

A comparison of two pollinators: the introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of NE Brazil

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Summary

1. Aspects of the flowering biology of wild cashew *Anacardium occidentale*, an andromonoecious, self-fertile tree, were studied in the north-east of Brazil, where this species is endemic. Comparison was made among two bee species, *Apis mellifera* and *Centris tarsata*, in their pollination of cashew flowers using a number of measures including single bee visits to marked flowers.

2. Cashew flowering is protandrous within a day. Male flowers greatly outnumber hermaphrodite flowers. Stigmas lose receptivity rapidly and pollen is quickly removed from anthers yet flowers remain intact for several days.

3. Only females of *C. tarsata* collected pollen from cashew flowers, and then only from male flowers. The similar foraging behaviour of the nectar collectors of the two bee species under investigation when visiting hermaphrodite cashew flowers suggests that they may both act as good pollinators.

4. We develop an index of efficiency of pollen removal from anthers (PRE_i) whereby the relative benefits of flower visitors to a component of a plant's male reproductive success can be quantified.

5. Comparisons of single bee visits to flowers with unvisited flowers and others receiving unlimited visits were used to show that: *C. tarsata* pollen collectors were more efficient than nectar collectors of either bee species at removing pollen from anthers; nectar collectors of both bee species had similar pollen removal efficiencies; *C. tarsata* was more efficient at depositing pollen on stigmas than *A. mellifera*; both bee species had statistically similar efficiencies at setting seed.

6. The indices of efficiency for some of the stages in the pollination of cashew suggest that *C. tarsata* flower visits may enhance plant reproductive success over flower visits by *A. mellifera* but that both bee species may be suitable for the pollination of commercially grown cashew.

7. Despite cashew's single ovule per flower, high nut set demands a high rate of pollinator visitation during the peak time of stigma receptivity. Provision of additional bee pollination in commercial orchards is recommended to obtain good nut yields.

Key-words: pollen deposition, pollen removal, pollinator efficiency, stigma receptivity.

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Introduction

Analysis of the comparative efficiency of flower visitors in effecting pollination is of theoretical interest in interpreting the adaptive value of floral traits, and can shed light on the coevolution between plants and their

pollinators. From an applied stance, too, evaluation of the role of flower visitors is necessary to enable objective decisions to be reached over the choice of pollinators to maximize crop pollination (Torchio 1990). There is a paucity of such information, for tropical crops in particular (Roubik 1995), and cau-

tion has recently been advised about the over-dependence of crop pollination on the polylectic honey bee *Apis mellifera* L. (Torchio 1990; O'Toole 1994). Particularly where the honey bee is an introduced species (e.g. the Americas), there may be indigenous flower visitors of native crop species or varieties that are at least as adequate as pollinators (e.g. Tepedino 1981).

Most attention has been devoted to the study of plant–pollinator relationships via a flower's female fitness components, no doubt partly because of their relative ease of measurement. Comparison among pollinators has then often entailed quantification of the number of pollen grains they have deposited on stigmas (e.g. Primark & Silander 1975; Herrera 1987; Waser & Price 1990) or the number of seeds they have set (e.g. Schemske & Horvitz 1984; Motten 1986; Young 1988), or both (Dieringer 1992), following a single pollinator visit to a virgin flower. Less attention has been given to plant–pollinator relationships via male components of a flower's reproductive success (e.g. Galen & Stanton 1989; Murcia 1990).

Some floral traits, such as the size of flowers and floral displays, have been considered to have been selected upon primarily through male function (Stanton, Snow & Handel 1986; Stanton & Preston 1988; Cruzan, Neal & Wilson 1988; Galen & Stanton 1989). Factors such as pollen production, floral mechanisms for pollen distribution, pollinator foraging characteristics and pollen removal, are the processes more closely related to pollen dispersal and therefore a flower's male function (Harder & Thomson 1989; Thomson & Thomson 1989; Young & Stanton 1990). But efficiency of pollen transport, the number and size of a plant's potential mates, efficiency of pollen deposition on stigmas, and the dynamics of fertilization and seed development also influence a flower's reproductive success (Galen & Stanton 1989; Young & Stanton 1990). Indeed, Broyles & Wyatt's (1990) careful study of plant reproductive success, employing genetic paternity analysis to determine the fate of pollen, suggests that flowers may not function to enhance predominantly a plant's male fitness components. These studies nonetheless highlight the need to consider both male and female reproductive functions of a flower in any study of plant reproduction and plant–pollinator interactions.

Despite earlier models defining pollinator efficiency in terms of some of the aforementioned factors using indirect measures (Spears 1983; Sudgen 1986), a challenge still comes in quantifying their efficiency directly, both in terms of pollen removal from anthers (a flower's male function) and pollen deposition on stigmas (a flower's female function) (Inouye *et al.* 1994; and see Snow & Roubik 1987; Wilson & Thomson 1991; Fishbein & Venable 1996).

Cashew *Anacardium occidentale* L. is an andromonoecious tree whose nut is an important commercial product of many tropical countries (Heard,

Vithanage & Chacko 1990; Reddi 1991). Although a largely cultivated crop, there are very few selected cultivars and most orchards still comprise seedling material (de Araújo & Rodrigues 1988; Wunnachit *et al.* 1992). Hence, wild and cultivated cashew have essentially identical floral traits. There is little information on cashew floral and pollination biology from its native range in Brazil (Haarer 1954; Lima 1988; Reddi 1991; Freitas 1994, 1995a); caging of cashew flowers where the species has been introduced outside its native range has shown that it requires insect pollination to set fruit (Northwood 1966; Free & Williams 1976; Phoon 1984). In Brazil, too, Freitas & Paxton (1996) found that cashew requires insect pollination, and that pollination is achieved mainly by stamen-derived pollen grains, given the low viability of staminoid (short stamen) pollen.

Many insect species have been suggested as potential pollinators of cashew, including ants, bees and wasps (for review, see Free 1993). It has even been claimed recently that bees rarely visit cashew flowers, and that flies are its major pollinators (Roubik 1995 p. 67). However, in north-eastern Brazil, bees are the most frequent flower visitors of commercially grown cashew, and they have been implicated as cashew's major pollinators (Freitas & Paxton 1996).

There is often a low nut set of commercially grown cashew, which in some instances is thought to be due to a lack of adequate pollination (Free 1993). However, there is as yet no information on the relative efficiency of various pollinators of cashew, nor on their abundance, with which to support this view, or to suggest methods for augmenting the pollination of commercially grown cashew.

