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Tropical Understory *Piper* Shrubs Maintain High Levels of Genotypic Diversity Despite Frequent Asexual Recruitment

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ABSTRACT

Many plant species have the capacity to regenerate asexually by resprouting from stem and leaf fragments. In the pan-tropical shrub genus *Piper*, this tendency is thought to be higher in shade-tolerant than light-demanding species, and to represent a trade-off with annual seed production. Here we use molecular markers to identify clones in five *Piper* species varying in light requirements. We test predictions that (i) asexual recruitment success is highest in shade-tolerant species, and (ii) that consequently, shade-tolerant species are characterized by lower genotypic diversity than light-demanding *Piper*. We found that two shade-tolerant *Piper* species recruited asexually more frequently (36–42% of sampled shoots were of asexual origin) than, two light-demanding and one shade-tolerant species (0–26%). Furthermore, as predicted, genotypic diversity was negatively correlated with the frequency of asexual recruitment in the population. Nonetheless, genotypic diversity of *Piper* was high compared with other clonal plants. The proportion of unique genotypes found per population ranged from 0.58 to 1.0 and the genotypic Simpson's diversity ranged from 0.93 to 1.0 for all five species. Our results suggest that even though asexual reproduction plays an important role in maintaining local populations of *Piper* in the understory, it does not seem to reduce genotypic diversity to levels that will threaten these species ability to respond to environmental change.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: AFLP; Barro Colorado Island; clonal reproduction; distinguishable genotypes; Panama; *Piperacea*; tropical wet forest; vegetative reproduction.

Piper is a pan-tropical genus containing over 1000 species (Burger 1971) occupying a range of forest habitats, from treefall gaps to deeply shaded forest understories. *Piper* is often a dominant element in the shrub layer of tropical forests, and plays a key role supporting frugivorous bat communities (Fleming 1985). *Piper* is also a favorite model system for investigating how ecologically similar species coexist, and partition rain forest environments (Field & Vazquez-Yanes 1993). As a consequence, many *Piper* species have been studied thoroughly in terms of their physiology (Walters & Field 1987, Chazdon *et al*. 1988, Williams *et al*. 1989), phenology (Marquis 1988, Thies & Kalko 2004), seed characteristics (Vázquez-Yanes & Smith 1982, Orozco-Segovia & Vázquez-Yanes 1989, Daws *et al*. 2002), reproductive strategies (Semple 1974, Fleming 1981, de Figueiredo & Sazima 2000) and natural enemies (Greig 1993b, Marquis *et al*. 1997).

Experimental studies have revealed strong seed and establishment limitation of sexual recruitment, particularly among shade-tolerant *Piper* species. Up to 76 percent of seeds are lost to predators at the pre-dispersal stage (Greig 1993b), and remaining seeds have exceptionally low emergence success. *Piper* have tiny photoblastic seeds (<< 1 mg; Vázquez-Yanes 1974, Vázquez-Yanes & Smith 1982, Orozco-Segovia & Vázquez-Yanes 1989, Daws *et al*. 2002) that fail to emerge when sown in the understory, even when surface litter is removed. Only 0.2 percent of 8000 seeds sown in the understory emerged for five *Piper* species studied in Panama (Lasso *et al*. 2009). Likewise, Fleming (1981) found only four surviving seedlings of 1600 seeds of *Piper amalago*, and Greig (1993a) observed only five surviving seedlings from 3000 seeds of five *Piper* species, experimentally sown into both gap and understory conditions. If seedling recruitment is strongly limited, as published evidence suggests, then an alternative regeneration pathway through asexual reproduction could be critical for *Piper* population persistence in the understory.

The most common methods of asexual reproduction observed in *Piper* are layering and fragmentation. In layering, new plants originate at nodes from branches and trunks that are trampled, or pinned to the ground by litter fall. The original shoot may eventually rot away leaving independent ramets. In fragmentation, new plants resprout from portions of stem or leaf tissue that remains alive on moist soil or leaf litter layers (Greig 1993b; Lasso *et al*. 2009). In both cases, physiologically independent ramets are produced that are difficult to identify as clones unless molecular markers are used.

A consequence of asexual reproduction, which has both benefits and costs, is that offspring are genetically identical to each other and to their parent. Such genetic similarity may be advantageous if a genotype is well adapted to a particular environment, but disadvantageous if the environment changes. Thus, an increase in the frequency of asexual recruitment could potentially lead to a decrease in the genotypic diversity of the population, which in the long term could limit a population's resilience to environmental change (Hamilton 1980, Burt 2000), and may jeopardize its resistance to biotic enemies (Hamilton *et al*. 1990). Even though, this assumption of reduced genotypic diversity has prevailed for some time, a series of reviews indicate that populations in which asexual reproduction occurs can be as genotypically polymorphic as sexual ones (Ellstrand & Roose 1987, Hamrick & Godt 1989, Widén *et al*. 1994, Silvertown 2008).

