Mating among relatives rate: $t_m - t_s$	0.10 (0.08-0.12)	0.20 (0.17-0.23)	0.11 (0.08-0.14)
Correlation of the estimate of t : r_t	0.07 (0.03-0.11)	0.12 (0.10-0.14)	0.08 (0.06-0.10)
Correlation of the estimate of p multilocus: $r_{p(m)}$	0.07 (0.05-0.09)	0.11 (0.09-0.13)	0.05 (0.03-0.07)
Correlation of selfing among loci: $r_{i(l)}$	0.56 (0.49-0.63)	0.39 (0.32-0.46)	0.63 (0.54-0.72)
Fraction of apparent selfing due to biparental inbreeding: $1 - r_t$	0.44	0.61	0.34
Number of pollen donors: N_{ep}	15.2 (11.1-33.3)	9 (7.7-10.0)	19.6 (14.3-16.7)
Coefficient of coancestry: Θ	0.146 (0.139-0.154)	0.144 (0.139-0.148)	0.134 (0.129-0.139)
Variance effective size: $N_{e(v)}$	3.40 (3.25-3.61)	3.48 (3.37-3.61)	3.70 (3.60-3.88)
Total variance effective size: $N_{e(v)t}$	30.6 (29.3-32.5)	24.4 (23.6-25.2)	29.6 (28.8-31.7)

[] - number of plants in the progeny; () standard errors in the progeny or minimum-maximum in the population.

outcrossed origin of most of the plants in the progenies in these populations. The difference between the multilocus and single-locus ($t_m - t_s$) outcrossing rates was high (minimum 10%) and significantly different from zero for all three populations, providing evidence of mating among relatives, especially in the Shanusi population. The fixation index of maternal parents was ($F_a = 0$).

According to the estimated value of the fraction of apparent selfing due to uniparental inbreeding ($r_{i(l)}$), the inbreeding in offspring is partially uniparental and partially biparental, with slightly more uniparental inbreeding in Cuiparillo and Paranapura, and slightly more biparental inbreeding in Shanusi. The correlation of paternity ($r_{p(m)}$) within the progenies was low (≤ 0.11), suggesting low frequency of full-sibs within progenies and that a large effective number of pollen donors mated with the sampled seed-trees (9 to 20 per population). Based on the standard error, the average co-ancestry coefficient (Θ) among plants within progenies was not significantly different between populations (Table 1). These values were also close to those expected in half-sib progenies ($\Theta = 0.125$), resulting in an estimate of variance effective population size ($N_{e(v)}$) close to the value expected in half-sib progenies ($N_{e(v)} = 4$). Assuming that the seed-trees are not relatives, not mated with

one another, and received a non-overlapping pollen pool, the average $N_{e(v)}$ can be multiplied by the number of progenies per population. This yields the total effective population size of 30.6, 26.6 and 29.6 in the Cuiparillo, Shanusi and Paranapura populations, respectively, and 86.8 for the three populations as a group.

Discussion

Outcrossing rates

The high outcrossing rates found for peach palm are similar to those found for other palm species, such as *Astrocaryum mexicanum* (Eguiarte *et al.*, 1992), *Euterpe edulis* (Conte *et al.*, 2008), *Acrocomia aculeata* (Abreu *et al.*, 2011) and *Cocos nucifera* (Akuba *et al.*, 2009), as well as other tropical tree species (Ward *et al.*, 2005). These high outcrossing rates found for peach palm may be explained by one or more of the following interrelated factors: i) the seeds were collected during the peak of the harvest season (W. Chávez Flores, pers. com., 2007), which would have increased the possibility of numerous plants contributing to cross-pollination, due to the large number of insects visiting the inflorescences during the peak of synchronous flowering three months earlier; ii) the flowering phenology

of the protogynous peach palm favors exogamy (Mora Urpí and Solís, 1980); and, iii) inbreeding depression killing selfed seeds between the fertilization and sampling stages for genotyping, as observed in other tropical tree species (Gribel and Gibbs, 2002; Hufford and Hamrick, 2003; Naito *et al.*, 2005; Del Castillo and Trujillo, 2008).

Mating among relatives

The rates of mating among relatives detected here probably arose from the agricultural practice of planting open-pollinated offspring of a few selected seed-trees in the same cultivated plot (Clement, 1988; Cole *et al.*, 2007). This practice produces spatial genetic structure (SGS) within cultivated peach palm and permits mating among relatives, although Adin *et al.* (2004) reported extremely high gene flow within and between the river systems around Yurimaguas, owing to farmers' seed sourcing practices, which reduces SGS overall. However, for cultivated plants, there is a higher expectation of mating among relatives, as it is common to have only a few seed sources per cultivated area due to farmers' selection practices (Louette, 2000). Still, mating among relatives may also be expected in natural populations of peach palm, as reported in other tropical tree species (Alves *et al.*, 2003; Carneiro *et al.*, 2007; Lemes *et al.*, 2007).

Correlated matings

The estimated effective number of pollen donors in peach palm was higher (9 - 20 per population) than that detected in other palm species, such as *Euterpe edulis* (maximum 9, Seoane *et al.*, 2005), which may be explained by the fact that the seeds were collected during the peak harvest season or by demographic differences, such as the large population sizes of cultivated peach palm compared with the very fragmented populations of *E. edulis*. In coconut, effective pollen donor number was very low (1-3), even though outcrossing rates were very high, and was attributed to low flowering synchrony within the plantation (Akuba *et al.*, 2009). High numbers of pollen donors increase the genetic diversity and variance effective population size within progenies. During other parts of the harvest season, however, the number of pollen donors is likely to be lower, so that estimates of variance effective population size within progenies are likely to decrease. This suggests the need to examine the mating system at the beginning and the end of the flowering season when inbreeding may be higher (Vencovsky and Crossa, 1999).

