Pollen-collecting by stingless bees on cacao flowers

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Summary. Stingless bees *(Trigona jaty)* routinely visit the flowers of *Theobroma cacao* (Sterculiaceae) in the Atlantic lowlands of Costa Rica. The bees collect pollen and behave as pollen thieves in flowers well exposed to direct sunlight in cacao plantations, and avoid flowers in heavy shade. Pollination rates are maximized, however, in heavy shade due to the high abundance of the small-bodied pollinating midges (Ceratopogonidae and Cecidomyiidae) found in such places. Pollen-thieving by stingless bees, therefore, may only impact on fruit set in cacao trees in direct sunlight, with only minimal to no impact in areas of cacao where natural pollinator activity is high.

Key words. Trigona jaty; pollination; cacao; sunlight; shade.

The thieving of nectar and pollen by nonpollinating organisms often comprises a major evolutionary force in breeding populations of some sexually-reproducing plant species[1–3]. A consequence of such foraging behavior by insects and other organisms to plants is often a reduced fitness since effective pollination is circumvented and floral rewards adapted for pollinating organisms often disrupted[4–6]. When a plant population is large and densely packed with sexually-competent individuals, the likelihood of attracting opportunistic nectar or pollen thieves greatly increases since an enhanced floral reward is available[3]. The degree to which such floral larceny[2] impacts upon the evolutionary structure of the plant population will be also a function of the abundance of thieving organisms and the temporal and spatial attractiveness of the flowers for otherwise generalist thief species. While members of the Sterculiaceae, a principally tropical group of trees and shrubs[7], have attractive flowers frequently visited by halictine and stingless bees, the principal pollinating agents of most species are believed to be small-bodied Diptera[8–10]. Herein I report that in commercial plantations of *Theobroma cacao* L. (Sterculiaceae) dense shade sharply reduces the intensity of pollen-collecting flowers by stingless bees (*Trigona jaty* (Smith)) while promoting the activity of small-bodied midges as pollinating agents[11]. Stingless bees collect pollen to provision their nests with food for the rearing of brood. Pollen thieving by these bees is maximized, however, in the absence of shade, a condition which also demotes the activity of pollinators owing principally to thermal and moisture stress[11, 12]. I censused the number of *T. jaty* visiting freshly-opened flowers of *T. cacao* on 80 trees consisting of two rows of 20 trees each of the 'Pound-7' variety bearing markedly albino (white) flowers, and two adjacent rows of the 'UF-613' variety bearing pinkish flowers. The 'Pound-7' variety is self-incompatible, and the 'UF-613' is self-compatible. Cross-pollination by natural agents between these two varieties is facilitated by these *T. cacao* being planted in adjacent rows. These trees are located in the La Lola Cocoa Farm near Siquirres, Limon Province, Costa Rica, and 52 of them (65%) are under shade cover from *Erythrina* trees (Leguminosae) and the remaining 28 trees (35%) in direct sunlight at the end of this plot (equal numbers of both varieties in shade and sunlight). Between 07.00 and 10.00 h, under sunny and dry weather conditions, I counted the numbers of bees on flowers and estimated the number of 'bee visits' within each tree for 3-min periods at each tree. Because approximately 3 min were spent observing flowers in each tree, about 25% of the time I shared observation time between two adjacent trees, depending largely upon the abundance of flowers encountered. Censusing bees was greatly facilitated by the ease with which an observer can approach *T. jaty* foragers without frightening them away, and by the size and coloration of this species making it readily distinguishable from other species. Immediately prior to this census (06.00–07.00 h) a census was taken of the number of freshly-opened flowers on each tree. During the bee census, there was a 60% reduction in the amount of available light within the foliage of the cacao trees under the *Erythrina* canopy relative to the canopy-less adjacent trees also examined for bee activity. On cacao trees shaded by *Erythrina* between 60 and 90% of the flowers are shaded during the morning hours indicated, i.e. with no contact with direct sunlight and partly concealed by shading cacao foliage, as compared to only 10–30% shading of flowers on canopy-less trees. All open flowers on each tree were examined for bees, and the behavior of bees at flowers also noted to corroborate previous observations on this species visiting cacao flowers in both Central and South America[13, 14].

