## SHORT COMMUNICATION

## Pollinators and pollen dispersal of *Piper dilatatum* (Piperaceae) on Barro Colorado Island, Panama

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Key Words: bees, clonal growth, fluorescent dye, pollination

The genus *Piper* is an important component of tropical forests worldwide. Many Piper species have been reported as self-compatible (Figueiredo & Sazima 2000), and many have the ability to reproduce asexually, forming clonal aggregations (Grieg 1993). Furthermore, the main dispersers of Piper (bats) transport whole infructescences to feeding roosts (Fleming & Heithaus 1981), tending to disperse closely related seeds in clumps. These characteristics of *Piper* biology are likely to result in populations with strongly marked spatial genetic structure, and raise the potential for inbreeding depression through self-fertilization. A few studies using allozymes to evaluate spatial genetic structure in Piper spp. support this view. These studies indicate that populations separated by more than 1 km are genetically distinct (high F<sub>ST</sub> values; Wright 1943) and that for some species inbreeding could be substantial (high values of FIS and FIT; Heywood & Fleming 1986, Mariot et al. 2002). However, the contributions of limited pollen and seed dispersal to generating spatial genetic structure remain unknown. Estimates of seed dispersal probabilities by Carollia perspicillata (Phyllostomidae) bats on Barro Colorado Island (BCI), Panama, and at Santa Rosa, Costa Rica, indicate that Piper dispersers move most seeds 50-300 m from the parent plant, with occasional longdistance events of > 1 km (Fleming 1981, Thies 1998). However, no studies have assessed how far Piper flower visitors move pollen. If seed dispersal is limited, and clonal reproduction is common, then long-distance pollen transfer may play a critical role in preventing inbreeding depression in *Piper* populations.

The objectives of this study were: (1) to determine the breeding system of a common *Piper* species; (2) to estimate the distance that pollen is moved through a sample population using fluorescent dye as a pollen analogue, and (3) to identify the pollinators of *Piper dilatatum* L. C. Rich. and to describe their daily visitation patterns. Research was carried out in tropical moist semi-deciduous forest on Barro Colorado Island, Panama (BCI; 9°09'N, 79°51'W). This forest receives 260 cm y<sup>-1</sup> rainfall and has a dry season between January and April (Leigh et al. 1982). Piper dilatatum is a shrub, usually found in gaps and clearings, 1.5-2 m tall, with white spicate inflorescences consisting of several thousand flowers (Croat 1978). The flowers are closely packed, minute and bisexual. Anthers are borne at the level of the stigma, so any part of a visitor that touches the pollen can touch the stigma too. Piper dilatatum is not completely dichogamous; one or two stamens open before the stigma matures, but the others open when it becomes receptive. Anthesis proceeds from the base to the tip of the spike over 8–12 d, though in the last 2-4 d most flowers are open. Inflorescence displays typically feature 2–30 spikes. Most plants flower from April-August, although some flower throughout the year (Thies & Kalko 2004). Two Carollia species of bat on BCI disperse the seeds (Kalko et al. 1996, Thies & Kalko 2004).

To determine if *P. dilatatum* is self-compatible, controlled pollinations were performed in the field on inflorescences isolated before anthesis. To accomplish this, we used plastic straws perforated with tiny holes to allow for gas exchange and closed them at both ends with nylon mesh. We isolated 3–6 inflorescences per plant and used 1–2 inflorescences for each of four treatments: (1) self-pollination, (2) cross-pollination, (3) no pollination, and (4) natural pollination. Outcross pollen was collected

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from plants found  $> 50 \, \text{m}$  away from pollinated plants and applied with a brush. It was not practical to measure the proportion of flowers that set seeds, so we measured the proportion of matured inflorescences, as the whole inflorescence aborts without sufficient pollination.

To estimate the distance that flower visitors move *Piper* pollen we used fluorescent dye as a pollen analogue for *P. dilatatum*. The study subpopulation was located in the approximately  $500 \times 120$ -m BCI laboratory clearing. No additional plants were found along streams and trails surrounding the clearing. Of 183 marked plants in the study area we chose four as sources of dye, each separated from one another by approximately  $100 \, \text{m}$ , and  $57 \, \text{others}$  as receivers-only, for a total of  $61 \, \text{receiving plants}$ .

During 17 d between 28 June and 24 July 2004, on days with no heavy rain, we coated five inflorescences of each source plant with dye. A different colour was chosen for each of the four source plants: pink, orange, yellow and green (Real Pros Sportfishing, Hepworth, Ontario, Canada). The colours were rotated once between plants to control for any pollinator preference in colour. Dye was applied at 08h00. The same source plants were used throughout the study. One source finished flowering on day 12 and was not replaced. At night all plants were checked with a commercial black light for dye deposition. The number of inflorescences in anthesis per plant with and without dye (and the colour of the dye) was recorded each day. Distances between plants were calculated by using a GPS and tape measure. Plants excluded from the study were not in flower or were in areas illuminated at night, preventing detection of dye.

