

Bumblebee pollination of understorey shrub species in a tropical montane forest in Costa Rica

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ABSTRACT. This study addresses the pollination ecology of shrub species in an upper montane forest in Costa Rica, where the diversity of insect visitors is much below that of the well-studied tropical forests of lower elevations. Data are presented on visitation rates by the tropical bumblebee *Bombus ephippiatus* to flowers of three species, pollen loads on bumblebee bodies, and pollen loads on stigmas of two of the species. High visitation rates to flowers of *Palicourea brenesii* were found, a species which produces copious amounts of nectar. Visitation rates to nectarless *Leandra subseriata* and *Deppea grandiflora* were much lower. Conspecific pollen comprised on average 90% of the pollen load on stigmas of *Leandra*, while *Palicourea* stigmas carried only 26 to 55% conspecific pollen. With only two ovules per flower, fruit and seed set in *Palicourea* were not limited by a shortage of conspecific pollen. In *Leandra*, 20–25% of the flowers received too few conspecific pollen grains for maximum seed set among 440 ovules per flower. Individual bumblebees showed no constancy in plant choice, judging from the high number of different pollen types (6–12) on their bodies. Workers returned frequently to the same *Palicourea* patch without visiting nearby *Palicourea* plants, which suggests the use of foraging paths.

RESUMEN. Se estudió la ecología reproductiva de arbustos en un bosque montano, donde la diversidad de insectos polinizadores es muy bajo que en la selva húmeda. Se estudiaron el grado de visita a las flores de tres especies de arbusto por el abejorro *Bombus ephippiatus*, las cargas de polen en los cuerpos de abejorros, y las cargas de polen en las estigmas de dos de las especies. Se encontraron altos grados de visita a las flores de *Palicourea brenesii*, que producen cantidades copiosas de néctar. Los grados de visita a las flores de *Deppea grandiflora* y de *Leandra subseriata* eran mucho más bajos. El polen conespecífico constituyó en promedio 90% de la carga del polen en las estigmas de *Leandra*, mientras estigmas de *Palicourea* llevaron

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solamente 26–55% de polen conoespecifico. Con dos óvulos por flor, *Palicourea* no se faltó polen para la formación de frutos y semillas. En *Leandra*, 20–25% de las flores recibieron demasiado pocos granos de polen para obtener el grado máximo de formación de semillas para los 440 óvulos. Los abejorros individuales no demostraron constancia en las especies visitadas, juzgando del alto número de tipos de polen (6–12) en sus cuerpos. Obreras volvieron con frecuencia al mismo grupo de *Palicourea* sin visitar plantas próximas de *Palicourea*, que indica uso de ‘camino de forraje’.

KEY WORDS: *Bombus ephippiatus*, flower constancy, *Depeea grandiflora*, foraging paths, *Leandra subseriata*, Melastomataceae, *Palicourea brenesii*, Rubiaceae, stigmatic pollen loads, visitation rates

INTRODUCTION

Successful sexual reproduction in animal-pollinated species depends to a large extent on the behaviour of flower visitors (Conner *et al.* 1995, Harder & Barrett 1993, Pellmyr & Thompson 1996, Thostesen & Olesen 1996, Webb & Bawa 1983). The number of visits a plant receives and the number of pollen grains deposited per visit affect female reproductive success through seed set (the number of ovules fertilized on the plant itself) and male reproductive success through pollen export (the number of ovules fertilized by pollen grains from that plant). Low visitation rates can lead to pollen limitation of fruit or seed set, a decrease in female reproductive success due to insufficient receipt of pollen (Burd 1994). A second component of pollinator behaviour, flower constancy, influences the effectiveness of a visit to a flower. When a pollinator exclusively visits flowers of one species, it will transfer more pollen to conspecific stigmas than an individual that visits several plant species during one foraging bout. The uptake of heterospecific pollen between visits to flowers of one species reduces the number of conspecific pollen grains that are deposited on the second flower if the different pollen types are transferred on the same body parts of the visitor. Furthermore, the presence of heterospecific pollen can form a barrier to pollen germination (stigma clogging), pollen tube growth, or subsequent fertilization by conspecific pollen (McLernon *et al.* 1996). Part of the pollen taken up from the anthers by a visitor that does not show flower constancy is likely to be deposited on stigmas of flowers of other species. This results in a decrease in male fitness compared to pollen transport by an animal that visits one species only.

We studied pollinator visitation rates and flower constancy in a tropical montane oak forest in the Cordillera de Talamanca, Costa Rica. Compared to the situation in lowland tropical forests, where a wealth of species of flower visitors is present (Bawa *et al.* 1985, Kato 1996), this system is very poor in visitor diversity. Almost all diurnal flower visits in the understory we observed were performed by one species, the bumblebee *Bombus ephippiatus* Say. *Bombus ephippiatus* is a neotropical montane bumblebee species (subgenus *Pyrobombus* Dalla Torre) that ranges from Mexico to Ecuador (Laverly & Plowright 1985).

In Costa Rica it is found from 1300 m asl in cloud forest in Monteverde (Cane 1993) to 3500 m on the páramo (Heithaus 1983). Other flower visitors, such as other bee species and hummingbirds, have been observed, but their visits comprised only 1% of all flower visits observed (Wesselingh *et al.* 1999). The absence of other major pollinating species in this system is likely to cause competition among plants to attract the same visitors. Problems with hetero-specific pollen transfer among species may occur depending on the degree of floral constancy of individual bumblebee visitors.