Trigona jaty foragers collect large quantities of cacao pollen during the sunniest hours of the morning[13,14] and an individual bee frequently visits several flowers in a tree before moving on to another tree or returning to the nest[15]. A bee alights on one of the five bulbous 'petal hoods' concealing the five sets of anthers. It then crawls inside the hood and collects pollen from both the freshly-dehisced anther sacs and the inner surfaces of the petal hood. Once completed, the bee exits the petal hood and moves on to an adjacent one and repeats the same behavior. Generally all five sets of anther sacs in a cacao flower are gleaned free of pollen. At least two other species of stingless bees occasionally visit cacao flowers in Costa Rica, but the smaller-bodied *T. jaty* is by far the most abundant bee species exhibiting this behavior[15]. A total sample of 25 *T. jaty* foragers collected from cacao flowers at various sites in Costa Rica revealed that virtually all pollen grains in the corbicula were from cacao flowers. A total of 2079 open flowers were present on the 80 trees studied (25.98 ± 25.91) of which 1172 were UF-613 (29.30 ± 30.54) and the remaining 907 on Pound-7 (22.67 ± 20.12). Analysis (chi-square test) of the abundance of open flowers on shaded vs exposed cacao trees by variety (table 1) revealed significantly more open flowers on exposed trees than expected ($p < 0.001$; $\chi^2 = 52.0$ for UF-613 trees, $\chi^2 = 16.6$ for Pound-7 trees). There

Table 1. The distribution of bees *(Trigona jaty)* on 'white' flowers versus 'pink' flowers* of *Theobroma cacao* (Sterculiaceae) cultivars 'Pound-7' and 'UF-613', respectively, on both shaded and exposed (sunlit) trees at La Lola

Total No. Pound-7 trees (white flowers)	No. open flowers N	$\bar{X} \pm SD$	No. bee visits observed N	$\bar{X} \pm SD$	Total No. UF-613 trees (pink flowers)	No. open flowers N	$\bar{X} \pm SD$	No. bee visits observed N	$\bar{X} \pm SD$
Shaded cacao trees (N = 52)									
26	531	20.42 ± 16.95	13	1.62 ± 0.74	26	644	24.76 ± 17.99	20	1.72 ± 0.78
Exposed cacao trees (N = 28)									
14	376	26.85 ± 25.168	34	3.50 ± 0.97	14	528	37.71 ± 45.38	26	3.25 ± 1.28

* Between these two cultivars, the coloration of the sepals and petals varies greatly, giving one kind (UF-613) distinctly pinkish flowers.

Table 2. Comparative abundance of bees *(Trigona jaty)* on shaded and exposed ('sunny') flowers of *Theobroma cacao* (Sterculiaceae) at 'Finca Experimental La Lola' in eastern Costa Rica. Data given are for both trees in direct sunlight ('exposed', absence of canopy shade cover) and in dense shade provided by *Erythrina* trees*

No. bees seen		No. bee visits**		No shaded flowers visited***		No. sunny flowers visited***	
Total	$\bar{X} \pm SD$	Total	$\bar{X} \pm SD$	Total	$\bar{X} \pm SD$	Total	$\bar{X} \pm SD$
Shaded cacao trees (N = 52)							
26	0.51 ± 0.72	33	1.65 ± 0.74	0	—	33	1.65 ± 0.74
Exposed cacao trees (N = 28)							
43	1.44 ± 1.32	60	3.26 ± 1.19	4	0.14 ± 0.35	56	3.00 ± 1.02
Total cacao trees (N = 80)							
69	0.85 ± 2.07	93	2.43 ± 1.27	4	0.14 ± 0.35	89	2.28 ± 1.09