In this paper, we investigated the role of two bee species, regular visitors to cashew flowers, in the pollination of cashew. We studied cashew flower reproductive biology associated with the foraging behaviour of these two flower visitors and, through the use of single bee visits to virgin flowers, we documented these two visitors' relative efficiencies in collecting pollen from anthers, depositing pollen on stigmas, and setting nuts. We also present the first report of *Centris (Hemisiella) tarsata* Smith, an indigenous solitary bee, as a potentially good native cashew pollinator.

Materials and methods

Observations and experiments were carried out during the cashew blooming seasons, July to September, of 1993 and 1994 in the county of Beberibe, the state of Ceará, in the north-east of Brazil (Fig. 1). At our field site, Frecheiras, honey bees *A. mellifera* and an indigenous solitary bee species, *C. tarsata*, systematically visited wild native cashew trees growing in sand dunes, a typical habitat for *A. occidentale* (Reddi 1987; Lima 1988).

Cashew presents male and hermaphrodite flowers in the same panicle (Fig. 2). Each panicle may contain

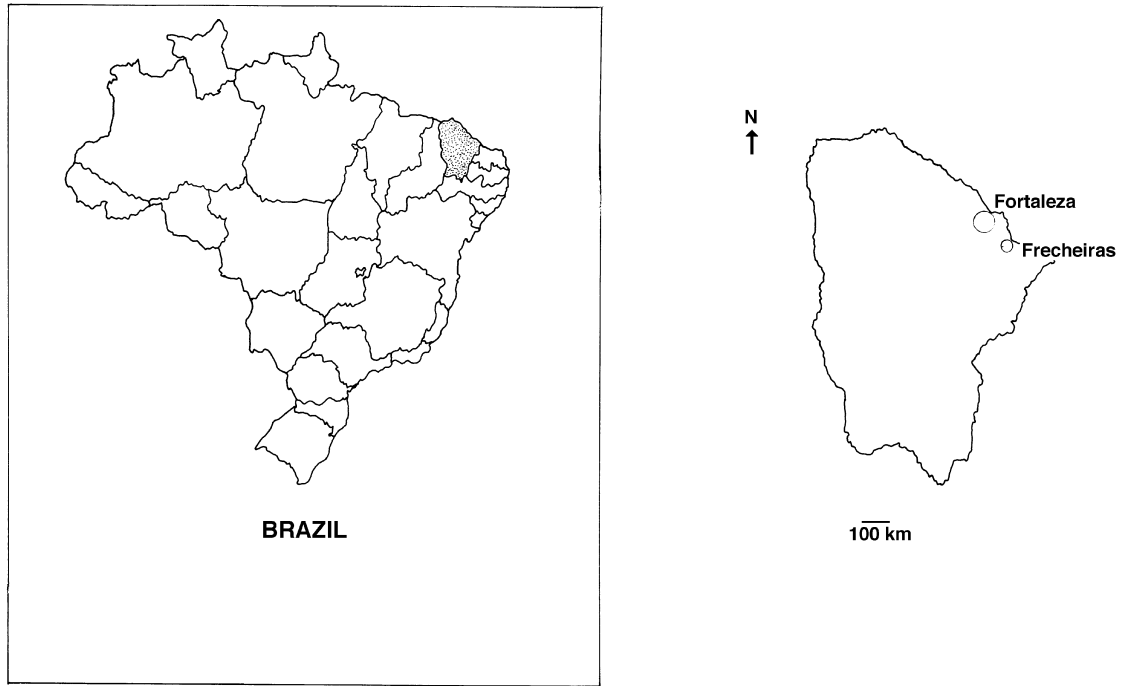


Fig. 1. Map of Brazil showing the state of Ceará (shaded) where experiments with cashew were undertaken. A more precise location of the locality of Frecheiras is shown on the right.

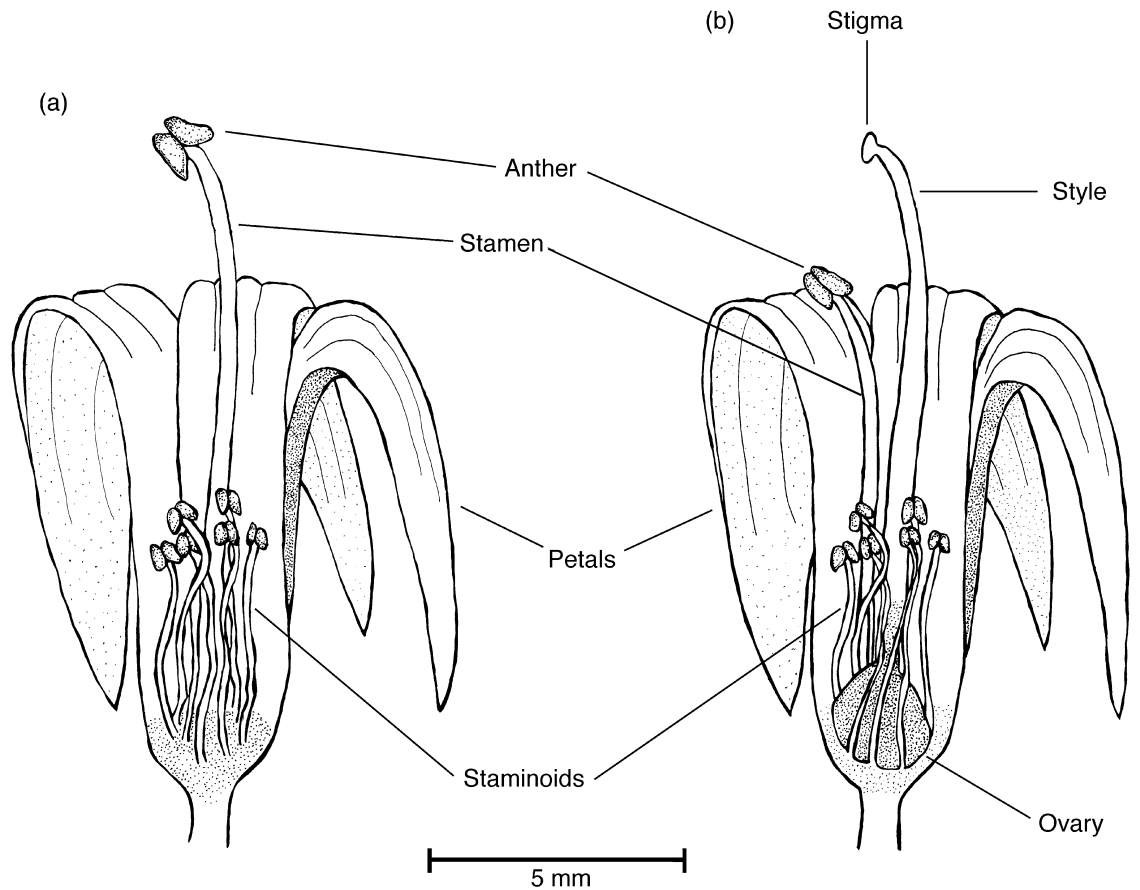


Fig. 2. Schematic diagram of a male (a) and hermaphrodite (b) cashew flower showing the relative positions of the stamen and staminoids of each and the stigma of a hermaphrodite flower.