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Experimental studies of *Piper* species consistently indicate greater resprouting capacity for shade-tolerant than light-demanding species (Greig 1993a, Lasso *et al.* 2009). This difference may result from variation in resource allocation patterns or from variation in the frequency of stem breakage resulting from greater debris fall in the forest understory (Aide 1987, Clark & Clark 1991). For *Piper*, field experiments carried out on 22 species in Costa Rica (Greig 1993a), and eight species in Panama (Lasso *et al.* 2009), indicate that shade-tolerant species have a higher capacity to resprout and regenerate by layering and fragmentation relative to congeneric light-demanding species. These studies have also shown that shade-tolerant species tend to have lower sexual reproductive success, suggesting that asexual regeneration may be critical for population persistence.

Less clear, is whether differences in resprouting capacity translate to actual differences in asexual recruitment rate, and if the magnitude of asexual recruitment is sufficient to significantly impact genotypic diversity. In two previous genetic studies of *Piper*, three species, *P. amalago*, *Piper pseudofuligineum*, and *Piper jacquemontianum*, showed extremely low values of genetic diversity (Heywood & Fleming 1986), whereas one species *Piper cernuum*, showed high values of genetic diversity (Mariot *et al.* 2002). But the relationship of genetic diversity to either light environment or regeneration capacity was not explicitly studied in these four *Piper* species.

The objective of this study was to connect experimental studies of resprouting capacity and sexual reproductive success in *Piper* with measurements of the frequency of asexual recruitment in the field based on the identification of clones using genetic markers. Specifically, we test the prediction that light-demanding species restricted to forest gaps and clearings recruit asexually less frequently than do species restricted to the forest understory. Additionally, we test the prediction that asexual recruitment in understory species is sufficient to significantly reduce genotypic diversity. By linking molecular assessments of asexual reproductive success with experimental assessments of asexual reproductive capacity, we provide an experimental approach aimed at assessing the importance of asexual recruitment in tropical forests.

METHODS

Study site and study species.—The study was conducted in tropical semi-deciduous forest on Barro Colorado Island (BCI), Panama (9°10′ N, 79°51′ W), which is described in detail elsewhere (Leigh 1999). Annual rainfall on BCI averages 2600 mm, with a pronounced dry season between January and April. The genus *Piper* is represented by 22 species on BCI (Croat 1978), including both light-demanding and shade-tolerant species. Here we focus on five species; three are commonly found in the understory (*Piper darienensis* C.DC., *Piper cordulatum* C.DC. and *Piper aequale* Vahl.) and two are commonly found in gaps and clearings (*Piper dilatatum* L.C.Rich., and *Piper marginatum* Jacq.).

Based on physiological data, fecundity data and the results of fragmentation experiments in the field (Lasso *et al.* 2009), these *Piper* species were classified according to three life-history strategies. *Piper marginatum* and *P. dilatatum* are pioneer species that live exclusively in gaps, produce large numbers of seeds, and show little asexual reproductive ability. *Piper darienensis* and *P. cordulatum* are shade-tolerant species with low annual seed output and a high capacity to resprout. *Piper aequale* is shade-tolerant, but resembles light-demanding species because it produces large numbers of seeds and has intermediate capacity to resprout.

Here, we use the term *individual* to refer to stems, or plants with apparent no connections to other plants. Once the outcome of molecular analysis is known, these can be classified as *genets* if they have unique genetic fingerprints or as *ramets* or clones if they share the same genetic fingerprint with other stems or individuals.

Sampling method.—Differences in the distribution of shade-tolerant and light-demanding species required different sampling strategies. For *P. dilatatum* and *P. marginatum*, individuals were concentrated close to the forest edge, and the size of gaps and clearings did not permit the establishment of large plots. In contrast, aggregations of *P. darienensis*, *P. cordulatum* and *P. aequale*, were more sparsely distributed; thus, larger areas needed to be sampled to include a sufficient number of individuals to obtained unbiased estimates of genotypic diversity.

Collections of the three shade-tolerant *Piper* species were made in two 1-ha plots located 754 m apart (Plot 1 next to Snyder Molino trail marker 2 and Plot 2 next to Lake trail marker 4). Collections of the two light-demanding species were made in three 35 m × 35 m plots located in the edge of forest surrounding buildings and laboratories. In each plot, we collected leaves from all stems not obviously connected to other stems and spatially separated by at least 5 cm from other stems. In total, we collected samples from 892 stems (range 26–182 stems per species per plot; Table 1). A grid was established in each plot to obtain *x* and *y* coordinates for each stem to calculate the geographic distance among them.

