

## SHORT COMMUNICATION

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

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(Accepted 22 June 2007)

**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  $F_{ST}$  values; Wright 1943) and that for some species inbreeding could be substantial (high values of  $F_{IS}$  and  $F_{IT}$ ; 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 long-distance 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 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 × 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 m, and 57 others as receivers-only, for a total of 61 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 logit-transformed the proportion of pollinated inflorescences and analysed  $\text{logit}(p)$  in relation to the logarithm of distance in order to linearise the relationship between  $\text{logit}(p)$  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  $\text{logit}(p)$  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

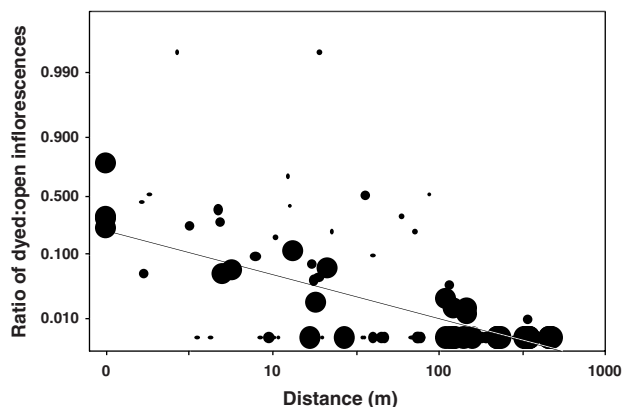
proportion ( $p$ ) at specified distances. To provide approximate standard errors around predictions, we first back-transformed  $\text{logit}(p) \pm 1$  SE and then determined the geometric mean of the difference between predicted proportions ( $p$ ) and those predictions based on  $\pm 1$  SE.

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



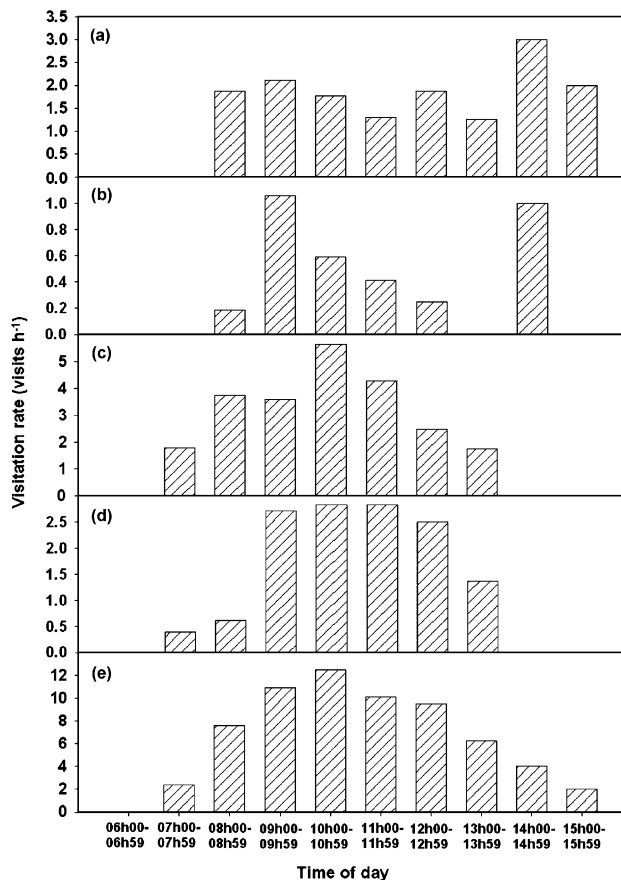
**Figure 1.** Regression of the probability of pollen deposition ( $p$ ) against distance (m) from a source plant in *Piper dilatatum*. Figure depicts  $p$  on logit-scale and distance on logarithmic scale. Regression equation:  $\text{logit}(p) = -1.34 - 1.63 \log(m)$ .  $F_{4,239} = 91.4$ ;  $R^2 = 0.605$ ;  $P < 0.001$ . 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
<i>Trigona muzonesis</i> Schwarz (Apidae)	333	40
<i>Trigona (Tetragona) dorsalis</i> Smith (Apidae)	185	22
<i>Trigona fuscipennis</i> Friese (Apidae)	34	4
<i>Trigona (Frieseomelitta) nigra</i> Cresson (Apidae)	2	0
<i>Paratetrapedia calcarata</i> Cresson (Apidae)	14	2
<i>Tetragonisca angustula</i> Latreille (Apidae)	56	7
<i>Megachile</i> spp. (Megachilidae)	10	1
<i>Plebia frontalis</i> 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. muzonesis* most abundant. *Trigona dorsalis* is a generalist pollinator, as are many syrphid flies which visit *Piper* (Fleming 1985). *Trigona dorsalis*, *T. muzonesis* 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  $3.64 \pm 2.83$  ( $\pm$  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 muzonesis* (c), *Trigona (Tetragona) dorsalis* (d), all visitors (e).