hundreds of flowers which open sequentially over several weeks. Flowers of both types contain one stamen and 9 short 'staminoids', whilst hermaphrodite flowers contain a stigma and style of approximately the same length as a male flower's stamen, a stamen slightly shorter than that of a male flower, and 9 short staminoids (Fig. 2). Cashew is fully self-compatible but staminoid-derived pollen is of very low viability (Freitas & Paxton 1996). Each hermaphrodite flower contains one ovule.

FLOWER BIOLOGY

Cashew flowering was studied in 20 inflorescences chosen at random among cashew trees. Flower buds were marked and the ratio of hermaphrodite to male flowers, their coloration and change of colour throughout their lives were recorded. Time of anthesis and pollen presentation of stamens in male and hermaphrodite flowers, and time of stigma presentation in hermaphrodite flowers were investigated in 500 flowers of both types.

Changes in stigma receptivity were also investigated by covering 50 panicles chosen at random among cashew trees with fine nylon and muslin bags, each panicle containing several hermaphrodite flower buds about to open. Then, groups of 30 hermaphrodite flowers from different panicles and trees were hand-pollinated at 0, 2, 4, 6, 22, 24, 26, 28, 30 and 46 h after anthesis with cross-pollen from other trees. Pollen was obtained from the stamens of male flowers which were collected every morning and whose anthers were allowed to dehisce in Petri dishes at 28 °C, though some flowers were kept at below 20 °C to delay anther dehiscence and ensure that fresh pollen was available for hand pollination performed in the afternoons. After hand pollination, the panicles were rebagged and hand-pollinated flowers were observed 7 days later to determine nut (= seed) set.

BEE FORAGING BEHAVIOUR

The general foraging behaviour of *A. mellifera* workers and males and females of *C. tarsata*, the only regular cashew flower visitors at Frecheiras, was studied to determine whether they collected pollen or nectar, touched stamens or stigmas, and whether there was a diurnal pattern to their foraging. Possible preferences for a flower's age or type (male vs. hermaphrodite) were investigated by following 400 *A. mellifera* and 400 *C. tarsata* flower visits to different panicles on randomly chosen trees. The age of visited flowers was estimated by inspection of their petals, anthers and stigmas according to Freitas (1994). Diurnal bee abundance was recorded at 9.00, 11.00, 13.00 and 15.00 h for five consecutive days by scoring the

total number of bee visits to randomly selected panicles across 5-min intervals.

POLLEN REMOVAL EFFICIENCY OF BEES

The mean number of pollen grains on the cashew stamen of male flowers receiving no insect visitation was obtained from 40 flowers, one flower per panicle chosen at random from randomly selected trees. Flowers were bagged from before they opened until the end of the afternoon upon which they first opened. At this time, stamens were collected to assess the number of pollen grains still adhering to them. Each stamen's anther was individually washed in a drop of 70% ethanol on a microscope slide to dislodge all pollen grains from it. Anthers were carefully checked after washing to ensure they did not still bear pollen grains. Following this step, a cover slip was placed on the top of each slide and the total number of pollen grains was counted under an optical Olympus CH microscope at $\times 100$ magnification.

The mean number of pollen grains remaining on a cashew flower's stamen after unrestrained visitation was obtained from the anthers of 40 non-bagged male flowers, one flower per panicle on randomly selected trees, at the end of the afternoon of the day on which anthesis occurred using the same method as described above. Finally, the mean number of pollen grains remaining after only one visit paid by an *A. mellifera* worker or *C. tarsata* individual was obtained by bagging flower buds on randomly selected trees with fine nylon and muslin bags, releasing them from their bags after flowers had opened and anthers dehisced, and watching the flowers until a single bee visited one male flower. Immediately after the bee visit, the stamen of the visited flower that had dehisced on that day was collected and placed in a 1.5 ml plastic microcentrifuge tube. For *A. mellifera* workers collecting nectar and *C. tarsata* individuals collecting pollen or nectar, pollen grains remaining on a total of 40 stamens per visitor type (one observation per panicle) were counted as described above.

The efficiency of bees in removing cashew pollen from stamens of male flowers was estimated by adapting the pollination efficiency index (PE_{*i*}) proposed by Spears (1983). A pollen removal efficiency index (PRE_{*i*}) was computed as:

$$\text{PRE}_i = \frac{R_i - N}{V - N}, \quad \text{eqn 1}$$

where R_i is the mean number of pollen grains removed per flower in a plant population receiving a single visit from species *i* (and/or collecting a particular floral resource); N is the mean number of pollen grains removed per flower in a plant population receiving no visitation; and V is the mean number of pollen grains removed per flower in a plant population receiving unrestrained visitation.

PRE_{*i*} will range between 0 (poor pollen remover) to 1 (excellent pollen remover).

POLLEN DEPOSITION EFFICIENCY OF BEES

Quantifying the number of pollen grains deposited on stigmas by a pollen vector was evaluated for *A. mellifera* and *C. tarsata* by enclosing 160 hermaphrodite flowers yet to open in fine nylon and muslin bags. Flowers were selected at random from 160 panicles on randomly chosen trees. Forty flowers were kept bagged at all times whilst the remaining flowers were released from their bags soon after anthesis. Hermaphrodite flowers were carefully observed until a bee visited them. Immediately after the first bee visit, the species identity of the bee was recorded, the stigma was removed from the flower and the number of cashew pollen grains deposited on it by the bee, the stigma pollen load per visit, was counted under an optical stereoscopic microscope Olympus TGHM at a magnification of $\times 40$, for 40 flowers visited by both bee species. The remaining 40 flowers received unrestricted visits. Their stigmas were collected at 16.00 h on the day of anthesis and the number of cashew pollen grains adhering to them was counted as described above.

As pollen grains were not detected on permanently bagged flowers (see Results), a bee species' efficiency at depositing pollen on stigmas is given simply as the ratio of the number of pollen grains deposited following one visit divided by the number of pollen grains following unrestricted visits.