* The cacao trees censused for floral bee visits are an equal mix of two distinctive cultivars, 'Pound-7' (with whitish flowers) and 'UF613' (with pinkish flowers), arranged in four rows of 20 trees each; bees visiting cacao flowers were censused on 12 March 1984 from 07.00 to 10.00 h under sunny, dry weather conditions. ** The summation of the number of flowers visited per bee in an individual cacao tree; the frequency distribution of which, for 1, 2, 3 and 4 flower visits per bee in a tree within 3-min observation periods, is 42, 21, 3 and 0, respectively for all trees combined. *** Bee visits to both shaded and exposed (sunlit) individual flowers were recorded for both shaded and exposed entire cacao trees, and these data are based upon considering only those flowers visited by bees. Of the 80 trees censused, 20 shaded cacao trees had bee visits, and 19 exposed trees had bee visits during this census.

was also a difference in the number of bee visits to flowers of exposed vs shaded Pound-7 trees; there were significantly more bee visits to flowers of exposed trees vs shaded Pound-7 trees; there were significantly more bee visits to flowers of exposed trees than expected ($p < 0.001$, $\chi^2 = 18.5$). Both the total numbers of bees seen and bee visits estimated were about twice as frequent on exposed (sunlit) trees than on shaded trees and close to 100% of these visits were confined to sunlit (sunny) flowers on both shaded and exposed trees (table 2). These differences are significant (for numbers of bees seen on shaded versus sunny trees, $t = 6.301$ with $df = 35$ and $p < 0.001$). Typically individual bees visit more than one flower in a tree within a 3-min period for 37% of the total bee visits recorded (21 and 3 visits to two and three flowers, respectively, or 24/65 two or more visits), but by far the majority of visits in such a small time interval are for one flower (about 63% of the time, table 2). Multiple flower visits are more than double in frequency on canopy-less cacao trees than on shaded trees (total of 32 versus 13 such visits for exposed and shaded trees, respectively). Had intervals of observing individual bees been extended, undoubtedly the frequency of multiple flower visits would have increased. Foraging *T. jaty* may spend several minutes visiting many flowers within a cacao tree[13]. Because the two varieties of *T. cacao* trees studied were planted in adjacent rows, facilitating the exchange of pollen from the self-compatible trees (UF-613) to the self-incompatible trees (Pound-7) any observed differences in pollination frequencies between midges and bees are the result of self-incompatibility (in Pound-7 trees) appreciably lowering pollination success rate.

My observations in Central America indicate that for at least two other species of *Theobroma* with vivid red flowers, most notably *T. speciosum* (introduced) and *T. simiarum*, bees are most numerous on those cauliflorous inflorescences positioned in direct sunlight during morning hours. Although stingless bees are pollinators of many tree species in Central American forests[16], the evidence for the interaction of *T. jaty* with cacao flowers overwhelmingly supports the role of this organism as a pollen thief rather than as an effective pollinator of this tree species[13,14]. Even though the precise positions of flowers with respect to exposure to direct sunlight were unknown, the data do support the contention that tree position (sunlit versus shaded trees) influences bee visits to cacao flowers. In large experimental cages enclosing entire self-compatible cacao trees (UF677 variety), these bees eventually habituate to cacao flowers but do not pollinate them as evidenced by an absence of fruit set[14]. There is no evidence suggesting that cacao pollinators, midges in the dipteran families Ceratopogonidae and Cecidomyiidae[17,18], directly compete with stingless bees for floral rewards. These pollinators may obtain nectar and fragrance oils from *T. cacao* flowers as well as from flowers of other *Theobroma* species[19]. Although some flower-visiting Ceratopogonidae in the temperate zone feed on pollen[20], I have no evidence for such behavior for midges visiting cacao flowers. By far the greatest taxonomic diversity and population densities of cacao-pollinating midges occur in the most shaded portions of cacao plantations[21–23], and the highest levels of fruit set resulting from pollination often occur on trees under these conditions[11]. Cacao and related species of *Theobroma* largely thrive in dense forest under natural conditions[24] and their flowers are adapted both physiologically and morphologically for pollination by small flying insects such as midges[25]. *Theobroma* pollen possesses features clearly indicating an insect-mediated pollination system[19,26].