DNA isolation and amplified fragment length polymorphism (AFLP) procedure.—Leaves were collected and kept on ice until they were processed later the same day. In the laboratory, leaves were surface cleaned with pure ethanol (95%) and dried in silica gel for 1 wk. Twenty mg of dry tissue were ground using FastPrep FP120 (Qbiogene). DNA was extracted using a DNeasy 96 plant extraction kit (Qiagen) following the manufacturer's protocol. DNA concentrations were estimated by running DNA samples on agarose gels (1.5%) with a Low DNA Mass™ Ladder (Invitrogen) of known concentrations.

We performed AFLP analysis following the method of Vos *et al.* (1995) with some modifications. The polymerase chain reaction conditions and genotyping procedures are described in detail in Lasso (2008). We used four primer combinations per species to obtain AFLP fingerprints. Three combinations were used across all species; *Eco*RI-ACG/*MseI*-CTG, *Eco*RI-AGG/*Mse*I-CCA, *Eco*RI-ATG/*Mse*I-CAG. The fourth primer pair used was as follows: *Eco*RI-ACG/*Mse*I-CTA for *P. darienensis* and *P. dilatatum*, *Eco*RI-AGT/*Mse*I-CTG for *P. aequale*, *Eco*RI-AGG/*Mse*I-CAG for *P. cordulatum*, and *Eco*RI-AGT/*Mse*I-CAT for *P. marginatum*. For *P. dilatatum* we used two additional primer pairs, *Eco*RI-ACG/*Mse*I-CTT and *Eco*RI-ACG/*Mse*I-CCA, because many of the loci

TABLE 1. *Asexual recruitment, genetic diversity and spatial dispersion in* Piper *populations at Barro Colorado Island, Panama. The number of stems sampled (*N*), the number of genets identified with amplified fragment length polymorphism (AFLP) markers (*G*), the proportion of distinguishable genotypes (*G/N*), Simpson's genotypic diversity (*D*), the percentage of asexual recruitment (Asex), and the Morisita's index of dispersion for genets (I_{genets}) and clones (I_{clones}), are presented.*

Species	Plot	*N*	*G*	*G*/*N*	*D*	Asex (%)	I_{genets}	I_{clones}
Shade-tolerant								
Piper darienensis	1	182	105	0.58	0.93	42	4.69	7.22
	2	167	98	0.59	0.98	41	3.43	4.50
Piper cordulatum	1	72	46	0.64	0.98	36	2.96	3.71
	2	60	38	0.63	0.93	37	2.55	5.12
Piper aequale	1	166	156	0.94	0.99	6	4.44	3.33
	2	59	55	0.93	0.99	7	5.90	2.29
Light-demanding								
Piper dilatatum	3	26	26	1.0	1.0	0	5.81	—
	4	43	39	0.91	0.99	9	3.87	6.40
	5	33	30	0.91	0.99	9	3.50	7.47
Piper marginatum	3	41	41	1.0	1.0	0	7.43	—
	4	43	32	0.74	0.97	26	2.75	6.15

were monomorphic. The four primer combinations yielded 559–770 clearly identifiable bands per species. After the filtering of loci, only 15–27 percent of fragments were selected as follows: 120 loci (87 polymorphic loci) for *P. darienensis*, 102 (68 polymorphic loci) for *P. cordulatum*, 124 (95 polymorphic loci) for *P. aequale*, 140 (49 polymorphic loci) for *P. dilatatum*, 153 (110 polymorphic loci) for *P. marginatum* (Lasso 2008). Fingerprint data were obtained by running the amplified samples in an ABI Prism 3130 capillary sequencer instrument, and presence or absence of fragments was scored using Genescan and Genotyper software (version 3.7, Applied Biosystems).

CLONE IDENTIFICATION, CLONAL SIZE, AGGREGATION AND DISPERSAL DISTANCES.—Each sample was either classified as a member of a clone (ramet) or as a unique genotype (genet) using the software package *Genotype* (Meirmans & Van Tienderen 2004). *Genotype* uses pairwise genetic distances to classify samples as ramets or as unique genotypes based on a user-specified threshold. In *Genotype*, the threshold determines the maximum dissimilarity allowed between two 'individuals' to still be considered ramets of the same clone. We calculated this threshold after inspecting the frequency distribution of pairwise genetic distances among three replicate samples from 11–24 known clones; threshold values ranged from 0 to 5 percent (Lasso 2008).