EFFICIENCY OF NUT SET (SPEARS' POLLINATION EFFICIENCY INDEX)

The efficiency of honey bees and *C. tarsata* at setting cashew nuts was evaluated using Spears' (1983) pollination efficiency index (PE_{*i*}), as:

$$PE_i = \frac{P_i - Z}{U - Z}, \quad \text{eqn 2}$$

where P_i is the mean number of seeds set per flower by a plant population receiving a single visit from species i ; Z is the mean number of seeds set per flower by a plant population receiving no visitation; and U is the mean number of seeds set per flower by a plant population exposed to unrestricted visitation.

The same procedure described above to investigate a bee's efficiency in transferring pollen grains to stigmas was employed to measure its PE_{*i*}; namely, the use of unvisited, single visited and unrestrained (multiple visited) flowers. The protocol differed only in that immediately after a single bee visit (P_i), the visited flower was labelled accordingly and rebagged to prevent further visitation. Forty flowers were kept bagged throughout their lives to act as controls (Z) and 40 flowers were marked but left unbagged for the entire

day on which they first opened to receive unrestrained visits (U) and then rebagged. Seven days after the end of the experiment and before any fruit drop, initial nut set was recorded for each treatment.

Means are presented \pm SEM throughout.

Results

FLOWER BIOLOGY

Flowers were presented in terminal panicles at an approximate proportion of 10% hermaphrodite flowers to 90% male flowers ($n = 500$ panicles). Both male and hermaphrodite flowers were white at anthesis with two pink stripes on each petal. As flowers aged, they progressively acquired a pink coloration, turning entirely pink by the second day and then red by the third day (see Free 1993; Freitas 1994).

Male flower anthesis commenced at 06.00 h, with $82.2 \pm 3.1\%$ of flowers open by 10.00 h ($n = 500$ flowers). In hermaphrodite flowers, anthesis and stigma presentation started around 10.00 h and $95.0 \pm 2.3\%$ of hermaphrodite flowers were open by 12.00 h, with only a few stigmas being first presented later in the day ($n = 500$ flowers; Fig. 3). Anther dehiscence of stamens occurred mainly after 09.00 h for male flowers and 10.00 h for hermaphrodite flowers, but male flowers contributed proportionally most of the stamen-derived (viable) pollen available (Fig. 3).

Hand pollination trials showed that stigmas were receptive up to 30 h after anthesis ($n = 300$ stigmas), but stigma receptivity varied with age ($G^2 = 126.8$, d.f. = 9, $P < 0.001$). Stigmas less than 4 h old were more receptive than older stigmas ($n = 30$ stigmas for each age group; Fig. 4). Stigma requirements for pollen at Frecheiras (sum of the percentage of all stigmas opening on one day that are presented at a given time of the day \times stigma receptivity according to stigma age) increased dramatically from 10.00 h to 12.00 h, maintained a steady level until 14.00 h, and then dropped rapidly as stigma receptivity decreased with flower ageing (Fig. 5).

BEE FORAGING BEHAVIOUR

Both bee species invariably touched the stamen of male flowers and the stigma, and occasionally the stamen, of hermaphrodite flowers during their visits. *Apis mellifera* workers and males of *C. tarsata* collected only nectar while females of *C. tarsata* collected pollen and nectar, though only nectar from hermaphrodite flowers. However, nectar-collecting females of *C. tarsata* often held large amounts of pollen in their scopal hairs (pollen collection apparatus), suggesting that individual bees may have switched between nectar and pollen collection within a foraging trip. Neither bee species normally contacted staminoids when collecting nectar from a flower and,

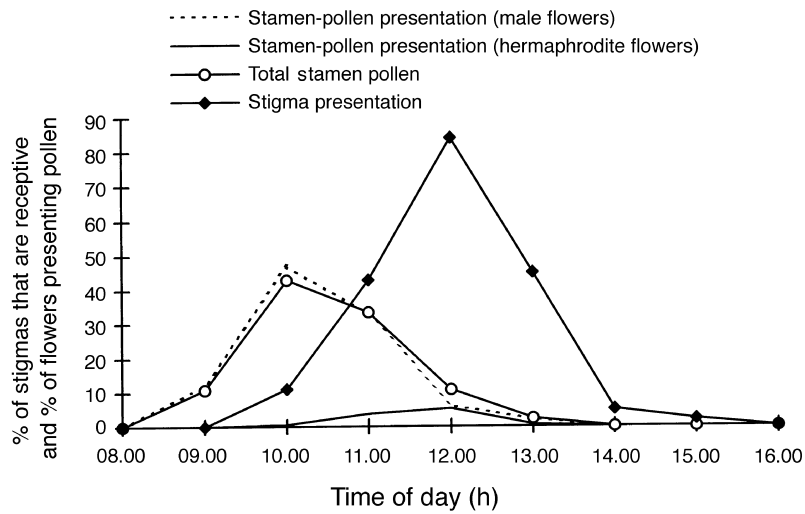


Fig. 3. Stigma presentation pattern of hermaphrodite cashew flowers throughout the day ($n = 500$) and pollen presentation patterns of the stamens of male cashew flowers ($n = 500$), hermaphrodite ($n = 500$) cashew flowers and total stamen-derived pollen, taking into account the proportional contribution of male and hermaphrodite flowers to the total amount of pollen presented by panicles.

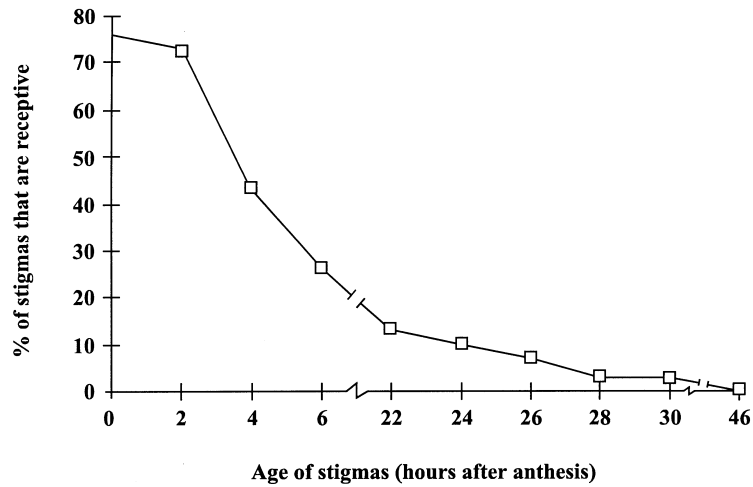


Fig. 4. Stigma receptivity of cashew flowers ($n = 30$ for each data point) to hand pollinated, stamen-derived cross-pollen at different times after flower anthesis. Note that the abscissa is not linear.