We investigated pollination by *Bombus ephippiatus* of the two most common understorey shrubs at our study site: *Palicourea brenesii* Standl. (Rubiaceae) and *Leandra subseriata* (Naudin) Cogn. (Melastomataceae). These species flower simultaneously, but are very different in habit. *Palicourea* has blue, tubular flowers that produce copious amounts of nectar, while the small, white *Leandra* flowers do not produce any significant amount of nectar. The poricidal anthers of *Leandra* require buzzing for pollen release.

We posed the following questions: (1) How often are flowers on each of the species visited? (2) Do individual bumblebees show flower constancy, i.e. do they limit their visits in one foraging bout to one or a few plant species? (3) Do bumblebees systematically visit all plants of a species in an area, or do they follow foraging paths? (4) What is the composition of the pollen loads deposited on flower stigmas? We discuss the implications of the behaviour of the main flower visitor for the reproductive ecology of the plant species involved, by comparing our findings with data we collected earlier on the reproductive system of these species (Wesselingh *et al.* 1999).

STUDY SITE

The study was conducted in a primary forest in the valley of the Rio Savegre on the Pacific slope of the Cordillera de Talamanca, near the village of San Gerardo de Dota. The site was located on the east side of the valley just above the river, at 2320 m asl. The forest was on a steep west-facing slope (up to 45°) and is classified as lower montane cloud forest (Kappelle *et al.* 1989). Due to the steep slope and several natural treefall gaps, the vegetation was relatively open with a high coverage of herbs (70%). The climate in the area is characterized by a dry season with very little rain from December until May, and almost daily rainfall during the rest of the year. Total annual rainfall is *c.* 2700 mm, with daily maximum temperatures inside the forest of around 15 °C, and an average nightly minimum of 10 °C (Kappelle 1996). The area forms part of the 'Reserva Forestal Los Santos'. Parts of the valley were cleared in the 1950s and are now in use as pasture land and orchards, but over 80% of the Rio Savegre watershed area still consists of primary forest (van Omme *et al.* 1997). The observations in this study were all performed in the dry season, from the end of February until the end of April 1996. Figure 1 shows the study site, with locations of the plants studied.

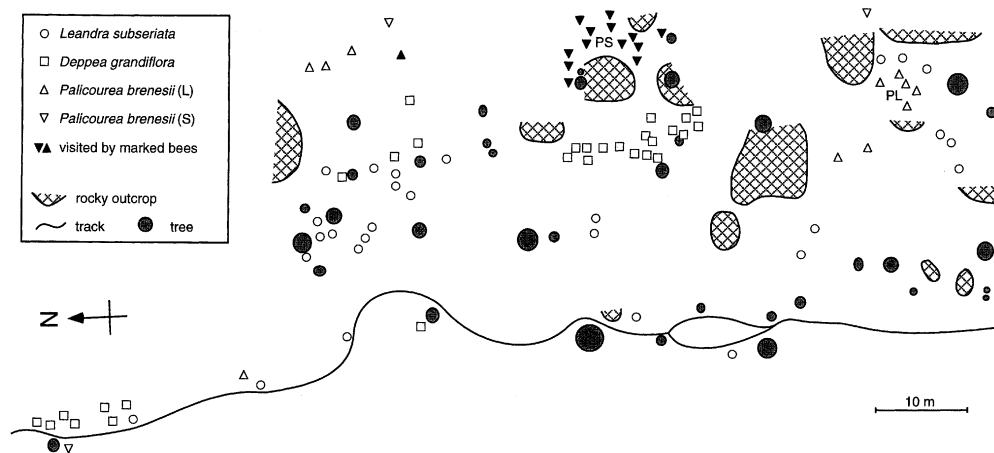


Figure 1. The study site in the Río Savegre valley, Costa Rica, with the locations of the plants studied. Only plants that flowered during the study period are indicated. For *Palicourea*, visitation rates were recorded at PS (short-styled morph) and PL (long-styled morph). Filled triangles indicate *Palicourea* plants visited by bumblebees that were marked earlier while visiting the short-styled patch (see Methods section: Foraging behaviour of marked bumblebees).

METHODS

Visitation rates

We collected data on visitation rates for *Leandra*, *Palicourea* and a third species, *Deppea grandiflora* Schltdl. (Rubiaceae), which has bright yellow flowers and produces low amounts of nectar. Visitation rates for each species were recorded at two different patches within the study site. Before observations started, the number of open flowers was counted. During 1 h, the arrival time of each bumblebee and the number of flowers visited were recorded. The observation hour was followed by a 15-min break, after which the observations were resumed. For *Deppea* and *Leandra*, observations were done at two different patches, each on at least three consecutive days. For the distylous *Palicourea* we observed a patch with short-styled flowers on 5 d, and a patch of long-styled flowers on 4 d. For all species we conducted observation bouts of several hours a day, starting at different times in order to obtain multiple observations for most of the daylight hours. Observations were corrected for effective time observed, which could be less than 1 h due to the 15-min breaks. From the data on number of flowers visited and the number of open flowers we calculated the number of visits per flower per hour.