To estimate the probability of pollen deposition (p) at various distances we used empirical logistic regression to model the proportion of inflorescences receiving dye per plant against distance from source plants (Kutner et al. 2005). We fitted a common slope for all four sources but allowed the intercepts to differ for each source (Kutner et al. 2005; example in Ballard et al. 2003). We logittransformed the proportion of pollinated inflorescences and analysed logit(p) in relation to the logarithm of distance in order to linearise the relationship between logit(p) and distance. To reduce the influence of plants with only a few open inflorescences, we used weighted regression, weighting by the square root of the number of inflorescences open on each plant (Kutner et al. 2005). We used Intercooled Stata 8.0 (Stata Corporation, College Station, Texas 77845, USA) for analyses. We used the robust option to allow for clustering (n = 61 unique plants in the analysis) in estimating standard errors. Statistically, intercepts were significantly different ( $F_{4,239} = 3.84$ ; P = 0.01), confirming the statistical model used. However, for illustration we present only one regression line, depicting the common slope with a mean intercept (weighted mean of the four source-specific intercepts). We calculated logit(p) and back-transformed pollination

**Table 1.** Results of self-compatibility experiment: number (n) of *Piper dilatatum* inflorescences (infl) achieving maturation into infructescences (infr) under different pollination treatments.

Treatment	n infl	n infr	infr/infl	n plants
Autogamy	24	0	0.00	13
Self-pollination	22	16	0.73	12
Cross-pollination	26	25	0.96	13
Natural pollination	23	17	0.74	14

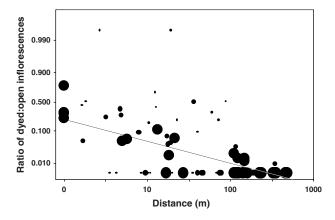
proportion (p) at specified distances. To provide approximate standard errors around predictions, we first back-transformed logit (p)  $\pm 1$  SE and then determined the geometric mean of the difference between predicted proportions (p) and those predictions based on  $\pm 1$  SE.

For the observation of potential pollinators we selected four 1-m-tall plants with 6–27 open inflorescences. Visitors were recorded for a total of 91 h at 1-h intervals between 06h00 and 16h00 on 10 d between 21 June and 20 July 2004. The number of inflorescences on the same plant that each insect visited was also noted. When possible, this count was performed on five individuals of each visitor species per hour. One individual of each visitor species was captured for identification. We found no nocturnal visitors engaged in pollination.

We found that 73% of inflorescences hand-pollinated with self-pollen matured into infructescences, while none of those in the no-pollination treatment did (Table 1). This eliminates the possibility of autogamy. Inflorescences treated with outcross pollen matured 96% of the time while only 74% of inflorescences matured in the control (naturally pollinated) group. Some self-pollination probably occurred in the outcross pollen treatment because we could not emasculate the tiny flowers, but this would only reduce the difference between the self- and cross-pollination treatments. These results suggest that while pollen from outcross sources may lead to higher fertility rates, *P. dilatatum* seed sets may contain high numbers of embryos produced by self-pollination.

In common with similar studies (Dudash 1991, Ghazoul *et al.* 1998, Waser & Price 1982, Young 2002), we found a decline in the probability of dye transfer with distance (Figure 1;  $F_{4,239} = 91.4$ ;  $R^2 = 0.605$ ; P < 0.001). The estimated probability of dye transfer between inflorescences of the same plant (distance = 0 m) was  $0.207 \pm 0.038$  ( $\pm$  SE). At 2.1 m from dye sources, probability of receiving dye fell by half ( $0.105 \pm 0.022$ ), while plants 33 m from sources had a  $0.021 \pm 0.005$  probability of dye reception. The estimated probability of dispersal at the greatest distance at which we observed dye movement (340 m) was  $0.004 \pm 0.001$ .

A diverse assemblage of insects visited *P. dilatatum* (Table 2). Peak activity occurred between 10h00 and 11h00, most visitation taking place from 08h00 to 14h00 (Figure 2). The visitors consisted of Hymenoptera



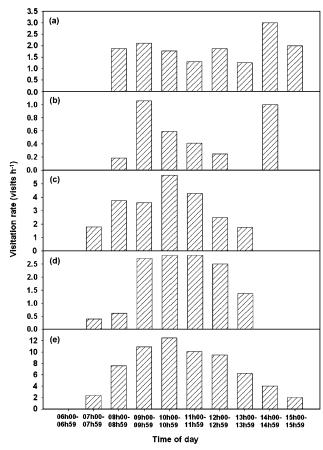
**Figure 1.** Regression of the probability of pollen deposition (p) against distance (m) from a source plant in *Piper dilatatum*. Figure depicts p on logit-scale and distance on logarithmic scale. Regression equation: logit(p) = -1.34-1.63 log(m). F<sub>4,239</sub> = 91.4; R<sup>2</sup> = 0.605; P < 0.001. Symbol sizes represent the number of inflorescences on each plant: 1-10 = small, 11-50 = medium, 51 or more = large.