**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.

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

Fluorescent dye 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.

## ACKNOWLEDGEMENTS

We thank the National Science Foundation Undergraduate Mentoring in Environmental Biology program, for financing this project and the Smithsonian Tropical Research Institute for logistic support. We especially thank Douglas Schemske, David Roubik, Rachel Gallery and Robert Horan for loaning equipment, advice, and help identifying insects.

## LITERATURE CITED

- ADLER, L. S. & IRWIN, R. E. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany* 97:141–150.
- BALLARD, G., GEUPEL, R., NUR, N. & GARDALI, T. 2003. Long-term declines and decadal patterns in population trends of songbirds in western North America. *Condor* 105:737–755.
- CAMPBELL, D. R., WASER, N. M., PRICE, M. V., LYNCH, E. A. & MITCHELL, R. J. 1991. Components of phenotypic selection: pollen export and lower corolla width in *Ipomopsis aggregata*. *Evolution* 45:1458–1467.
- CROAT, T. 1978. *Flora of Barro Colorado Island*. Stanford University Press, Stanford. 943 pp.
- DUDASH, M. 1991. Plant size effects on female and male function in hermaphroditic *Sabatia angularis* (Gentianaceae). *Ecology* 72:1004–1012.
- FIGUEIREDO, R. & SAZIMA, M. 2000. Pollination biology of Piperaceae species in southeastern Brazil. *Annals of Botany* 85:455–460.
- FLEMING, T. H. 1981. Fecundity, fruiting pattern, and seed dispersal in *Piper amalago* (Piperaceae), a bat-dispersed tropical shrub. *Oecologia* 51:42–46.
- FLEMING, T. H. 1985. Coexistence of five sympatric *Piper* (Piperaceae) species in a tropical dry forest. *Ecology* 66:688–700.
- FLEMING, T. H. & HEITHAUS, E. R. 1981. Frugivorous bats, seed shadows, and the structure of tropical forests. *Biotropica* 13:45–53.
- GHAZOU, J., LISTON, K. & BOYLE, T. 1998. Disturbance-induced density-dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. *Journal of Ecology* 86:462–473.
- GREIG, N. 1993. Regeneration mode in neotropical *Piper*: habitat and species comparisons. *Ecology* 74:2125–2135.
- HEYWOOD, J. & FLEMING, T. H. 1986. Patterns of allozyme variation in three Costa Rican species of *Piper*. *Biotropica* 18:208–213.
- KALKO, E. V. K., HERRE, E. A. & HANDLEY, C. 1996. Relation of fig fruit characteristics to fruit-eating bats in the New and Old World tropics. *Journal of Biogeography* 23:565–576.
- KUTNER, M. H., NACHTSHEIM, C. J., NETER, J. & LI, W. 2005. *Applied linear statistical models*. McGraw-Hill Irwin, New York. 1396 pp.
- LEIGH, E., RAND, A. & WINDSOR, D. (eds.). 1982. *The ecology of a neotropical forest: seasonal rhythms and longer-term changes*. Smithsonian Institution, Washington, DC. 503 pp.
- MARIOT, A., DI STASI, L. C. & DOS REIS, M. S. 2002. Genetic diversity in natural populations of *Piper cernuum*. *Journal of Heredity* 93:365–369.
- SEMPLE, K. 1974. Pollination in Piperaceae. *Annals of the Missouri Botanical Gardens* 61:868–871.
- THIES, W. 1998. *Resource and habitat use in two frugivorous bat species (Phyllostomidae: Carollia perspicillata and C. castanea) in Panama: mechanisms of coexistence*. Ph.D. Dissertation. Eberhard-Karls-University of Tübingen, Germany.
- THIES, W. & KALKO, E. V. K. 2004. Phenology of neotropical pepper plants (Piperaceae) and their association with their main dispersers, two short-tailed fruit bats, *Carollia perspicillata* and *C. castanea* (Phyllostomidae). *Oikos* 104:362–376.
- THOMSON, J. D., PRICE, M. V., WASER, N. M. & STRATTON, D. A. 1986. Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*. *Oecologia* 69:561–566.
- WASER, N. M. 1988. Comparative pollen and dye transfer by pollinators of *Delphinium nelsonii*. *Functional Ecology* 2:41–48.
- WASER, N. M. & PRICE, M. V. 1982. A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* 63:2268–2272.
- WRIGHT, S. 1943. Isolation by distance. *Genetics* 28:114–138.
- YOUNG, H. 2002. Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). *American Journal of Botany* 89:433–440.