Pollen loads on bumblebees

Bumblebee workers were caught with an insect sweep net after visiting at least one flower of *Palicourea brenesii* in the long-styled cluster on 8 April 1996, and at the short-styled cluster on 21 April 1996. We made no attempt to catch bumblebees on the other two plant species, because visitation rates were so low that we would not have been able to catch enough animals in the time

available. The bumblebees caught were anaesthetized with ethyl-acetate and all pollen was removed from their bodies (head, thorax and abdomen) with a small cube of glycerine jelly, prepared after Beattie (1972), but without the addition of phenol. We did not collect pollen from different body parts separately, and excluded the contents of the corbiculae. The gel cube was put immediately on a glass slide and melted with a lighter held under the slide. A cover slip was put on top of the melted gel. After the gel had hardened again, the sides of the cover slip were covered with transparent nail polish to prevent infection by fungi. The slides were transported to the laboratory in Amsterdam and types and numbers of pollen grains were recorded with a microscope. A reference collection of pollen from flowers in the area (including cultivated species) was made to make it possible to identify the pollen types found on the bumblebees' bodies.

Foraging behaviour of marked bumblebees

To make an estimate of the number of individual bumblebees involved in visiting *Palicourea* and whether or not they also visited neighbouring patches of the same species, we attempted to catch all bumblebees visiting the short-styled *Palicourea* patch on the afternoon of 21 April. Workers were marked on the thorax with a dot of red acrylic paint, and males with green. No anaesthetic was used. The bumblebees were immobilized with a soft plug in a tube with a coarse mesh on one end, through which the paint could be applied. After the paint had dried, the bees were released. The next day, observations were performed at the short-styled and long-styled patch simultaneously. We used the same observation methods as described above for visitation rates, but now we also noted whether the bumblebees were marked and with what colour. On 23 and 24 April, all other *Palicourea* plants in the area were observed in 30-min shifts, to see which of these were also visited by bumblebees marked at the short-styled cluster.

Stigmatic pollen loads

Stigmas were collected at random on *Leandra* ($n = 17$) and *Palicourea* ($n = 18$ for each style morph) after the corollas had wilted, and preserved in 70% ethanol. In the laboratory in Amsterdam the stigmas were softened in a 10% ammonia solution for 6 h, and squashed on a glass slide with a piece of glycerine jelly, the same as used for the bumblebee pollen loads. This procedure stained the pollen grains and made them visible through the stigmatic tissue. The ethanol solution in which the stigmas had been kept was centrifuged, and the pellet was resuspended and also mounted in glycerine jelly. The total number of pollen grains and the number of conspecific pollen grains were counted. The discernible pollen types of heterospecific pollen were noted, but it was not always possible to count these types individually. Stigmas of *Deppea* were not analysed, because *Deppea* pollen is a-specific (type 'small') and could not be distinguished from other, heterospecific pollen types.

Statistical analyses

Differences among plant species in bumblebee visitation rates were tested with the non-parametric Friedman test, because the design was unbalanced due to varying numbers of observation days per hour local time. We used the mean number of visits per flower for each hour as the variable. We considered the hours of day (08h00–17h00) as blocks. Multiple comparisons among treatment means were carried out with the simultaneous test procedure (STP: Sokal & Rohlf 1995), which is based on pairwise Mann–Whitney U-tests.

Because of lack of normality, differences among pollen grain numbers and percentages of total pollen load on bumblebees visiting the two *Palicourea* style morphs were tested non-parametrically with a two-tailed Mann–Whitney U-test. The same test was used for differences in stigmatic pollen loads between the two *Palicourea* morphs. To correct for multiple comparisons in the latter analysis, we used the sequential Bonferroni-correction (Holm 1979, Rice 1989) on the P values that were obtained by z-transformation, corrected for ties.

RESULTS

Visitation rates

Visitation rates were highest around midday for all plant species, but large differences in visitation rates existed among species and among days (Figure 2). The cumulative visitation rate (visits per flower per day) was 0.63 for *Leandra* and 1.03 for *Deppea*. *Palicourea* received 45.6 visits per flower per day for the long-styled morph, and 48.5 for the short-styled morph, 45 to 75-fold higher than the other two species. The Friedman test confirmed this: the difference among species in visitation rate was highly significant ($\chi^2 = 24.1$, $df = 3$, $P < 0.0001$). Multiple comparisons showed that both *Palicourea* morphs had significantly higher visitation rates than *Deppea* and *Leandra* ($P < 0.01$). The *Palicourea* morphs did not differ significantly from each other, and the difference between *Deppea* and *Leandra* was close to significance ($U = 69$, critical value at $\alpha = 0.05$ is 69.6).

Palicourea visitors collected nectar, *Leandra* visitors buzzed the anthers to collect pollen, and *Deppea* visitors collected nectar. Many of the visiting bumblebees had filled corbiculae, which indicates that they had either actively collected pollen from flowers, or gathered pollen that had been deposited to their bodies, earlier on in the foraging bout.

Pollen loads on bumblebees

Pollen loads on bodies were highly variable among individual bees, both in number of pollen grains and in composition of the load (Figure 3). Total loads ranged between 72 and 1503 pollen grains. The number of discernible pollen types on an individual bee averaged 8.7 (range 6–12). The number of *Palicourea*

brenesii pollen grains was very low, on average 5.4 at the long-styled and 8.1 at the short-styled patch, 1.2% and 1.6%, respectively.