**Table 2.** Potential pollinators of *Piper dilatatum*. Number (n) of visitors to inflorescences of four plants over 91 h of observation.

Species	n	% of total
Syrphids	148	18
Halictids	41	5
Trigona muzonesis Schwarz (Apidae)	333	40
Trigona (Tetragona) dorsalis Smith (Apidae)	185	22
Trigona fuscipennis Friese (Apidae)	34	4
Trigona (Frieseomelitta) nigra Cresson (Apidae)	2	0
Paratetrapedia calcarata Cresson (Apidae)	14	2
Tetragonisca angustula Latreille (Apidae)	56	7
Megachile spp. (Megachilidae)	10	1
Plebia frontalis Friese (Apidae)	11	1
All	834	100

and Diptera, particularly bees of the genus Megachile (Megachilidae), stingless bees (Apidae) and syrphid flies, all documented Piper pollinators (Figueiredo & Sazima 2000, Fleming 1985, Semple 1974). The most common visitors were Trigona species with T. (Tetragona) dorsalis and T. muzoenesis most abundant. Trigona dorsalis is a generalist pollinator, as are many syrphid flies which visit Piper (Fleming 1985). Trigona dorsalis, T. muzoenesis and Megachile spp. also visit several shade-tolerant and light-demanding species of Piper on BCI (E. Lasso, pers. obs.). On average, visitors landed at  $3.64 \pm 2.83 \ (\pm \text{SD})$ inflorescences per visit (Table 3). Halictid bees stopped at more inflorescences on the same plant in a single visit than any others  $(4.45 \pm 2.89, n = 22)$ ; Paratetrapedia calcarata  $(2.07 \pm 1.03, n = 15)$  and Megachile spp.  $(1.5 \pm 0.84,$ n = 6) visited the fewest.

Visits from *Megachile* spp. and *P. calcarata* would be more likely to result in out-crossing than those from halictids, which are probably responsible for much self-pollination. Observations on many hymenopteran



**Figure 2.** Daily visitation pattern of visitors to four *Piper dilatatum* plants (from top to bottom): syrphids (a), halictids (b), *Trigona muzoenesis* (c), *Trigona (Tetragona) dorsalis* (d), all visitors (e).

 $\begin{tabular}{ll} \textbf{Table 3.} & Number of inflorescences per plant visited per visitor. \\ n=number of insects observed, visits=number of inflorescences visited per plant per insect, Mean=arithmetic mean number of inflorescences visited per insect. \\ \end{tabular}$ 

Species	n	Visits	Mean $\pm$ SD
Syrphids	80	300	$3.75 \pm 3.18$
Halictids	22	98	$4.45 \pm 2.89$
Trigona muzonesis	115	426	$3.70 \pm 2.75$
Trigona (Tetragona) dorsalis	87	312	$3.59 \pm 2.88$
Trigona fuscipennis	17	66	$2.83 \pm 1.75$
Trigona (Frieseomelitta) nigra	6	9	$6.50 \pm 7.78$
Paratetrapedia calcarata	15	31	$2.07 \pm 1.03$
Tetragonisca angustula	12	34	$3.88 \pm 2.06$
Megachile spp.	2	13	$1.50 \pm 0.84$
Plebia frontalis	2	13	$6.50 \pm 2.12$
All	358	1302	$3.64 \pm 2.83$

visitors indicate they move mostly up and down inflorescences, only occasionally between inflorescences and plants (Semple 1974). Our study examined only part of the season during which *P. dilatatum* flowers; the community of visiting insects may change at other times of the year.

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Fluorescent dve does not always mimic pollen perfectly. sometimes differing in dispersal distance (Thomson et al. 1986) or variance in dispersal distance (Campbell et al. 1991). Therefore measures of dispersal with dyes should be interpreted cautiously. Still, visible mixing of pollen and dye on pollen combs of bees makes it reasonable to interpret dye as a pollen analogue (Adler & Irwin 2006, Waser 1988). Our most significant finding – that a large proportion of P. dilatatum pollen is distributed among the inflorescences of the same plant (Figure 1) is supported by our observations of pollinator behaviour: several inflorescences in the same plant are visited before the visitor leaves. We also show that self-pollination can lead to self-fertilization because over 70% of inflorescences hand-pollinated with self-pollen set seeds. Given these three pieces of evidence we conclude that most pollinator activity in *P. dilatatum* favours geitonogamy.

Limited pollen movement, the fact that this species can propagate asexually (E. Lasso & J. Dalling, unpubl. data) and its self-compatibility suggest that most of the seeds of *P. dilatatum* are probably the result of self-pollination and that their populations are likely to have strongly marked spatial genetic structure, as did the study populations of Heywood & Fleming (1986) and Mariot *et al.* (2002). While pollen can move over 100 m, it may not do so with sufficient frequency to prevent inbreeding depression.

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