More so than variations in floral pigmentation, my data show that shade figures prominently in determining both the foraging behavior of pollen-thieving stingless bees and the likelihood of flowers to be ransacked by these organisms. Although cacao flowers seem not to be physically-damaged as a result of stingless bee pilfering[4], a reduction in the availability of pollen in freshly-opened flowers after pollen-collecting diminishes the likelihood of successful pollination by midges. Diptera involved in the pollination systems of Sterculiaceae often exhibit markedly diurnal cycles in floral visitation activity[27] and bee-ransacked flowers in the morning may have less pollen for midges active near dusk that same day. But the degree to which this condition influences levels of natural pollination is determined by whether or not flowering trees are heavily shaded. *Trigona* bees, on the other hand, while abundant in tropical wet forests[28], may preferentially forage in sunny places in tropical habitats as a means of optimizing energy allocated to foraging in relation to floral rewards[29].

In this study I have not demonstrated a direct causal relationship between positioning of flowers relative to sunlight and bee floral-foraging. However, these data strongly suggest that such a relationship exists. Additionally, when small numbers of *T. jaty* are confined to silk-bolting cloth cages[11] enclosing generously-flowering (self-compatible) cacao tree branches in the morning, two behaviors results: 1) bee flight activity is markedly subdued in cages placed in heavy shade, and 2) bees congregate in sun-flecked places on the sides of cages (N = 6 'trials' with 4–8 bees each between 09.00 and 10.00 h at La Lola).

The findings have important implications for conclusions regarding the impact of nectar and pollen-thieving organism on the breeding systems of plant species. The impact of pollen thieving by bees may vary spatially within a plant population, to the extent that such behavior may have very little consequence to the activity and effectiveness of pollinating organisms in some floral reward patched.

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1 Faegri, K., and Van der Pijl, L., The Principles of Pollination Ecology. Pergamon Press, London 1966.
2 Inouye, D.W., Ecology *61* (1980) 1251.
3 McDade, L.A., and Kinsman, S., Evolution *34* (1980) 944.
4 Barrows, E.M., Biotropica *8* (1976) 132.
5 Hernandez, H.M., and Toledo, V.M., Ann. Mo. bot. Gdn *66* (1979) 512.
6 Young, A.M., Biotropica *15* (1983) 78.
7 Conquist, A., An integrated System of Classification of Flowering Plants. Columbia Univ. Press, New York 1981.
8 Posnette, A.F., Trop. Agricult. (Trinidad) *21* (1944) 115.
9 Entwistle, P.F., Pests of Cocoa. Longman, London 1972.
10 Wellensiek, S.J., Archf Koffiecult. Indonesië *6* (1932) 87.
11 Young, A.M., J. appl. Ecol. *20* (1983) 801.
12 Proctor, M., and Yeo, P., The Pollination of Flowers. Taplinger, New York 1972.
13 Soria, S. de J., Revta Theobroma (Brasil) *5* (1975) 12.
14 Young, A.M., J. appl. Ecol. *18* (1981) 149.
15 Young, A.M., Unpublished observations.
16 Wille, A., A. Rev. Ent. *28* (1983) 41.
17 Winder, J.A., PNAS *24* (1978) 518.
18 Young, A.M., Proc. ent. Soc. Wash. (D.C.) *87* (1985) 49.
19 Young, A.M., Schaller, M., and Strand, M., Am. J. Bot. *17* (1984) 466.
20 Downes, J.A., Trans. R. ent. Soc. Lond. *106* (1955) 439.
21 Kaufmann, T., Turrialba (Costa Rica) *25* (1975) 90.
22 Hernandez, J., Diss. Univ. Wisconsin, Madison 1965.
23 Young, A.M., J. appl. Ecol. *19* (1982) 47.
24 Addison, G.O., and Tavares, R.M., Bolm téc. Inst. agron. N. (Belem, Brasil) *25* (1951) 3.
25 Bystrak, P.G., and Wirth, W.W., USDA Tech. Bull. *1591* (1978).
26 Taylor, A.S., Turrialba (Costa Rica) *10* (1965) 1.
27 Taroda, N., and Gibbs, P.E., New Phytol. *90* (1982) 735.
28 Wilson, E.O., The Insect Societies. Harvard, Cambridge, Mass. 1971.
29 Kevan, P.G., and Baker, H.G., A. Rev. Ent. *28* (1983) 407.