Significant differences after Bonferroni-correction were found between bees foraging on short-styled and long-styled plants. Bumblebees foraging at the short-styled patch carried significantly more *Leandra* pollen ($U = 8$, corrected $P < 0.05$) and more pollen of the 'small' type ($U = 7$, corrected $P < 0.05$) than the bumblebees caught at the long-styled site. Asteraceae pollen was common in the pollen loads of both groups of bumblebees.

Foraging behaviour of marked bumblebees

On 21 April 1996, we caught and marked nine bumblebees, seven workers and two males, at the short-styled *Palicourea* patch. After that, no more unmarked bumblebees visited the site, only the previously marked animals. The following day, 234 bumblebee approaches to the same patch were recorded during 6 h of observation (08h45–16h15). Of these, 171 were made by marked workers and 50 by unmarked bumblebees. In 13 cases it could not be assessed with certainty whether the bee was marked or not. Assuming that all visiting bumblebees behaved in the same way, and that all seven marked workers were present the day after marking, then the total number of individuals visiting this cluster was not more than ten (seven marked workers carried out 171/234 = 73% of the visits). From 171 visits in 360 min of observation it can be calculated that a marked worker visited the short-styled patch on average every 14.7 minutes, or four times an hour. This number could be even higher if some of the marked workers did not return to the patch. The two marked males were not seen again.

At the long-styled patch, about 15 m away from the short-styled patch, 176 bumblebee approaches were recorded on that same day, but none of these animals was marked. Figure 1 shows which of the other observed *Palicourea* plants at the study site were visited by the marked bumblebees on 23 and 24 April 1996. The bees limited their visits to *Palicourea* plants to the patch where they were marked and the immediate vicinity, with the exception of one long-styled plant that stood about 10 m west of the patch.

Stigmatic pollen loads

Total stigmatic pollen load differed between short-styled and long-styled *Palicourea* flowers, with long-styled flowers having almost twice as many grains on their stigmas (Table 1). However, this difference was not significant after Bonferroni correction. The number of conspecific grains did not differ between the two morphs, but because of the difference in total load, the percentage conspecific pollen on short-styled stigmas was twice as high as on long-styled flowers. Thus, long-styled stigmas carried a significantly higher fraction of heterospecific pollen. In contrast, the stigmatic loads of *Leandra* consisted of 90% conspecific pollen.