The frequency of sexual reproduction was assessed for each plot by dividing the number of unique genotypes found in the plot by the number of samples from that plot and from there we calculated the frequency of asexual recruitment (1 − frequency of sex recruitment). To estimate the size of clones or dispersal distances by asexual means, we calculated the pairwise geographic distances among ramets from plot coordinates using GenAlEx 6.0 (Peakall & Smouse 2006). To assess the spatial distribution of clones we calculated Morisita's index of dispersion (I_δ)(Morisita 1959) after splitting sampling plots in 16 quadrats (see Fig. 1)

$$I_\delta = \frac{q\sum_{i=1}^{q} x_i(x_i-1)}{N(N-1)},$$

where q is the number of quadrats, x_i is the number of individuals in the ith quadrat and N is the number of plants in the whole plot. Values of $I_\delta > 1$ indicate patchy distribution, $I_\delta < 1$ uniform distribution and $I_\delta = 0$ random distribution.

CLONAL GENOTYPIC DIVERSITY INDICES.—Different metrics of genotypic diversity have been calculated for clonal plants, the most commonly used being 'proportion distinguishable' (PD) and Simpson's diversity index (*D*) (Ellstrand & Roose 1987). We calculated these same two indices using the software *Genodive* (Meirmans & Van Tienderen 2004). The PD was calculated as *G* (number of genotypes)/*N* (number of samples), which is the proportion of distinguishable genotypes or the proportion of individuals in the plot that were recruited sexually. The Simpson genotypic diversity index corrected for sample size (Pielou 1969) was calculated as

$$D = \frac{n}{n-1} \times \left(1 - \sum_{i=1}^{s} p_i^2\right),$$

where n = sample size and p_i = frequency of genotype i. To test whether the populations sampled were of sufficient size to provide unbiased estimates of genotypic diversity, we plotted jack-knife estimates of diversity against sample size for each species and plot combination. In all cases, diversity estimates were asymptotic. To determine whether genotypic diversity is negatively associated with asexual recruitment, we carried out a Pearson correlation analysis between the Simpson genotypic diversity index calculated for each plot and the percent of individuals that were recruited asexually in each plot.