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Microbial methylation of benzenethiols and release of methylthiobenzenes

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Summary. Three phylogenetically diverse microorganisms methylated several different chloro- and nitro-substituted benzenethiols to yield the corresponding methylthiobenzenes. These products were identified by gas chromatography–mass spectrometry. In several cases large percentages of the methylthio products were released by intact cells into the medium, suggesting that microbial methylation of xenobiotic thiols may be a significant biotransformation in many ecosystems.
Key words. Methylthiobenzenes; benzenethiols; xenobiotics; ecosystems.

In recent years it has been recognized in animal and plant systems that many xenobiotics, including drugs and pesticides, are converted to sulfur containing metabolites[3–9], some of which are free thiols or their disulfide derivatives. We have recently discovered that many phylogenetically distinct microorganisms contain the enzyme glutathione-S-transferase that catalyzes formation of thioether conjugates between glutathione and a variety of electrophiles[10]. In one of these organisms, *Tetrahymena thermophila,* metabolism of the fungicide pentachloronitrobenzene is initiated by glutathione-S-transferase and a major excreted metabolite is pentachloromethylthiobenzene[11]. Excretion of the thioanisole follows methylation of pentachlorobenzenethiol by a S-adenosylmethionine-dependent thiol methyltransferase, an enzyme we have purified and characterized from the above organism, from the yeast *Saccharomycopsis lipolytica,* and from the green alga *Euglena gracilis*[12]. Given the nonspecific substrate specificities of the thiol methyltransferases in these organisms[12], it is possible that many methylthio metabolites are released into the environment as a result of microbial transformation of xenobiotic thiols.

During these studies we wondered whether intact microbial cells could take up exogenous thiols and methylate them via the thiol methyltransferase system. To study this possibility whole cells of *E. gracilis, T. thermophila* and *S. lipolytica* were incubated with various aromatic thiols bearing chloro- or nitro-substitutions. These thiols were chosen because they are good substrates for the thiol methyltransferase as measured in vitro[12], and because of the ease of detection of methylated chloro- and nitro-metabolites using gas chromatography coupled to electron capture detection.

Cells were grown as previously described[10,11], harvested in exponential growth by gentle centrifugation, and washed and suspended in 10 mM Tris-Cl, pH 7.4, for *T. thermophila* and *S. lipolytica* or 10 mM potassium phosphate, pH 6.1, for *E. gracilis*. The cell concentrations were determined by hemacytometer counts. Reaction mixtures contained cells in a volume of 5 ml. Aromatic thiols were prepared or obtained commercially as described elsewhere[12]. Thiols were dissolved in acetone and added to the incubation mixtures at concentrations of $1–5 \times 10^{-5}$ M (0.1% v/v final acetone concentration), and the mixtures shaken gently for 3 h at 25 °C. At the end of the incubation cells were pelleted in a clinical centrifuge and resuspended in fresh buffer. Both the resuspended cells and reaction medium were extracted with 5 ml hexane (pesticide grade, Burdick and Jackson Laboratories, Muskegan, MI) twice after adding 50 µl of 10 N NaOH; for determinations of total product the centrifugation step was omitted. The hexane layer was analyzed for the methylthio products by gas chromatography on a 1.8 m column packed with SP2250 on 100/120 Supelcoport (Supelco Inc., Bellefont, PA). The oven temperature was 180 °C, the detector temperature was 250 °C and the injection port temperature was 200 °C. A nitrogen flow rate of 25 ml/min was used. Control reaction mixtures without added thiol or with boiled cells added were treated identically. Quantitation of methylthio products was accomplished by measuring peak areas, compared to peak areas of authentic methylthio derivatives prepared as described elsewhere[12].

The production of methylthiobenzene derivatives of three substituted benzenethiols by whole cell incubations with *T. thermophila* is shown in the figure. The major product in each case (compared to boiled cell controls) had a retention time identical to the authentic methylthio derivative run at the same time. The amount of each methylthiobenzene recovered was dependent on the length of incubation, the number of cells present