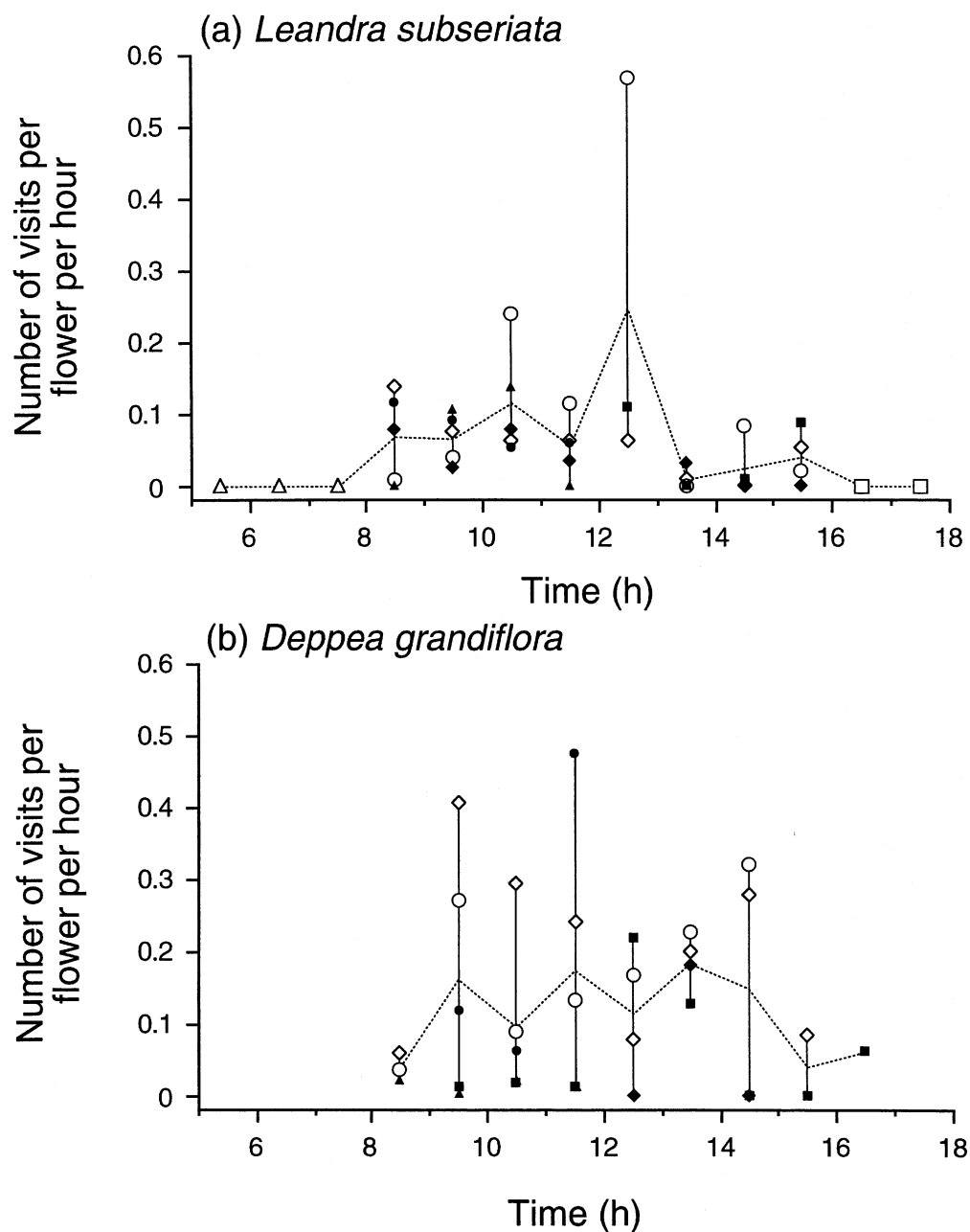
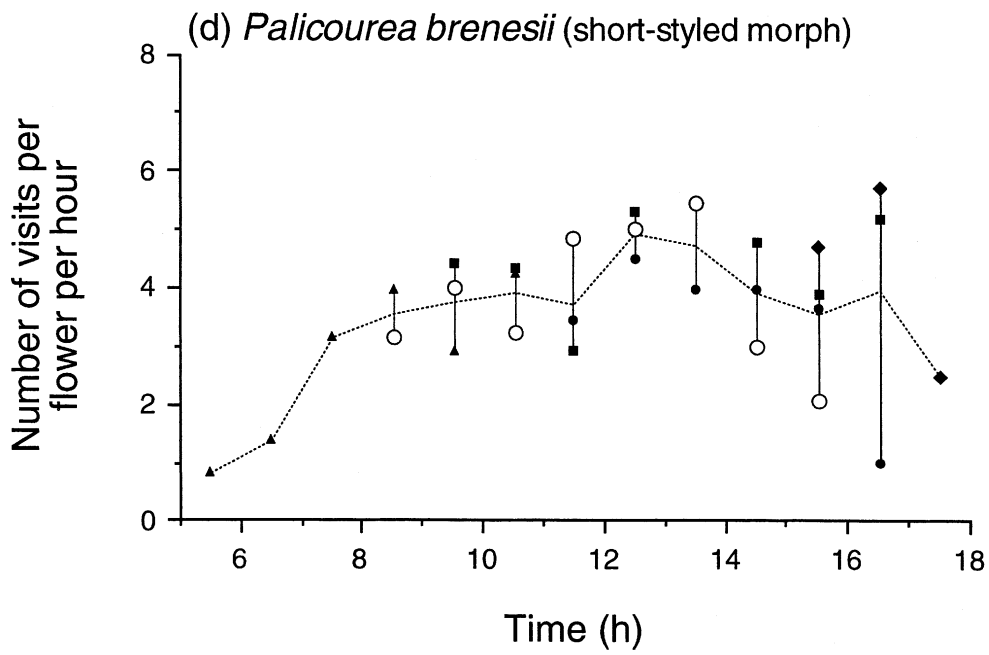
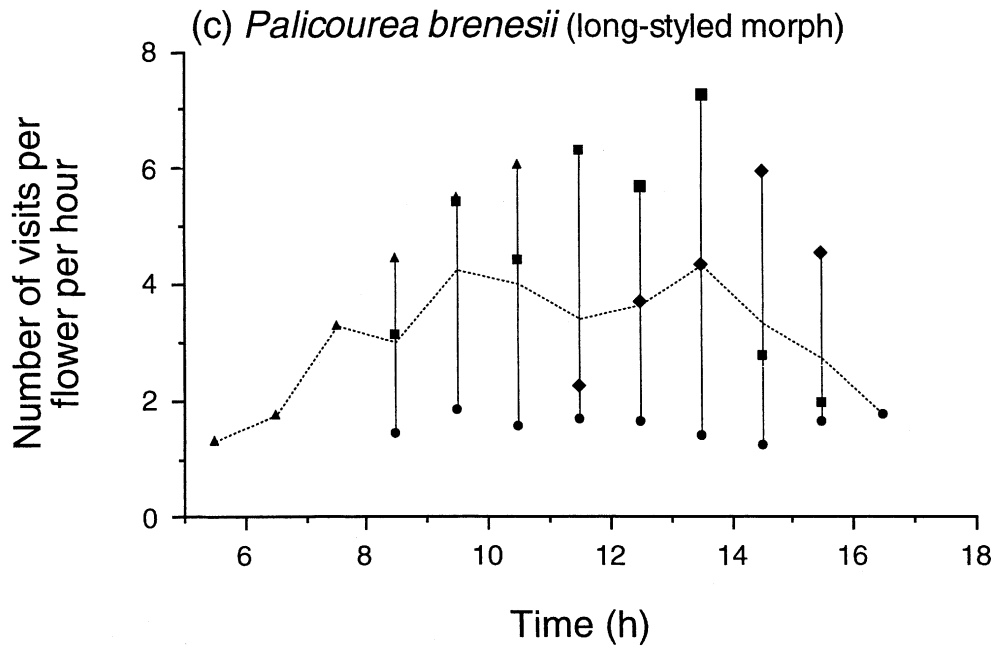


Figure 2. Visitation rates throughout the day of *Bombus ephippiatus* on (a) *Leandra subseriata*, (b) *Deppea grandiflora*, and (c) the long-styled and (d) the short-styled morph of *Palicourea brenesii*. Indicated are the number of visits per flower for each observation hour, with a different symbol for each observation day. As a visual aid, a dotted line is drawn connecting the means for each hour. Note the difference in scale of the vertical axis between graphs (a) and (b) vs. (c) and (d).