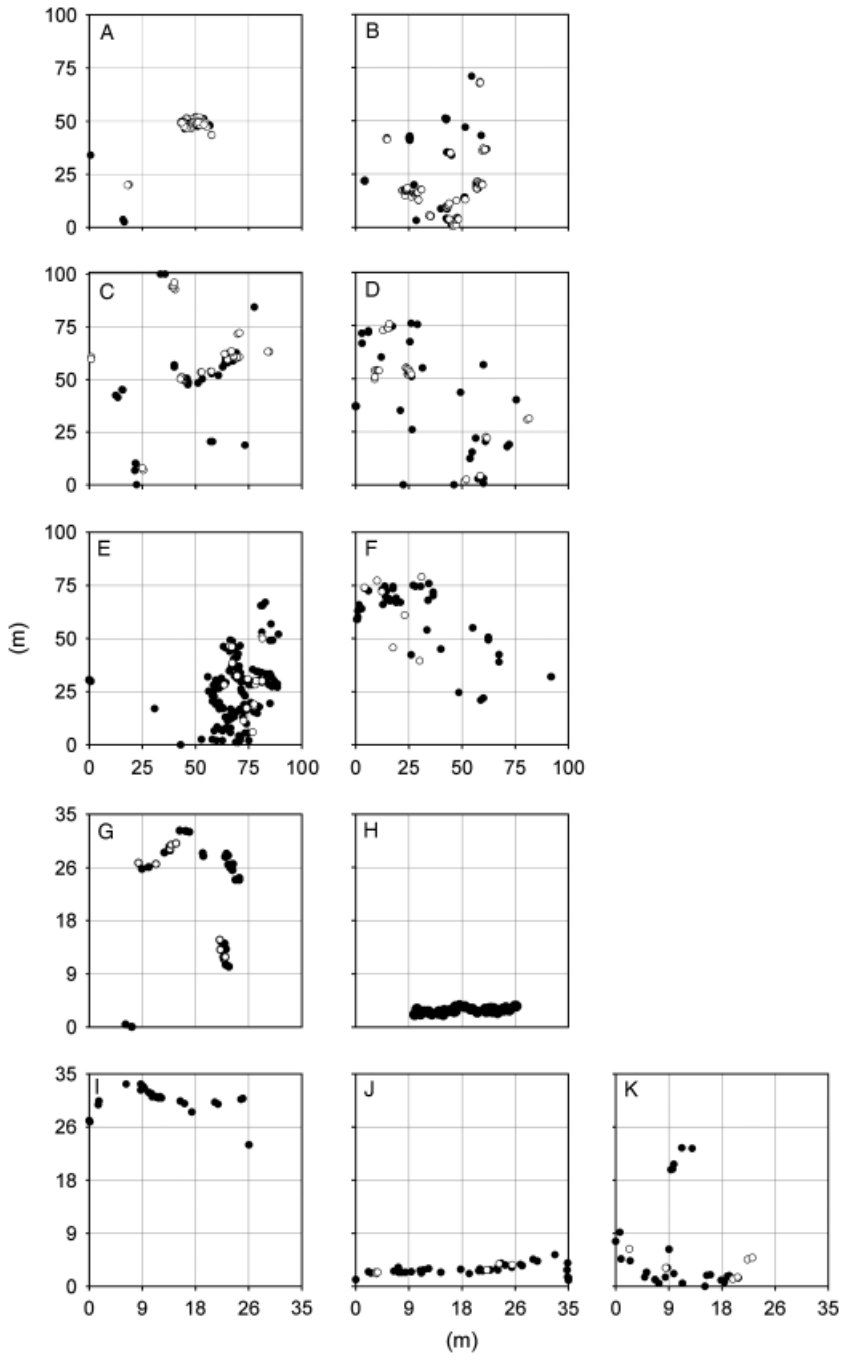

FIGURE 1. Location of all genets (black circles) and clones (white circles) in 1 ha plots established in the understory and 35 m × 35 m plots located in clearings. (A) and (B) *Piper darienensis* Plot 1 and 2, respectively; (C) and (D) *Piper cordulatum* Plots 1 and 2, respectively; (E) and (F) *Piper aequale* Plots 1 and 2, respectively; (G) and (H) *Piper marginatum* Plots 3 and 4, respectively; (I), (J) and (K) *Piper dilatatum* Plots 3, 4 and 5, respectively.

CORRESPONDENCE BETWEEN SPECIES' RESPROUTING ABILITY AND MOLECULAR DATA.—In our previous work, we created an index of asexual reproductive ability (ARAI) for *Piper* species included in this study (Lasso *et al*. 2009). ARAI was calculated by averaging the percentage of cuttings and pinned-down branches that survived in growing house and field experiments. To determine how this index of species' ability to reproduce asexually corresponds with the estimated level of asexual recruitment from fieldwork, we computed a Pearson correlation analysis and a two sample paired *t*-test between species ARAI scores and the percentage of individuals recruited asexually estimated from the molecular data.

RESULTS

Asexual recruitment was more frequent in shade-tolerant species than in light-demanding species ($X^2 = 7.08$, df = 2; $P = 0.03$). The frequency of asexual recruitment was remarkably similar across the two sample plots for each species. For the two shade-tolerant species, 41–42 percent of individuals of *P. darienensis*, recruited asexually in the plots sampled, while in *P. cordulatum* 36–37 percent of individuals recruited asexually (Table 1). *Piper aequale*, had much lower values of asexual recruitment than shade-tolerant species, ranging from 6 to 7 percent asexual recruitment across the plots. These values are similar to the two light-demanding species, for which three of four populations had asexual recruitment of 0–9 percent. One population of the light-demanding *P. marginatum* recruited 26 percent of individuals asexually (Table 1).

Individuals belonging to the same clone tended to be tightly aggregated in localized patches (Fig. 1). Both clones and genets exhibited clumped distributions as Morisita's index of dispersion was always > 1 (Table 1). For all species, most clones were < 10 m apart (Fig. 2A), although a few clones of the shade-tolerant species were distributed over remarkably large distances; up to 45 m in *P. darienensis* and 78 m in *P. cordulatum* (Fig. 2A). These two cases could probably represent long-distance dispersal events or just the remnants of a clone that once occupied a larger area since the probability that these genotypes could arise through random recombination during sexual reproduction is very low (probability of identity = 3.5×10^{-5} and 1×10^{-4} for *P. darienensis* and *P. cordulatum*, respectively). One possible mechanism for long-distance dispersal is through surface runoff during heavy rains, especially in slopes. Populations of *P. aequale* had fewer clones, but they tended to be more widespread (Fig. 2A). For all species, clones were typically small with 2–6 ramets (Fig. 2B), though two shade-tolerant species had several clones represented by 7–15 ramets (Fig. 2B) and *P. darienensis* had one clone containing 45 ramets.