Due to the high outcrossing rate and low paternity correlation, the estimates of the mean coefficient of co-ancestry within progenies were close to those expected in progenies of half sibs (0.125). This suggests that under random outcrossing in this progeny trial one can expect some biparental inbreeding. However, this inbreeding may be reduced by selection of superior genotypes within progenies, which is likely to reduce within-plot coancestry. Since this

trial is being prepared for use as a source of improved seeds (seed nursery), this will be important for both improvement and conservation, as suggested by Cornelius *et al.* (2006) for a similar trial in Peru.

The Yurimaguas progeny trial is composed almost exclusively of plants from cross-pollination. Consequently, estimation of genetic parameters and selection for heart-of-palm production can be based on classical quantitative genetic models applied to exclusively outcrossed species, which presuppose random mating, absence of inbreeding in the parental generation ($F_a = 0$), and progenies composed exclusively of half-sibs (Resende, 2002), considering that the level of inbreeding detected here is very low.

Effective population size

The estimated total variance effective size ($N_{e(v)}$) of the population samples (maximum 30.6) of these three river basins in the Pampa Hermosa landrace is lower than what is considered acceptable for genetic conservation at the population level (minimum 50, Frankel and Soulé, 1981). However, the high gene flow among these populations (Adin *et al.*, 2004; Alves-Pereira *et al.*, 2012; and Rodrigues DP (2007) PhD Thesis, Universidade Federal do Amazonas, Manaus, AM, Brazil) suggests that conservation should be planned at the landrace level, where the estimated $N_{e(v)} = 86.8$ is well above the minimum for short term conservation. This estimate is quite close to the tall coconut recommendation (90; Konan *et al.*, 2008), which has similar outcrossing values (Akuba *et al.*, 2009).

Implications for genetic conservation

Effective size of populations is generally a fraction of the census population, often as low as 10% (Frankham *et al.*, 2004). In this study, it was slightly more than 24% (mean $N_{e(v)}/N = 86.6/359$), likely due to high pollen donor number at peak flowering season. The progeny trials at INPA (Alves-Pereira *et al.*, 2012; Clement *et al.*, 2012) and Embrapa (Kalil Filho *et al.*, 2010; Clement *et al.*, 2012) in Brazil, and in Peru (Cornelius *et al.*, 2006) are adequately dimensioned to handle genetic conservation based on these estimates. The major peach palm germplasm collections in Brazil (375 accessions, with a maximum of 9 plants/accession) and Costa Rica (1,200 accessions with 9 plants) do not have the requisite numbers even of this important landrace, and have much smaller numbers of other landraces; all other collections are smaller (Mora Urpí *et al.*, 1997; Graefe *et al.*, 2013). The main Brazilian collection, at INPA, Manaus, for example, has nearly sufficient census numbers of the Pampa Hermosa, Putumayo and Pará landraces (Clement *et al.*, 2001), but is extremely deficient in all other landraces and wild populations.

In situ conservation is even more problematic. Wild peach palm (*B. gasipaes* var. *chichagui*) across southern Amazonia occurs in quite small populations in most places (10-20 plants) in ecosystems that are increasingly frag-

mented (Clement *et al.*, 2009). It is unlikely that these populations have the high $N_{e(v)}$ found in this study because of low census numbers, but even if they did they are doomed to extinction in fragmented ecosystems because of the lack of gene flow among populations that would keep inbreeding at acceptable levels. Only a few Indigenous Lands and even fewer National Parks and other conservation units contain meta-populations of sufficient size to maintain evolutionary flexibility, although climate change is expected to negate this by the end of the century (Clement *et al.*, 2009).

On farm conservation is less problematic than either *in situ* or *ex situ* conservation. Demand for locally popular fruit types remains strong in the pre-Columbian distribution of peach palm (Clement *et al.*, 2004; Graefe *et al.*, 2013) and there are millions of trees in the Neotropics (Clement, 2008), although only about 10% meet consumer preferences. The expansion of the heart-of-palm agribusiness also provides a conservation bonus, at least for the landraces, such as Pampa Hermosa, that are the genetic bases for the expansion. There are probably 43,000 ha planted in the Neotropics (Clement, 2008), of which at least half is derived from the Pampa Hermosa landrace. Combined with the Peruvian progeny trials (Cornelius *et al.*, 2006), these plantations make Pampa Hermosa the best-conserved peach palm landrace.

As climate change accelerates during the 21st century, both *in situ* and *ex situ* conservation of crop and wild relative genetic resources will become both more critical to support agricultural adaptation and more difficult to achieve in the face of alternative demands for human and financial resources for other activities of mitigation and adaptation. Our estimates of the numbers of plants necessary for adequate *ex situ* population conservation are similar to those in coconut and certainly in most outcrossing tropical trees, which means that *ex situ* conservation will become continually more difficult for minor crops and other underutilized species. The implications are that on farm conservation will become continually more important or the variety of species that supply the human enterprise will become continually narrower.

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