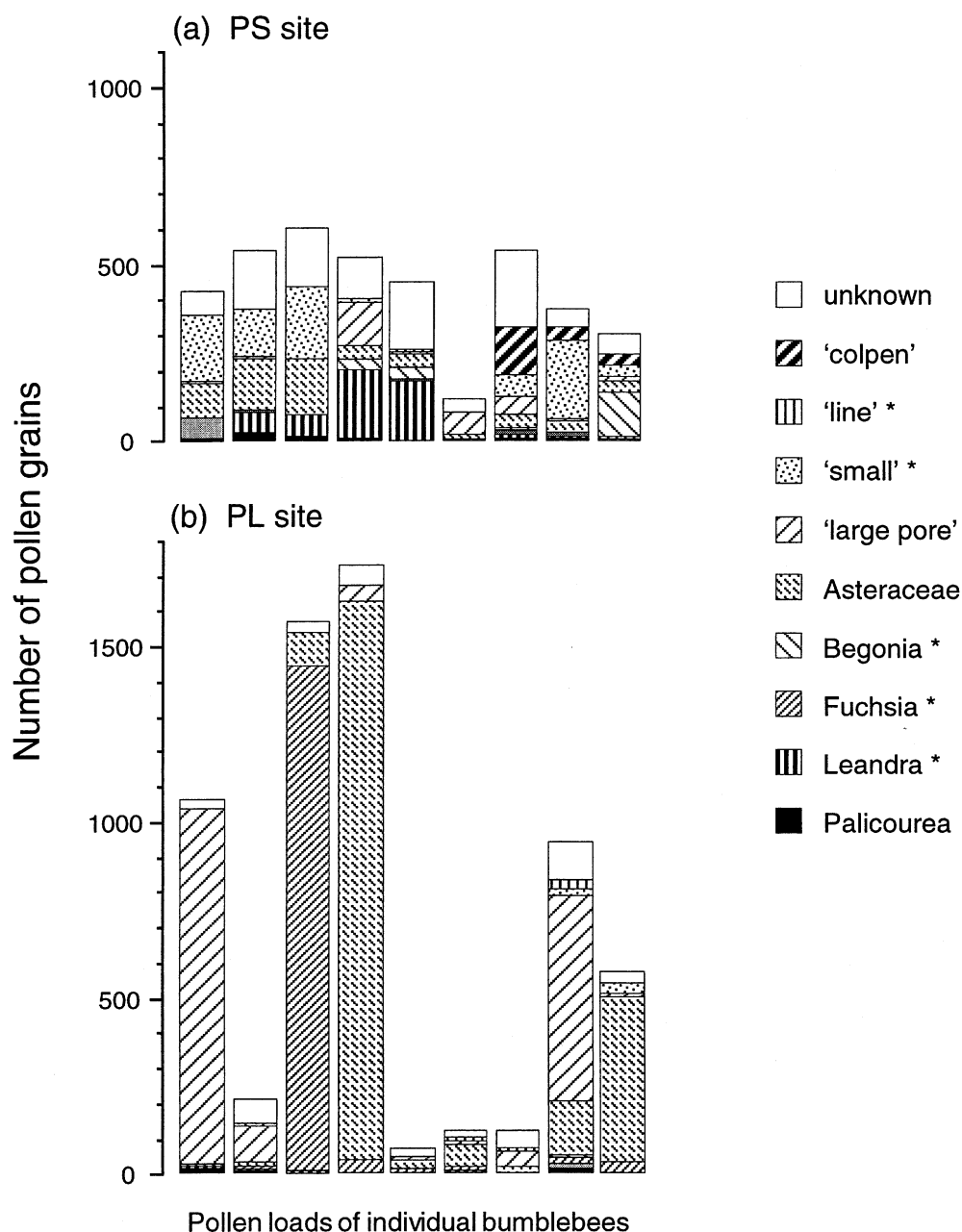


Figure 3. Size and composition of pollen loads on bodies of nine *Bombus ephippiatus* workers each that were caught after they had just started foraging on flowers in (a) the short-styled patch PS or (b) the long-styled patch PL of *Palicourea brenesii*. Pollen type names between quotation marks indicate an unidentified type that was either not represented in the pollen reference collection or could be produced by more than one plant species. In the category 'unknown', very rare pollen types (a few grains observed on less than three bumblebees) and grains that could not be identified, were lumped. An asterisk after the pollen type name denotes a significant difference in number of pollen grains of that type between the workers at the two patches.

Table 1. Pollen loads on stigmas of both flower morphs of *Palicourea brenesii* and *Leandra subseriata*. The total number of pollen grains, the number and percentages of species-specific grains and recognizable heterospecific pollen types are shown. The column between the *Palicourea* morphs gives the original P values (after z-transformation) for the Mann–Whitney U-test for differences between the morphs, with bold type for values that remained significant after sequential Bonferroni-correction.