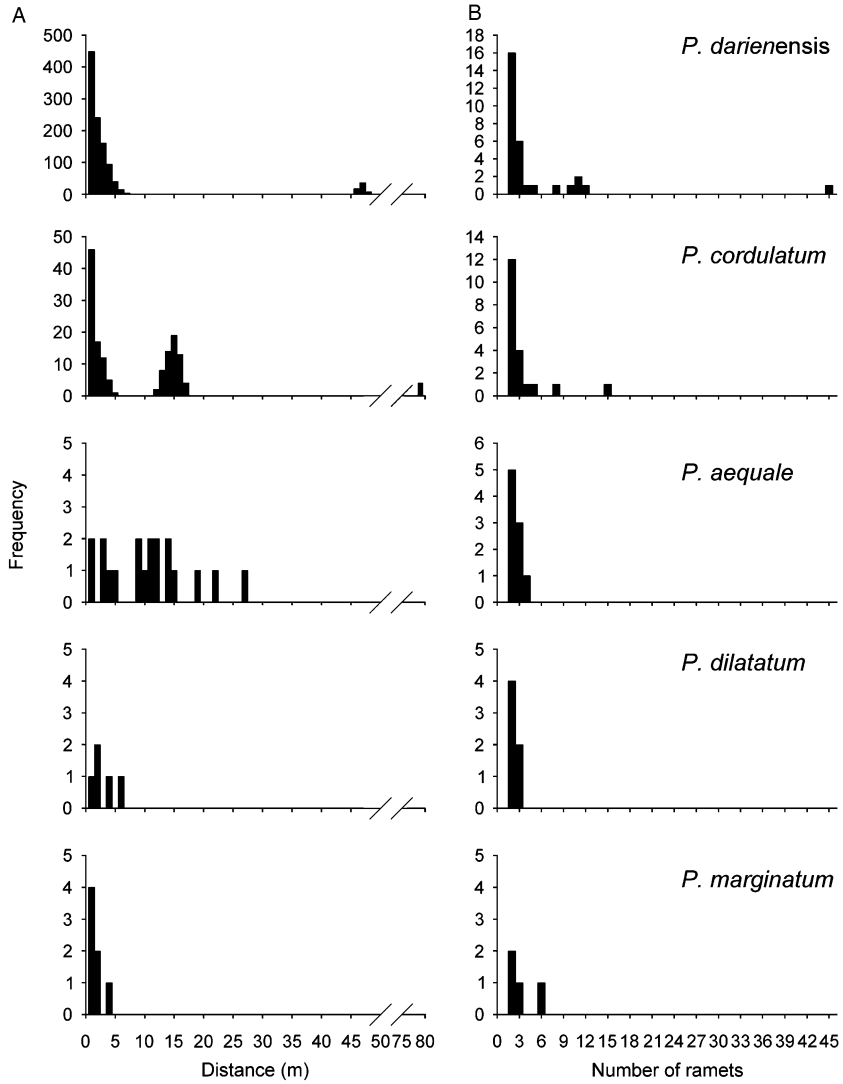

FIGURE 2. Frequency distribution of (A) the pairwise geographic distances among ramets that belong to the same clone and (B) the number of ramets per clone.

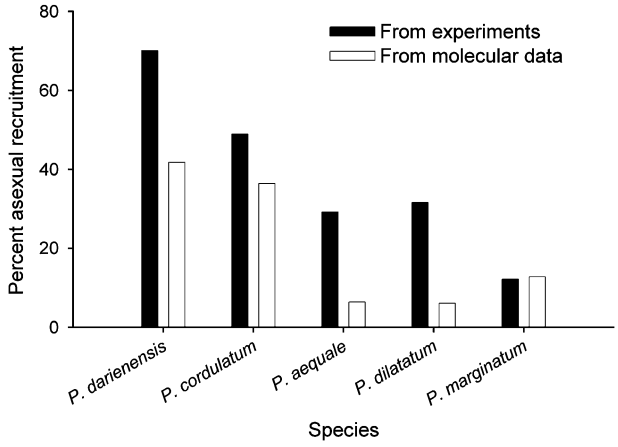

FIGURE 3. Correspondence between estimates of species ability to reproduce asexually from experiments (percent of fragments that resprout and survive) and measurements of the percentage of individuals in the population that recruited asexually obtained using amplified fragment length polymorphism markers.

Genotypic diversity in *Piper* decreased as asexual recruitment increased (Pearson correlation = − 0.83; $P = 0.0014$). Values of genotypic diversity were in the range of $D = 0.93$–1.0 (Table 1).

Estimates of asexual recruitment frequency obtained from molecular data were positively associated with estimates of species' abilities to reproduce asexually based on transplant experiments (one-tailed Pearson correlation = 0.85; $P = 0.03$). Nonetheless, transplant experiments significantly overestimated asexual recruitment success ($t = 3.34$; df = 5; $P = 0.029$; Fig. 3).

DISCUSSION

HABITAT DIFFERENCES IN ASEXUAL RECRUITMENT.—Our data indicate that asexual recruitment occurs in all *Piper* species regardless of shade tolerance. We did, however, find a marked difference in the frequency of asexual recruitment between shade-tolerant *Piper* species (42% clonal) and *Piper* species from gaps and clearings (< 26% clonal). These differences are in agreement with findings from previous fragment survival and seedling recruitment experiments (Greig 1993a, Lasso *et al.* 2009). Thus, our data support the notion that asexual recruitment is an important alternative regeneration pathway for species growing in the light-limited understory, where sexual reproductive success is strongly impacted by pre-dispersal seed predation (Greig 1993b), and where both seed production and seedling establishment success are low (Fleming 1981, Greig 1993a, Lasso *et al.* 2009). Whether asexual reproduction is important for other understory genera remains an open question. Several characteristics of the shade-tolerant syndrome, and of the understory environment may, however, select for traits that promote asexual recruitment in other understory species as well.