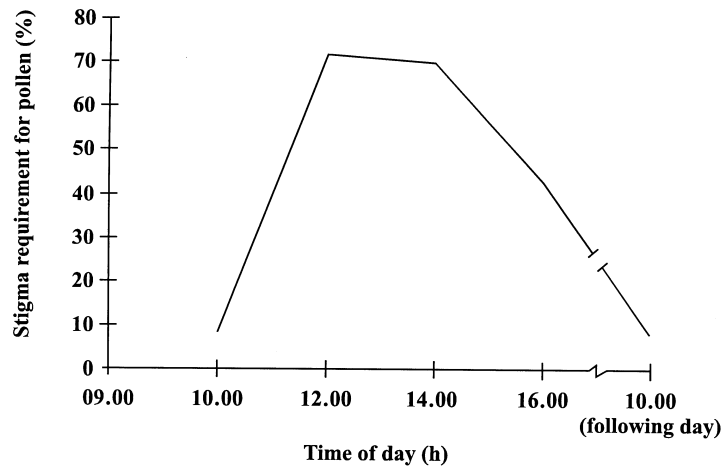


Fig. 5. Variation in pollen requirements of a cohort of cashew stigmas that became receptive on the same day (sum of the percentage of all stigmas opening on one day that are presented at a given time of the day \times stigma receptivity according to stigma age) at Frecheiras. Note that the abscissa is not linear.

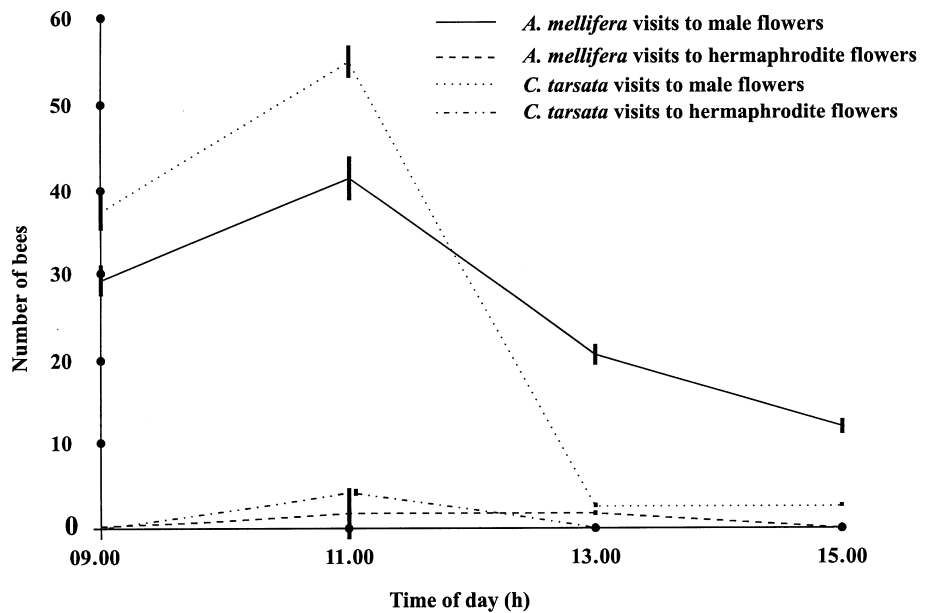


Fig. 6. Numbers of *A. mellifera* ($n = 107$) and *C. tarsata* ($n = 97$) bees visiting male and hermaphrodite cashew flowers throughout the day at Frecheiras. Bees were recorded for 5-min intervals on randomly selected panicles.

if so, then only with their frons or mouthparts, which did not subsequently contact stigmas (Freitas 1997).

The two bee species visited cashew flowers at similar times of the day when pollen grains were presented and stigmas were most receptive (Fig. 6). This suggests that both species are potential pollinators of cashew. Also, there was no difference in the frequency of visits to male vs. hermaphrodite flowers among bee species (Fisher's exact test $P = 0.722$); $\approx 90\%$ of all visits were to male flowers (Fig. 7), roughly in proportion to their abundance in panicles. *Centris tarsata* had a slightly higher frequency of visits to the same day's flowers vs. 1-day-old flowers compared with *A. mellifera* (Fisher's exact test $P = 0.012$); however, both species predominantly visited same-day flowers

($n = 400$ visits for each bee species; Fig. 7), again suggesting that they pollinate cashew.

CASHEW POLLEN REMOVAL EFFICIENCY OF BEES

At dehiscence, stamens of male flowers had 827 ± 18 pollen grains (Freitas 1995a). For the experiment comparing the number of pollen grains remaining on anthers of stamens following bee visitation to male flowers ($n = 200$ flowers), there were significant differences among the various treatments (Kruskal-Wallis ANOVA $\chi^2 = 172.7$, d.f. = 4, $P < 0.001$). Stamens of flowers unrestricted to insect visitors were the most depleted of pollen grains (96% of pollen grains

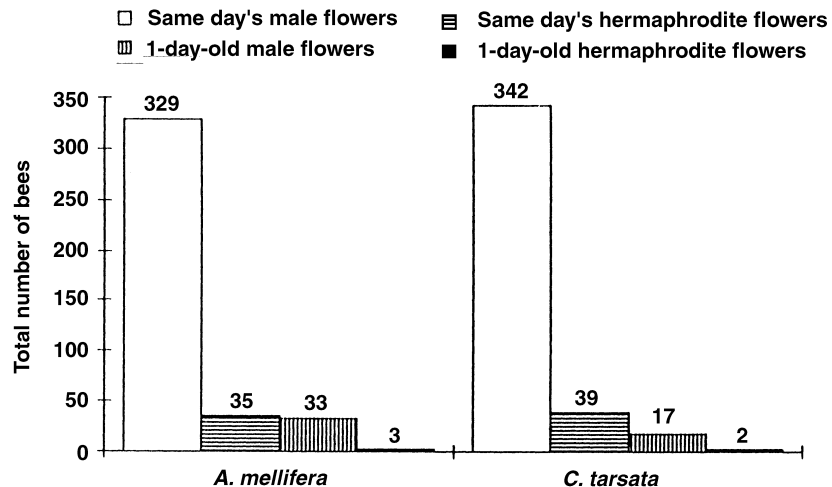


Fig. 7. Total number of *A. mellifera* ($n = 400$) and *C. tarsata* ($n = 400$) bees that visited either flowers that had opened on the same day the visit was made or flowers that had opened on the day prior to the visit (1-day-old flowers).