	<i>Palicourea brenesii</i>		<i>Leandra subseriata</i>	
	Short-styled (n = 18)	P	Long-styled (n = 18)	(n = 17)
	median (range)		median (range)	median (range)
<i>Number of grains</i>				
Total	193 (5–389)	0.0368	340.5 (30–546)	627 (11–1761)
Conspecific	59 (2–250)	0.4383	80 (6–140)	613 (8–1676)
<i>Heterospecific</i>				
<i>Fuchsia (paniculata)</i>	9 (0–54)	0.1243	17.5 (1–108)	0 (0–3)
Asteraceae	3 (0–14)	0.0143	12 (0–102)	1 (0–60)
<i>Percentage of total</i>				
Conspecific	51.4 (8.6–96.6)	0.0029	23.9 (12.7–60.3)	95.2 (61.7–99.0)
<i>Heterospecific</i>				
<i>Fuchsia (paniculata)</i>	7.7 (0.0–42.2)	0.6578	7.6 (1.3–31.6)	0 (0.0–0.29)
Asteraceae	1.7 (0.0–12.9)	0.0619	4.1 (0.0–24.9)	1.6 (0.0–9.1)

DISCUSSION

Bombus ephippiatus workers showed a profound lack of flower constancy: each individual animal sampled carried at least six different pollen types on its body. The lack of specialization may reflect the low availability of flowers per species and the patchy distribution of plants. Low flower densities are known to reduce both the number of approaches to a patch and the number of flower visits in a patch (Kunin 1997). *Palicourea* has large inflorescences with nearly 200 buds (Wesselingh *et al.* 1999), but only three or four flowers per inflorescence are open on a given day. Individual bumblebees of temperate species are known to specialize on one—‘major’—flower species with only occasional visits to other—‘minor’—species (Heinrich 1979). Nevertheless, mixed pollen loads were found in high frequency by Macior (1994) in a subalpine meadow in the USA, and by Kwak & Velterop (1997) in chalk grasslands in The Netherlands. In general, only 5% of individuals of temperate bumblebees have pollen of four or more species in their corbicular pollen loads (Heinrich 1976), while about half of the loads are pure. In contrast, only one-third of the bumblebees in our study carried pollen loads that had over 50% pollen of one species (Fig. 3), the others had fairly evenly distributed loads among pollen types, with no sign of majoring in just one plant species.

Both the large differences in pollen loads among bumblebees foraging at different locations (Figure 3) and the behavioural observations on marked

workers suggest that individual bumblebees had different foraging paths (Heinrich 1976). They seemed to follow a fixed route, whereby they returned to the same flowers at regular, short intervals. We were unable to follow bumblebees after they stopped foraging at a patch, because the insects would fly away swiftly and be lost from sight immediately. *Palicourea* flowers produced much nectar (R. A. Wesselingh and M. Witteveldt, *pers. obs.*), and our data on visitation rates showed that this species was highly favoured by bumblebees. *Palicourea* visitation in our study was much higher than the visitation rates by hummingbirds recorded by Feinsinger *et al.* (1991) for *Palicourea lasiorrachis* (one visit per flower per hour) in lower elevation cloud forest in Monteverde, Costa Rica. Still, individual bumblebees did not visit all *Palicourea* patches at the study site, but restricted their visits to just a few, as the observations on marked bumblebees showed. It is likely that, once established, foraging routes are fairly fixed (Comba 1999), with only limited scouting off-route to find new resources. This behaviour can strongly affect the genetic population structure of the plant species visited, and lead to small effective population sizes.

Bumblebee behaviour and plant performance

We found large differences among plant species in visitation rates. Daily visitation rate multiplied by flower life span gives the expected number of bumblebee visits that a flower received in a lifetime. Lifetime flower visitation rates were 75.2 for *Palicourea* (life-span 1.6 d), 3.7 for *Leandra* (life-span 5.8 d), and 8.9 for *Deppea* (life-span 8.6 d; Wesselingh *et al.* 1999). Although we have no data on the number of visits required for full seed set, *Palicourea* is unlikely to be visitation-limited. *Leandra* and *Deppea* still achieved a fairly high per-flower lifetime visitation rate, thanks to their long flower life spans.

Although pollen loads on bumblebees contained a very low percentage of *Palicourea* pollen grains (less than 2%), the percentage of conspecific grains found on stigmas was considerably higher. This may be due to the fact that we did not discriminate between different body parts when collecting pollen from bumblebees. Pollen from the tubular *Palicourea* flowers will be concentrated on the bumblebee's head, while, for instance, pollen of the buzz-pollinated *Leandra* flowers will be deposited mainly on the ventral side of the body. A second explanation is that we caught the bumblebees when they had just arrived at a *Palicourea* patch. The number of conspecific pollen grains on a pollinator's body increases with the number of visits to flowers of the same species, and flowers that are visited late in such a sequence receive a higher proportion of conspecific pollen (Rademaker *et al.* 1997).