In the understory, high rates of debris fall increase the chances that individuals of shade-tolerant species resprout from fallen stems and stem fragments (Aide 1987, Gartner 1989, Clark & Clark 1991, Guariguata 1998, Paciorek *et al.* 2000). Continually moist conditions in the litter layer may also be critical to fragment survival in the period before rooting systems develop. Furthermore, because resprouting success is correlated to the capacity to store resources in vegetative organs (Sagers 1993, Verdaguer & Ojeda 2002, Bond & Midgley 2003), shade-tolerant species may be better able to resprout than light-demanding species because they store greater quantities of nonstructural carbohydrate reserves in stems and roots (Kobe 1997, Poorter & Kitajima 2007, Myers & Kitajima 2007). Thus differential resprouting ability among species groups may be an indirect consequence of traits that confer shade tolerance. Nonetheless, the two species with the lowest proportion of sexually recruited individuals, *P. darienensis* and *P. cordulatum*, were also the species with the lowest annual seed production, lowest seed viability and lowest seedling emergence rates in the field (Lasso *et al.* 2009). *Piper aequale*, although a shade-tolerant species, resembles more light-demanding species with high annual seed production and a low resprouting capacity (Lasso *et al.* 2009). Our molecular data confirm that this species groups with the two light-demanding species, *P. dilatatum*, and *P. marginatum*.

Our results fall within in the lowest range of reported frequencies of asexual recruitment (*i.e.*, $1 - G/N \times 100$) for tropical species obtained using molecular markers. Murawski & Hamrick (1990) reported frequencies of 29–89 percent asexual recruitment in populations of *Aechmea magdalenae* on Panama. Bush & Mulcahy (1999) reported frequencies of 58–81 percent asexual recruitment in populations of *Poikilacanthus macranthus*, an understory montane forest shrub from Costa Rica. Miwa *et al.* (2001) reported 79 percent asexual recruitment in *Melaleuca cajuputi*, a pioneer tree from Taiwan. Because those studies were conducted on species from diverse taxa and habitats, and were not accompanied by measures of the species' reproductive ecology, generalizations about the importance of asexual reproduction for regeneration in tropical forests and how it relates to other life-history traits has not been possible. Our data, the first systematic assessment of frequency of asexual recruitment in a series of closely related species indicates that, at least for *Piper*, asexual recruitment is more frequent in the understory than in gaps and clearings, and in species with lower sexual success from seeds.

RECONCILING EXPERIMENTAL AND MOLECULAR ESTIMATES OF THE FREQUENCY OF ASEXUAL RECRUITMENT.—In the absence of molecular data, field experiments that have measured fragment survival (Gartner 1989, Kinsman 1990, Greig 1993a) or estimated of the frequency of asexual recruitment based on the presence of callus tissue growth (Sagers 1993) may also help clarify the importance of asexual recruitment. With this in mind, we compared our previous estimates of asexual reproduction obtained from experimental approaches (*i.e.*, tracking cutting survival) to those we obtained using molecular markers. We found that our previous assessments of species' ability to reproduce asexually based on field experiments (Lasso *et al.* 2009) significantly over-estimate the frequency of asexual recruitment compared with data using molecular markers. This may be because high survivorship of fragments will translate to high asexual recruitment only if the frequency of fragment formation is high. For example, about 80 percent of *P. darienensis* and

P. cordulatum stem fragments and pinned-down branches survived in previous experiments (Lasso *et al*. 2009), but the molecular data indicate that < 50 percent of the individuals (disconnected shoots) in both populations recruited asexually. Therefore, conclusions from previous studies, in which levels of asexual recruitment were determined only from fragment survival, should be revisited.

Estimates of asexual recruitment success based on callus tissue growth may also be unreliable. For example, Bush & Mulcahy (1999) who documented that 99 percent of field-sampled plants of the shrub *P. macranthus* had callus tissue; found that 58–81 percent individuals had actually recruited asexually when they used molecular markers to identify clones. Sagers (1993) suggested that 92 percent of newly established *Psychotria horizontalis* plants originated asexually based on the presence of callus tissue growth, but no molecular studies have confirmed this frequency of asexuality. Given that experimental approaches overestimate actual asexual recruitment success, with the molecular data available for tropical shrubs we can only conclude that asexual recruitment is probably taking place in many understory species, possibly more often in shade-tolerant species than in light-demanding species. Our data and previous data on tropical plants indicate that none of the species are completely clonal and seedling recruitment is probably taking place in all species.