Table 1. Mean number of pollen grains remaining and removed per stamen (assuming 827 ± 18 grains per anther at dehiscence) after no visit, unrestricted visits or single visits by *A. mellifera* and *C. tarsata* bees to male cashew flowers, and the bees' pollen removal efficiency index (PRE_i). Sample size = 40 flowers for each category of visit

Category of visit	Mean number of pollen grains remaining per stamen (\pm SEM)	Mean number of pollen grains removed from a stamen (\pm SEM)	PRE _i (\pm SEM)
Restricted (<i>N</i>)	772 ^a \pm 14	55 \pm 23	
Unrestricted (<i>V</i>)	35 ^c \pm 4	792 \pm 18	
Single visit by: (<i>R_i</i>)			
<i>A. mellifera</i> nectar collector	268 ^b \pm 12	559 \pm 22	0.68 ^b \pm 0.05
<i>C. tarsata</i> nectar collector	275 ^b \pm 16	552 \pm 24	0.67 ^b \pm 0.05
<i>C. tarsata</i> pollen collector	42 ^c \pm 2	785 \pm 18	0.99 ^a \pm 0.04

Means followed by the same letter within a column (columns 2 or 4) do not differ at $P < 0.05$ [*a posteriori* non-parametric multiple comparison test (Zar 1984) and Tukey-*B*-tests, respectively].

removed, $n = 40$ stamens), but they did not differ statistically from those of flowers receiving single visits by females of *C. tarsata* collecting pollen (95% of pollen grains removed, $n = 40$ stamens; Table 1). Nectar collectors of both bee species removed similar, though lower, numbers of pollen grains per visit from stamens of male flowers than *C. tarsata* pollen collectors (*A. mellifera*: 68% of pollen grains removed; *C. tarsata*: 67% of pollen grains removed; $n = 40$ stamens for each bee species).

Apis mellifera and female *C. tarsata* nectar and pollen collectors showed significant differences in their indices of pollen removal efficiency, PRE_i (ANOVA $F = 359.2$, d.f. = 2, 117, $P < 0.001$; distributions conform to the assumptions of ANOVA). *Centris tarsata* pollen collectors showed the highest value, with a PRE_i of 0.99. *Apis mellifera* nectar collectors and female *C. tarsata* nectar collectors had lower indices (0.68 and 0.67, respectively), and did not differ from each other in their pollen removal efficiencies (Table 1).

CASHEW POLLEN DEPOSITION EFFICIENCY OF BEES

Only nectar collectors of *A. mellifera* and nectar collectors of *C. tarsata* visited hermaphrodite cashew flowers on the day they opened. *Centris tarsata* females for-

aging for pollen restricted their visits to male flowers alone and therefore their efficiency of pollen deposition on stigmas could not be measured. However, as they may have switched between pollen and nectar collection on the same foraging trip, the pollen deposition efficiency of nectar collectors of *C. tarsata* may also represent that of pollen collectors of *C. tarsata*.

For the experiment comparing the number of pollen grains deposited on stigmas, there were significant differences among treatments (Kruskal-Wallis ANOVA $\chi^2 = 126.7$, d.f. = 3, $P < 0.001$; Table 2). Predictably, the greatest number of pollen grains was found on stigmas of flowers which received unrestricted insect visitation (range 13–65 grains; $n = 40$ stigmas). All single-bee visits resulted in pollen deposition on stigmas. However, *C. tarsata* deposited significantly more pollen grains (range 4–38 grains; $n = 40$ stigmas) than did *A. mellifera* (range 1–22 grains; $n = 40$ stigmas) following single visits (Table 2). Stigmas of bagged flowers did not bear any pollen grains ($n = 40$ stigmas; Table 2).

Calculation of the proportion of pollen grains deposited on stigmas following single visits in relation to the number deposited after unrestricted visits, the pollen deposition efficiency, revealed that *A. mellifera* had a pollen deposition efficiency per single visit of 0.24 whilst that of *C. tarsata* was significantly higher (ANOVA $F = 20.1$, d.f. = 1, 78, $P < 0.01$; distributions

Table 2. Mean number of pollen grains deposited per stigma after unrestricted visits or single visits by nectar collectors of *A. mellifera* or *C. tarsata* to hermaphrodite cashew flowers. Sample size = 40 flowers for each category of visit

Category of visit	Mean number of pollen grains deposited per stigma (\pm SEM)	Efficiency of pollen deposition on stigmas (\pm SEM)
Restricted	0 ^d	
Unrestricted	33 ^a \pm 2	
Single visit by:		
<i>A. mellifera</i> nectar collector	8 ^c \pm 1	0.24 ^b \pm 0.03
<i>C. tarsata</i> nectar collector	16 ^b \pm 2	0.48 ^a \pm 0.07

Means followed by the same letter within columns 2 or 3 do not differ at $P < 0.05$ [*a posteriori* multiple comparison test (Zar 1984) and ANOVA, respectively].

conform to the assumptions of ANOVA) at 0.48 (Table 2).

SPEARS' POLLINATION EFFICIENCY INDEX FOR CASHEW

There was significant heterogeneity in the number of nuts set among treatments: no visits, unrestricted visits and single visits by *A. mellifera* and *C. tarsata* nectar collectors ($G^2 = 137.1$, d.f. = 3, $P < 0.001$; Table 3). The number of nuts set after unrestricted visits (44 nuts from 50 flowers) was significantly greater than the number of nuts set following single visits by nectar collector bees (*A. mellifera* visits: 19 nuts from 50 flowers; *C. tarsata*: 26 nuts from 50 flowers), which was also significantly greater than the number of nuts set when flowers received no visits (0 nuts from 50 flowers; Table 3). Though single visits by *C. tarsata* set a greater number of nuts numerically than did those of *A. mellifera*, no statistical difference ($t = 1.193$, d.f. = 198, $P > 0.05$) was found between these two bee species following single visits (Table 3).

Spears' pollination efficiency index (PE_i), which considers the mean number of nuts set by single visits in relation to the mean number of nuts set per flower by unrestricted visits, also showed no significant difference among bee species (ANOVA $F = 1.979$, d.f. = 1, 98, $P > 0.05$, distributions conform to assumptions of ANOVA; Table 3) though *C. tarsata*'s PE_i of 0.59 was 37% greater than that of *A. mellifera*'s, at 0.43.