Pollen quantity and quality for Palicourea brenesii

Visitation rates to *Palicourea* were very high, but the pollen loads of bumblebees arriving at our focal *Palicourea* patches contained few *Palicourea* pollen grains. Still, the average load of conspecific pollen grains on stigmas was

high (Table 1), considering that *Palicourea* fruits have only two ovules. *Palicourea* is distylous, so pollen grains may come from the same plant, from a different plant of the same style morph, or from the opposite style morph. No size difference exists between pollen grains of the two morphs, so we could not study pollen flow between morphs. Nevertheless, considering the low number of *Palicourea* pollen grains carried by arriving bumblebees (average 5.4 pollen grains at the long-styled patch, 8.1 grains at the short-styled patch), many of the pollen grains deposited on stigmas are likely to be from other plants in the same patch, which are all of the same morph. We have strong indications that patches consist of several ramets of the same genet. *Palicourea* plants reproduce clonally by underground runners that put up new ramets (R. A. Wesselingh, *pers. obs.*). Style morphs in our study are not distributed randomly, as in other populations of other *Palicourea* species (Ree 1997), but are clumped in patches that quite probably consist of just one genetic individual. This means that pollen transferred within a patch contributes to geitonogamous self-pollination. In a previous study, seed set after hand selfing was high in long-styled flowers (59%), but only 4% in short-styled flowers (Wesselingh *et al.* 1999). Natural fruit set is around 32% (Wesselingh *et al.* 1999), and this suggests that some heteromorphic pollen is being deposited, especially on short-styled stigmas. Although the results suggest that long-styled flowers should be better able to set fruit, given the high incidence of geitonogamous pollen transfer, natural fruit set is lower for long-styled than for short-styled plants (28% vs. 38%; Wesselingh *et al.* 1999). Both pollination and resource availability could play a role in the observed fruit set limitation.

Long-styled flowers of *Palicourea* received more pollen, as found for the majority of distylous species studied (Ree 1997), but this load consisted of only 26% conspecific pollen, compared to 55% for short-styled stigmas. The protruding stigma of long-styled flowers is much more likely to contact other parts of bumblebee bodies. In particular a bumblebee's ventral side will touch the stigma frequently when the insect crawls over a flower (R. A. Wesselingh, *pers. obs.*), while most of the *Palicourea* pollen will be found on the insect's head.

At present, we can conclude that *Palicourea brenesii* attracted many bumblebees, and received fairly limited, but sufficient amounts of conspecific pollen. Bumblebees appeared to use foraging paths that comprised several species, and the same individuals returned to a patch regularly. This leads to limited, unidirectional pollen flow between patches (Handel 1983), and a preponderance of within-patch (geitonogamous) pollen transfer. The low number of open flowers on a *Palicourea* inflorescence may have evolved to limit the extent of geitonogamous pollen deposition. Vegetative reproduction, however, counteracts this by increasing the number of inflorescences of the same genotype. Pollination quality posed more of a problem than pollination quantity for this species. A study of the population genetic structure, the spatial distribution of genotypes, and outcrossing rates would help to clarify the observed patterns.

Pollen quantity and quality on Leandra subseriata

The pollen loads on *Leandra* stigmas were quite pure: 90% of the pollen was conspecific. The number of ovules in *Leandra* is much higher than in *Palicourea* (mean 440, range 230–665; R. A. Wesselingh, unpubl. data), and the average number of conspecific grains counted on stigmas generally exceeded the number of ovules. In another study of *Leandra*, we found a bimodal pattern in natural seed set, with a peak around 75% and a second, much lower peak at 10% (R. A. Wesselingh, unpubl. data). In that data set, 18% of the plants had a seed set below 33%. These probably were flowers that received only one visit or no visits at all. We found the same pattern in our stigmatic load data. Four out of 24 stigmas (17%) had less than 30 conspecific pollen grains, while the rest had 270 grains or more. So almost 20% of the flowers that produced fruits appeared to be pollen-limited. *Leandra* is fully self-compatible, with no sign of inbreeding depression in germination and seedling growth rates (R. A. Wesselingh and M. Witteveldt, unpubl. data), but fruit abortion rates were high (75%) in flowers that were shielded from pollinator visitation (Wesselingh *et al.* 1999). This increases the actual number of flowers that is affected by pollen limitation. Natural fruit set was close to 90%, so if all aborted fruits were abscised because of a lack of pollination, around 25% of all flowers will have experienced some degree of pollen limitation. Thus, flowers of *Leandra subseriata* received high quality pollen loads when pollinated—also because self pollen is of equal value as outcross pollen—but a quarter of the flowers received insufficient pollen for either fruit set or maximum seed set.

In conclusion, *Palicourea brenesii* and *Leandra subseriata* experienced different pollination environments. While *Palicourea* was frequently visited for its nectar and received highly mixed pollen loads, *Leandra* was visited much less frequently, but by—pollen-collecting—bumblebees that deposited nearly purely conspecific pollen loads. Both species are self-compatible to some degree, but do not display strong autogamy, i.e. fruit set and seed set in the absence of pollinators (Wesselingh *et al.* 1999). Rather, a number of species we studied in this system engaged in vegetative reproduction through formation of tillers, or root growth from nodes of branches that touch the ground (Wesselingh *et al.* 1999; R. A. Wesselingh, *pers. obs.*). Clonal growth increases the number of inflorescences a plant produces, and this is likely to result in an increased attractiveness to pollinators, at the expense of increased geitonogamous selfing (Klinkhamer & de Jong 1993). The habit of individual bumblebee workers to follow foraging paths results in non-random mating and an increase in reproductive isolation among patches, and, in concert with vegetative reproduction, reduces effective population size. When more species have to compete for the same pollinator(s), one would expect to find divergence in flowering time. In this system, however, species may actually benefit from the attractiveness of other flowering plants through facilitation (Petanidou *et al.* 1995). The combined presence of different flowering species at the same time can sustain a

viable pollinator population (pollinator quantity), which compensates for losses in pollinator quality due to a lack of flower constancy.

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