CONSEQUENCES OF ASEXUAL RECRUITMENT ON GENOTYPIC DIVERSITY.—Previous values of genotypic diversity reported for *Piper* are in agreement with our findings and prediction that shade-tolerant species should be less genetically variable than light-demanding species. The diversity indices found in the light-demanding species *P. cernuum* (Mariot *et al*. 2002) are very high compared with the values found in other shade-tolerant *Piper* species studied in Costa Rica: *P. amalago*, *P. pseudofuligineum*, and *P. jacquemontianum* (Heywood & Fleming 1986). In these, three sympatric populations from Costa Rica only three polymorphic loci were detected in more than 20 loci analyzed, indicating that this population may have been founded recently by a small number of individuals or may have suffered a recent bottleneck in the number of individuals (Heywood & Fleming 1986) and that asexual recruitment is probably not to blame for their reduced diversity. Even though we found that genotypic diversity decreases in populations with higher asexual recruitment, our results are consistent with previous findings for other clonal plants that show that asexual species are genetically diverse (Ellstrand & Roose 1987, Hamrick & Godt 1989, Widén *et al*. 1994, Silvertown 2008).

It has been suggested that the genotypic diversity of tropical clonal species should be lower than in temperate clonal species, because the more favorable year-round growing conditions could lead to more extensive clonal spread (Bush & Mulcahy 1999). Current data, including ours, are in disagreement with this prediction. Values of Simpson's genotypic diversity reported for tropical species so far are in the range of 0.72–1.0 (Murawski & Hamrick 1990, Bush & Mulcahy 1999, Miwa *et al*. 2001), whereas the range observed in temperate species is 0.13–1.0 (Ellstrand & Roose 1987, Widén *et al*. 1994, Honnay & Jacqyemyn 2008). Indicating that either clonal spread is not more frequent in the tropics than in temperate zones or/and that other factors maintain high levels of genotypic diversity in spite of asexual recruitment.

What determines the genotypic diversity of clonal populations? Simulation models suggest that sexual recruitment rates of only 1 percent per year can be sufficient to maintain genotypic diversity (Watkinson & Powell 1993). Beyond the balance between sexual and asexual reproduction, some additional factors may influence genotypic diversity in a local population, including the number of founding individuals, the age of the population, the life span of ramets, habitat heterogeneity, size of the clones and frequency of somatic mutations. For example, populations with more founding individuals should have more unique genotypes and therefore higher genotypic diversity. If individual genets are long-lived, then the genotypic diversity of the initial population may persist through clonal growth (Yeh *et al*. 1995), even in the absence of novel genotype recruitment. Another mechanism that could maintain genotypic diversity is density-dependent mortality driven by natural enemies (pathogens and herbivores). This is a well-known mechanism acting on seeds, seedlings and saplings that thins clumps of plants (Janzen 1970, Connell 1971, Augspurger 1983, Sánchez-Hidalgo *et al*. 1999). Density-dependent processes may operate even more strongly on clonal aggregations sharing exactly the same genotype. Conversely, large clones may maintain genotypic diversity if genets with independent ramets spread the risk of mortality among individuals, thus reducing the probability of that genotype disappearing from the population (Charlesworth 1980, Cook 1983). Habitat heterogeneity may also select for different genotypes, and therefore contribute to the maintenance of higher genotypic diversity. Finally, the accumulation of somatic mutations could potentially add genotypic diversity to the population (*e.g.*, Mes *et al*. 2002, van der Hulst *et al*. 2003, Houliston & Chapman 2004, Paun *et al*. 2006). Although no estimates of the frequency of somatic mutation exists for tropical systems, data from elsewhere suggest that it is frequent and could potentially lead to evolutionary changes (Gill *et al*. 1995, Lian *et al*. 2003, Michel *et al*. 2004, Nagamitsu *et al*. 2004, O'Connell & Ritland 2004, Vaughan *et al*. 2007). How populations of *Piper* and other clonal species maintain their genotypic diversity remains to be investigated, however, the high level of genotypic diversity found suggests that even populations with frequent asexual recruitment are probably well equipped to respond to environmental change.

Asexual reproduction seems to play an important role in maintaining local populations of *Piper* in the understory. Nevertheless, sporadic windows of opportunity for sexual reproduction, resulting from occasional favorable conditions of light and moisture conditions probably permit regeneration from seedlings, which seem to be sufficient to maintain high levels of genotypic diversity. For gap species, for which forest regrowth limits the period when conditions are favorable for recruitment, sexual reproduction probably provides the only opportunity for long-distance transport of genotypes to new suitable habitat. Regardless of the reproductive mode used by *Piper* species from different habitats, all seem capable of sustaining high levels of genotypic diversity that enhances their resilience in a changing world.

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