Discussion

CASHEW POLLINATION SYNDROME

Insect visitors, in particular bees, appear to be important pollinators of cashew in its native range (Freitas & Paxton 1996). Our data point to a pollination syndrome of cashew in which a large floral display attracts and directs these insects to pollinate hermaphrodite flowers which individually have a short receptive

period. A much greater number of male than hermaphrodite flowers may ensure both that panicles are attractive to pollinators and that pollen grains have access to stigmas for pollination at the optimal time of day; anthers of male flowers dehisce just before and during anthesis of hermaphrodite flowers, coinciding with the time of highest insect visitation to flowers, greatest stigma receptivity and greatest stigma requirement for pollen.

Since cashew was originally a plant which inhabited mainly sandy coastal areas where harsh environmental conditions limit the distribution of most plant species and flower-visiting insects (Reddi 1987), an economical but long-lasting and attractive pollination unit may have been advantageous to cashew. Retaining old flowers on panicles for many days to enhance floral display, and producing small numbers of new flowers daily for a long period of time, may have been a better strategy of resource allocation and assured pollination in such a harsh environment than discarding most flowers every day and producing an entirely new display next morning (Ashman & Schoen 1994); the mating costs of producing a large floral display thereby can be minimized. The tiny size of cashew flowers, their long life span, and reduced amount of pollen and nectar (Heard, Vithanage & Chacko 1990; Reddi 1991; Freitas 1995a) reinforce this hypothesis, as do the feeding anthers (staminoids) to attract pollinators (Fægri & van der Pijl 1979). Economical pollen production in small male flowers, as shown by cashew, may also be a way to increase pollen dispersal of a plant by using several insect pollen vectors which visit numerous flowers on a panicle across successive days as each flower opens (Harder & Thomson 1989; Harder & Barrett 1995).

To what extent cross- vs. self-pollination is important in cashew reproduction is uncertain. As suggested by Free (1993), a delay in anther dehiscence of hermaphrodite cashew flowers may diminish the chances of self-pollination, although the great number of male flowers per tree may increase the level of geitonogamy (De Jong, Waser & Klinkhamer 1993; Harder & Barrett 1995).

Table 3. Total and mean number of fruits set per flower after no visit, unrestricted visits or single visits by nectar collector *A. mellifera* or *C. tarsata* to hermaphrodite cashew flowers, and their pollination efficiency index (PE_i). Sample size = 50 flowers for each category of visit, and SEMs obtained assuming a binomial distribution

Category of visit	Total number of nuts set	Mean number of nuts set per flower (\pm SEM)	PE _i (\pm SEM)
Restricted (Z)	0 ^c	0	
Unrestricted (U)	44 ^a	0.88 \pm 0.04	
Single visit by: (PE _i)			
<i>A. mellifera</i> nectar collector	19 ^b	0.38 \pm 0.07	0.43 \pm 0.10
<i>C. tarsata</i> nectar collector	26 ^b	0.52 \pm 0.07	0.59 \pm 0.08

Numbers followed by the same letter within the second column do not differ at $P < 0.05$ (G -test).

POLLINATION EFFICIENCY OF BEES: A
FLOWER'S MALE REPRODUCTIVE COMPONENTS

Pollen removal by flower visitors can be used as a measure of their efficiency in pollination through the male reproductive function of a plant (Inouye *et al.* 1994). Our use of a pollen removal efficiency index (PRE_{*i*}) allows for objective, quantitative comparison among flower visitors in their ability to remove pollen from flowers. The very high PRE_{*i*} recorded for pollen collectors of *C. tarsata*, which was higher than that for *A. mellifera*, may be attributed to their specialized pollen-collecting movements. Pollen-collecting *C. tarsata* females can effectively remove almost all the pollen from a male cashew flower's stamen in a single visit and therefore *C. tarsata* potentially may be a good pollinator from the plant's perspective when pollen collectors also visit hermaphrodite flowers to collect nectar.

The comparisons made between *A. mellifera* and *C. tarsata*, both pollen and nectar collectors, showed that the PRE_{*i*} can be used not only to compare pollen efficiency removal between different species but also between individuals within a species showing distinct foraging behaviours. Behavioural differences between individuals of a species dictate the amount of pollen they remove, and may affect the male reproductive success of a flower, too.

Though PRE_{*i*} may provide a measure of a flower visitor's effectiveness as a pollinator through its male reproductive function, the viability of those pollen grains removed by the visitor, and the change in viability of those grains during vector transport to stigmas, will also determine the visitor's efficiency (Inouye *et al.* 1994). Further, although the amount of pollen removed from a flower can be used as an indicator of the opportunity for male reproduction (Cruzan, Neal & Wilson 1988), high pollen removal rates do not necessarily translate into high rates of viable conspecific and compatible pollen deposition on receptive stigmas (Wilson & Thomson 1991). Pollen removed from a flower by an insect visitor may not reside at a location on the visitor which will allow the pollen to be transferred to a stigma of the same or another flower, or an efficient pollen remover may lose or sequester much pollen between subsequent flower visits. *C. tarsata* did not visit hermaphrodite flowers to collect pollen and so pollen-collectors of *C. tarsata* only deposited pollen on stigmas when they switched to collecting nectar and visited hermaphrodite flowers. Their efficiency in pollination with respect to a plant's male reproductive function will then have depended on the frequency with which pollen collectors switched to collecting nectar, data which is lacking for this and most other bee species. Tracking the fate of pollen removed by a vector, either directly (Nilsson, Rabakonandrianina & Pettersson 1992) or via paternity analysis (Broyles & Wyatt 1990; Chase *et al.* 1996) may allow a more accurate evalu-

ation of a vector's efficiency in pollination via the male function of a flower.

POLLINATION EFFICIENCY OF BEES: A
FLOWER'S FEMALE REPRODUCTIVE
COMPONENTS

Rates of pollen deposition on stigmas by flower visitors can be measured readily, and can provide one measure of the pollination efficiency of visitors through the female reproductive function of a plant (Herrera 1987; Snow & Roubik 1987). Single visits to cashew flowers by *C. tarsata* resulted in the deposition of twice as many pollen grains on stigmas as did single visits by *A. mellifera*. In this respect, *C. tarsata* is also a more efficient pollinator of cashew from the perspective of the plant than *A. mellifera*.

Theoretically, a cashew flower needs only one viable pollen grain to fertilize its single ovule and set a nut. But it seems that larger stigma loads are necessary to guarantee a high rate of successful pollination (and see Herrera 1987); 33 pollen grains per cashew stigma were necessary to achieve around 88% nut set (see Tables 2 and 3, and Freitas 1995a). If cashew pollen rapidly loses its viability, then some of the pollen that is deposited on a stigma by a flower visitor may be inviable, accounting for the discrepancy between the large amount of pollen deposited on stigmas and the low rate of fertilization of ovules. In addition, cashew pollen recorded on stigmas may be derived from both stamen-anthers and staminoid-anthers; pollen from the two sources is morphologically identical, yet pollen from the latter source has a very low viability (Freitas & Paxton 1996). A more accurate measure of a flower visitor's efficiency in pollination, as measured by pollen deposition rates to stigmas, should take these factors into account.

Another measure of the pollination efficiency of a flower visitor through the female reproductive function of a plant is provided by Spears' (1983) pollination efficiency index, PE_{*i*}, which considers fruits set by a single flower visit rather than pollen grains deposited on stigmas by visitors (Schemske & Horvitz 1984; Young 1988). Despite the higher rates of pollen deposition on stigmas by *C. tarsata*, and its PE_{*i*} being 37% greater than that of *A. mellifera*, there was no statistically significant difference between the two bee species' PE_{*i*}'s. Where plant offspring fitness is a function of the source of the pollen (e.g. cross- vs. self-pollen), PE_{*i*} may be a poor estimator of a flower visitor's pollination efficiency through a plant's female reproductive function. However, PE_{*i*} has the advantage that it provides an index of pollinator efficiency which takes into account the viability of pollen grains deposited on stigmas because PE_{*i*} incorporates the products of fertilization, the seeds or nuts, in its determination. Set against this advantage, where the deposition of large amounts of pollen per stigma through multiple visitation of flowers leads to decreases in seed

set, single visit data may not give an accurate view of a pollinator's role in pollination via a flower's female function (Young 1988); PE_i may then prove unsuitable as a measure of a pollinator's effectiveness at setting seed. This caveat is unlikely to apply to cashew, though, as its stigmas are only receptive for a brief duration.

POLLEN REMOVED VERSUS POLLEN DEPOSITED

Though nectar-collecting bees of *C. tarsata* and *A. mellifera* removed approximately the same number of pollen grains from cashew anthers, *C. tarsata* deposited a greater proportion of these pollen grains onto stigmas. Heterogeneity among flower visitors in the amount of pollen removed from anthers vs. that deposited on stigmas has been noted in other plant-pollinator systems (e.g. Snow & Roubik 1987; Wilson & Thomson 1991). Because both *A. mellifera* and *C. tarsata* showed similar behaviour when probing for nectar in cashew flowers, their differences in pollen deposition on stigmas may reflect a greater pollination ability by *C. tarsata* of cashew. Set against this, pollen-collector *C. tarsata* females may be very poor pollinators, acting as 'male plant antagonists' (*sensu* Wilson & Thomson 1991) within the assemblage of cashew pollinator species, removing pollen from stamens but depositing little or none of it on stigmas. To what extent this is true depends upon their frequency of switching from pollen collection to nectar collection.

However, both bee species had very low ratios of pollen grains deposited on stigmas to pollen grains removed from anthers. Such low ratios, around 1%, seem to be typical for other bee-mediated plant-pollinator systems (Harder & Thomson 1989; Young & Stanton 1990), and may reflect the fact that a high proportion of the pollen removed from an anther by a flower visitor is either lost (e.g. groomed by a bee from its body) or is sequestered onto locations on the visitor's body (e.g. the scopal hairs of the hind tarsi of *C. tarsata* females) from where it can no longer be transferred to a stigma.

APPLIED POLLINATION OF CASHEW

Centris tarsata appears to have been a more efficient cashew pollinator through a flower's male function than *A. mellifera*, and at least as good a pollinator through a flower's female function. For a number of other plant species too, flower visitors other than *A. mellifera* have been proposed as being more efficient pollinators (for reviews, see Torchio 1987; O'Toole 1994; Roubik 1995). In some cases, this may reflect a process of coevolution between a plant and its specific flower visitor (Ramirez 1970; Pellmyr & Huth 1994). This is unlikely to hold for the system under inves-

tigation here as both *A. mellifera* and *C. tarsata* are polylectic.

Additional factors determining the importance of a specific pollinator are its abundance and its frequency of flower visits. The measures of PRE_i and PE_i quantify the 'quality' of visits (*sensu* Herrera 1987) and not the 'quantity' of such visits; the latter can greatly influence the comparative effectiveness of pollinators (Waser & Price 1990; Fishbein & Venable 1996) at the population level. For example, individuals of *C. tarsata* are approximately twice as common on cashew panicles at Frecheiras as *A. mellifera* workers during the diurnal peak of bee visitation to cashew flowers (B. M. Freitas, unpublished data), again suggesting that *C. tarsata* effects a greater proportion of the pollination of wild cashew than *A. mellifera*.

Attempting to increase the abundance of wild bee species, such as *C. tarsata* in cashew orchards, faces many difficulties, not least because little is known of the factors that regulate wild bee abundance. *Centris tarsata* is relatively scarce in commercial cashew orchards, possibly in part because of the presence of other flower species that are more attractive to *C. tarsata* within the neighbourhood of orchards. The presence and effect of competing flower species for cashew pollination in Brazilian cashew orchards has already been demonstrated (Freitas 1994; Freitas 1995b). Additionally, the ground-nesting (fossorial) habits of many bees, including *C. tarsata*, mean that it is difficult physically to transfer them to orchards requiring pollination. *Centris* species have never before been managed as pollinators. Commercial use of pollinators other than *A. mellifera* is currently practised mainly with species that regularly nest non-fossorially within a moveable cavity (e.g. a hollow box, paper straw or wooden tube) (reviewed in Torchio 1987, 1990). On the other hand, *C. tarsata* has the great advantage of being unequivocally less defensive towards man than Africanized *A. mellifera* bees currently used to pollinate cashew orchards in Brazil (Freitas 1994). It warrants further studies on both its pollination ability in cashew orchards and its management for population increase.

Whichever bee species is managed for pollination of commercial cashew orchards, both *A. mellifera* and *C. tarsata* would need to visit a cashew flower more than once to assure a high level of nut set. Because of the short period of receptivity of cashew stigmas and the high stigma requirement of hermaphrodite flowers for pollen soon after anthesis, cashew growers are recommended to ensure that there are appropriate numbers of pollinators visiting flowers within the 4 h of peak stigma receptivity.

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